

**GENETIC AND MOLECULAR ANALYSIS OF BLAST
RESISTANCE IN RICE (*Oryza sativa* L.)**

A thesis submitted to

Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722

Dist. Ahmednagar (M.S.), India

by

Kumbhar Shailesh Dasharath

(Reg. No. 09/18)

In partial fulfilment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

AGRICULTURAL BOTANY

(GENETICS AND PLANT BREEDING)

DEPARTMENT OF AGRICULTURAL BOTANY

POST GRADUATE INSTITUTE

MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI – 413 722

DIST. AHMEDNAGAR (M.S.), INDIA

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2013

CANDIDATE'S DECLARATION

I hereby declare that this thesis or a part thereof has not been submitted by me or any other person to any other University or Institute for degree or diploma.

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C E R T I F I C A T E

This is to certify that the thesis entitled “**Genetic and molecular analysis of blast resistance in rice (*Oryza sativa* L.)**” submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra (India) in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **AGRICULTURAL BOTANY (GENETICS AND PLANT BREEDING)** embodies the results of a *bona fide* research work carried out by **Shri. Kumbhar Shailesh Dasharath** under my guidance and supervision and no part of the thesis has been submitted for any other degree or diploma or publication in any other form.

The assistance and help received during the course of this investigation and sources of literature referred have been acknowledged.

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C E R T I F I C A T E

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LIST OF ABBREVIATION

%	:	Percentage
Σ	:	Summation of
σ^2_e	:	Environmental variance
σ^2_g	:	Genotypic variance
σ^2_p	:	Phenotypic variance
/	:	Per
-	:	Minus
$\sim$	:	Approximate
+	:	Plus
<	:	Less than
=	:	Equal to
>	:	More than
$\times$	:	Multiplication
$\leq$	:	Less than or equal to
$\geq$	:	More than or equal to
$^{\circ}\text{C}$	:	Degree Celsius
a.m.	:	Ante meridiem
AFLP	:	Amplified fragment length polymorphism
APS	:	Ammonium per sulphate
ARS	:	Agricultural Research Station
B_1	:	Susceptible bulk
B_2	:	Resistant bulk
BC_1	:	First back cross generation
BC_2	:	Second back cross generation
bp	:	Base pair
BSA	:	Bulk segregants analysis
C.D.	:	Critical difference
C.V.	:	Coefficient of variance
CAPS	:	Cleaved Amplified Polymorphic Sequences
cm	:	Centimeter
cm^2	:	Square centimeter
cM	:	Centi morgan
Cov.	:	Covariance
CTAB	:	Cetyl Tri-methyl Ammonium Bromide

ddwater	:	Double distilled water
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxynucleoside triphosphates
EDTA	:	Ethylene diamine tetra acetic acid
<i>et al.</i>	:	et alli (Co-workers)
etc.	:	Etcetera
F ₁	:	First filial generation
F ₂	:	Second filial generation
F ₃	:	Third filial generation
Fig	:	Figure
g	:	Gram
G.A.	:	Genetic advance
GCV	:	Genotypic coefficient of variation
h ² (b.s.)	:	Heritability in broad sense
i. e.	:	that is
ISSR	:	InterSimple Sequence Repeat
Kg/ ha	:	Kilogram per hectare
L:B ratio	:	Length to breadth ratio
M	:	Molar
M.S.	:	Maharashtra
MAS	:	Marker assisted selection
mg	:	Miligram
min.	:	Minute
ml	:	Mililitre
mm	:	Millimeter
mM	:	Millimolar
ng	:	Nano gram
nm	:	Nanometer
no.	:	Number
P ₁	:	First parent (Susceptible)
P ₂	:	Second parent (Resistant)
PAGE	:	Polyacrylamide gel electrophoresis
PCoA	:	Principle co-ordinate analysis
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic coefficient of variation
PDI	:	Per cent Disease Intensity
pH	:	Potenz (-log [H ⁺])

QTLs	:	Quantitative trait loci
R	:	Resistant
RAPD	:	Randomly amplified polymorphic DNA
RDN	:	Radhanagari
RFLP	:	Restriction fragment length polymorphism
rpm	:	Revolution per minute
S	:	Susceptible
S.E.	:	Standard error
SCAR	:	Sequence Characterized Amplified Regions
SES	:	Standard Evaluation System
Sg	:	Segregating
SSR	:	Simple Sequence Repeat
STS	:	Sequence Tagged Sites
TBE buffer	:	Tris-borate-EDTA buffer
TE buffer	:	Tris-EDTA buffer
U	:	Unit
UPGMA	:	Unweighted Pair Group Method using Arithmetic Averages
UV	:	Ultra-violet
V	:	Volt
v/v	:	Volume by volume
Var	:	Variance
Var.	:	Variety
Viz.,	:	Namely
w/v	:	Weight by volume
µg	:	Microgram
µl	:	Microlitre
2D	:	Two dimension

ABSTRACT

Genetic and Molecular Analysis of Blast Resistance in Rice (*Oryza sativa* L.)

by

KUMBHAR SHAILESH DASHARATH

A candidate for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

Post Graduate Institute

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2013

Research Guide	:	Dr. J. V. Patil
Department	:	Agricultural Botany
Discipline	:	Genetics and Plant Breeding

The present investigations entitled, “Genetic and molecular analysis of blast resistance in rice (*Oryza sativa* L.)” was conducted at Agricultural Research Station, Radhanagari, Dist. Kolhapur; Agricultural Research Station, Lonavala, Dist. Pune and State Level Biotechnology Centre, MPKV, Rahuri during the period 2010 to 2012 with an objective to study genetic and molecular diversity and to identify QTLs and the linked molecular marker(s) for blast resistance in rice.

Sixty-five rice genotypes were evaluated in randomized block design with two replications at two locations viz., ARS, Radhanagari and ARS, Lonavala during *Kharif* 2010 to assess genetic diversity. Observations were recorded on twelve agro-morphological characteristics viz., days to 50 per cent flowering, days to maturity, plant height, panicle length, spikelets per panicle, spikelet fertility, tillers per plant, test weight, grain length, grain breadth, length : breadth ratio and seed yield per plant. Appreciable amount of variability was observed for all the characters except spikelet fertility (%). The GCV and PCV were highest for the character length : breadth ratio, followed by test weight. Heritability in broad sense was very high for all the traits. Genetic advance was highest for spikelets per panicle, followed by plant height. The D^2 value ranged from 4.21 to 24015.45, exhibiting substantial diversity among the genotypes under study. Sixty-five

Abstract cont...

S. D. Kumbhar

genotypes were grouped into 10 clusters. Cluster I was largest, followed by cluster III and cluster IV. The inter-cluster distance (D^2) ranged from 184.69 to 19165.63. The variance of cluster means revealed that test weight, grain length and length : breadth ratio had contributed major part of divergence.

To study the diversity for pathological traits related to blast disease in rice these genotypes were evaluated in randomized block design with two replications at blast “hot spot” location, ARS, Lonavala in uniform blast nursery during *kharif* 2010. Observations were recorded for infection type, diseased leaf area, lesion density, lesion size and per cent disease intensity. High variability was observed for all the pathological characters under study. The GCV and PCV were highest for the character lesion size, followed by lesion density. Heritability in broad sense was very high for all the traits, whereas genetic advance was highest for per cent disease intensity.

The D^2 value ranged from 0.03 to 1496.95, exhibiting substantial diversity among the genotypes under study. Sixty-five genotypes were grouped into 7 clusters. Cluster I was largest, followed by cluster II and cluster IV. The inter-cluster distance (D^2) ranged from 35.28 to 1273.78. The variance of cluster means revealed that per cent disease intensity, lesion density and diseased leaf area had major contribution towards total divergence.

The 50 genotypes selected from 65 genotypes studied for field evaluation, by discarding 15 most similar genotypes based on the field diversity analysis, were assessed for molecular evaluation. Among 25 ISSR and 17 SSR markers used 13 ISSR and 15 SSR markers were polymorphic, producing 103 and 187 amplicons, respectively among which 100 ISSR (97.08 %) and 178 SSR (95.18 %) amplicons were polymorphic. The similarity coefficient between the genotypes ranged from 0.31 to 0.90 for ISSR and 0.11 to 0.87 for SSR analysis. The UPGMA based clustering analysis using Dice similarity coefficient grouped 50 genotypes in three and four major clusters in ISSR and SSR analysis, respectively.

The pooled analysis of ISSR and SSR data reported the range of similarity coefficient from 0.25 to 0.85. Landraces reported more diversity, whereas the improved varieties had more similarity among them. Highest similarity was reported between the improved varieties Pawana and RTN 1, followed by Phule Maval and Phule Samruddhi. Clustering analysis based on the pooled analysis grouped 50 genotypes in five major clusters. Among the three methods used for molecular diversity studies, pooled analysis was more effective in distinguishing rice genotypes based on their genotypic constitution, parental relationship and evolutionary mechanisms.

Based on morphological, pathological and molecular diversity studies the genotypes Phule Samruddhi, Bhogavati, RDN 99-12, RDN 98-2-3-5-14, LK 248, Vivek Dhan 82, Siddhagiri, Diwani, Shyamjeer and Pusa Basmati 1 are suggested for future breeding programme.

F₂ population of a cross between a blast susceptible genotype EK 70 and a resistant RDN 98-2-3-5-14 was evaluated for blast at “hotspot” location, ARS, Lonavala during *kharif* 2011. The randomly selected 150 F₂ plants showed 111 resistant and 39 susceptible plants. The chi-square (χ^2) test to the expected segregation ratio of 3:1 for single gene model was non-significant indicating the resistance in RDN 98-2-3-5-14 is governed by single dominant gene.

Bulk segregants analysis method was employed to identify molecular markers associated with blast resistance in rice. Among the 25 SSR markers used, 14 were polymorphic among the parents, whereas two markers viz., RM204 and RM215 were polymorphic between susceptible and resistant parent and respective bulks indicating their possible association with blast resistance in rice.

The marker RM204 reported fragment of ~174 bp in 33 plants, of ~110 bp in 41 plants and both fragments in 73 F₂ plants. The chi-square (χ^2) analysis for a single gene model and simple regression analysis with phenotypic data indicated that the marker RM204 was significantly linked with the blast resistant gene in present population. The marker RM215, although was polymorphic in bulk segregants analysis, was found not associated with blast resistance in present investigation. The marker RM204 can be used for molecular analysis of segregating generations, breeding lines and varieties for blast resistance in rice. This approach will take less time and resources than field evaluation and could prove useful in marker assisted selection in the absence of appropriate testing conditions.

CHAPTER I

INTRODUCTION

Rice is the world's largest food crop, providing the caloric needs to millions of people daily. The genus *Oryza* is classified under the tribe Oryzeae, subfamily Oryzoideae, of the grass family Poaceae (Gramineae). There are two distinct types of domesticated rice, *Oryza sativa* or Asian rice and *Oryza glaberrima*, African rice and more than 20 wild species distributed throughout the tropics and subtropics. The Asian cultivated rice (*O. sativa*) is an economically important crop that is the staple food for more than half of the world population. All members of the *Oryza* genus have $n = 12$ chromosomes and though interspecific crossing is possible within each complex, it is difficult to recover fertile offspring from crosses across complexes. *Oryza sativa* is distributed globally with a high concentration in Asia (Vaughan *et al.*, 2003).

Rice is grown in 114 countries worldwide, constituting nearly 11 per cent of world's cultivated land (Rai, 2006). India, China, Indonesia, Bangladesh, Thailand, Vietnam, Myanmar, Philippines, Brazil and Pakistan are the major rice producing countries of world contributing 85 per cent and 89 per cent of world area and production, respectively (Rai, 2006). India is world's second largest producer and consumer of rice, next to China, producing 143.9 million tonnes paddy and around 100 million tonnes rice from 42.70 million hectare area with the average productivity of 3.37 tonnes per hectare (Anonymous, 2012 a). Rice is grown in almost all the states of India. West Bengal is the largest rice producing state in India contributing nearly 15.59 per cent to the national production followed by Uttar Pradesh and Andhra Pradesh. In Maharashtra rice, is cultivated on an area of about 15.14 lakh hectare with an annual production of around 27.12 lakh tonnes with average productivity of 1.79 tonnes per hectare (Anonymous, 2012 b). Rice production in India accounts for almost 22 per cent of the total world production. However, to meet the demand of the increasing population and maintaining self sufficiency, the present production level needs to be increased up to 140 million tonnes by 2025 (Anonymous, 2004). Predicted increase in water scarcity and decrease in arable land, the constant battle against new emerging pathogens and

pests and reduced quality due to possible adverse effects from climate change will pose greater challenges for rice breeders and agricultural scientists to satisfy the growing demand (Khush, 2005).

Rice, though traditionally a rainy season crop in India, is also increasing in acreage in winter season. It is now grown almost round the year in one or the other part of country and is broadly classified as *Aman*, *Aus* and *Boro* depending on the season of cultivation. With its long history of cultivation and selection under diverse environments, rice acquired wide adaptability enabling it to grow in a range of environments, from deep water to swamp, irrigated and wetland conditions as well as on dry hill slopes. Probably far more than any crop, rice can grow under diverse geographical, climatic and cultural conditions. It grows at more than 3000 m elevation and even 3 m below sea level.

Rice is subjected to attack by large number of pests and pathogens causing number of serious diseases which under epidemic conditions become epiphytotic and thereby causing serious losses. Among diseases, rice blast caused by *Magnaporthe grisea* (Hebert) Barr. (Barr, 1977) (anamorph : *Pyricularia oryzae*) (Rossman *et al.*, 1990) is one of the most devastating and destructive diseases of rice worldwide (Zeigler *et al.*, 1994). Under heavy dew, arial plant parts are affected. Leaf surface becomes speckled with oval lesions and plants are liable to lodging if stems are infected. If the panicle is infected, it results in severe yield loss (Ou, 1985). It is one of the earliest known destructive rice diseases widely distributed in every region of the world where rice is grown. Blast fungus infects both temperate and tropical rice grown under different ecosystems (i.e. upland, lowland, irrigated and rainfed) and causes more damage in areas where high input rice cultivation is practiced. Infection normally begins when a blast spore germinates and penetrates the rice leaf, resulting in lesions on the rice plant and ends when the fungus sporulates repeatedly for an extended time, dispersing many new airborne spores. In India the damage due to this disease is estimated as high as 75 per cent (Padmanabhan, 1959). An estimated quantity of 157 million tonnes of rice was lost between 1975 and 1990 worldwide due to blast alone (Baker *et al.*, 1997).

Disease control worldwide has relied on the combination of chemical fungicides and integrated management. For the last several years, plant breeding has achieved significant progress towards the enhancement of host resistance to this disease but the development of durably resistant cultivar is yet to be realized. The use of resistant rice cultivars is a powerful tool to reduce the use of environmentally destructive pesticides. Using classical plant breeding techniques, plant breeders have developed a number of blast resistant cultivars adapted to different rice growing regions worldwide. However, the rice industry remains threatened by blast disease because of the instability of the rice blast fungus. Recent advances in rice genomics provide additional tools for plant breeders to develop rice production systems that could be environmentally benign.

Plants protect themselves against pathogen by a combination of weapons like structural defence mechanisms that act as physical barrier and inhibit the pathogen from gaining entries and biochemical reactions that take place in the cells and tissues. The combination of structural characteristics and biochemical reactions employed in the defense of plant are different in different host-pathogen system. In addition, plants have evolved diverse recognition systems to detect proteins produced during infection by specific strains of pathogens. These pathogen proteins, designated as effectors proteins, are recognized by plant disease resistance (R) proteins in a specific manner first described genetically as the gene-for-gene interaction (Flor, 1971). Good amount of variability has been reported in rice crop for various yield and blast resistance traits. In view of this fact, basic work on breeding for resistance and screening of different genotypes at hot spot is the long term solution to avoid the outbreak and endemic spread of this blast disease.

In a rice improvement programme, it is the germplasm which virtually determine the success and nature of end product. The development of superior rice population involves the intelligent use of available genetic variability both indigenous as well as exotic to cater the need of various farming situations of rice. Breeding strategy is primarily influenced by the choice of germplasm. Any wrong choice of germplasm to initiate the selection process results in the wastage of resources. The pace and magnitude of genetic improvement generally depends on

the amount of genetic variability present in the population. Majority of economically important characters including grain yield, grain quality traits and disease and pest resistance are amenable for genetic improvement through *inter se* breeding among genetically diverse parents. Murthy and Arunachalam (1966) stated that multivariate analysis with “Mahalanobis D^2 statistics” is a powerful tool to know the clustering pattern to establish the relationship between genetic and geographical divergence and to determine the role of different quantitative characters towards the maximum divergence.

It is essential to find out the relative magnitude of additive and non-additive genetic variances, heritability and genetic gain with regard to the characters of concern to the breeder. The systematic breeding programme involves the steps like creating genetic variability, practicing selection and utilization of selected genotypes to evolve varieties. The large spectrum genetic variability in segregating populations depends on the level of genetic diversity among genotypes which offer better scope for selection. Robinson *et al.* (1949) emphasized that heritability of the characters is the main concern to the breeder, since it indicates the possibility and extent to which improvement is possible through selection. It has been suggested that heritability together with genetic advance will bring out the genetic gain expected from selection (Johnson *et al.*, 1955). Estimates of GCV, PCV, heritability and genetic advance will play an important role in rice improvement programmes.

Resistance to the pathogen is a classic gene-for-gene system, where a major resistance gene is effective against *Magnaporthe grisea* strains containing the corresponding avirulence gene (Flor, 1956; Silue *et al.*, 1992). Blast resistance is governed by a specific interaction of a particular resistance (R) gene in rice with a particular avirulence gene in the pathogen. Since the initial definition of the plant resistance (R) genes by Flor (1942), many R genes have been identified. Rice containing a *Pi* gene confers resistance to a *Magnaporthe grisea* race in a gene-for-gene manner. So far, about 73 major blast resistant (R) genes have been identified, many of them have been mapped on different rice chromosomes, and tightly linked DNA markers have been identified (Ballini *et al.*, 2008). The DNA markers have been used effectively to identify resistance genes and MAS has been applied for

integrating different resistance genes into rice cultivar lacking the desired traits. Most R genes recognize pathogen effectors produced by pathogens to inhibit defense, although there are a few exceptions (e.g., Lee *et al.*, 2009). Some of these effectors thus correspond to the initial definition by Flor of the avirulence gene. Depending on the presence/absence of these R genes and of the matching avirulence product, the interaction will be incompatible (plant is resistant) or compatible (plant is susceptible).

Molecular markers are essential for mapping genes of interest, marker-assisted breeding and cloning genes by map-based cloning. Commonly used molecular markers include restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs) and cleaved amplified polymorphic sequences (CAPs) (Mohan *et al.*, 1997; Kumar, 1999). Among these, PCR based SSR markers are preferable, because they are simple, co-dominant, multi-allelic, chromosome specific, highly informative and have no need of restriction digestion (Parida *et al.*, 2006). SSR markers are valuable tools for introgression and pyramiding of the blast resistance genes into blast susceptible cultivars. Breeding of rice cultivars with durable resistance is a priority in crop improvement and SSRs have been used for this purpose (Zeigler *et al.*, 1994; Hittalmani *et al.*, 2000).

Microsatellites or SSRs are repeat sequences of 2-6 nucleotides (motif), which are abundant, dispersed throughout the genome and show higher level of polymorphism. Primer pairs are designed based on the unique flanking sequence for amplification of a particular microsatellite repeat. SSR markers are co-dominant, multi-allelic and are being routinely used in mapping and tagging, genome analysis and diversity studies. Due to their easy detection and co-dominant nature they have become the marker of choice for genetic mapping and genome analysis (McCouch *et al.*, 1997). More than 20,000 microsatellite markers have been identified and mapped in rice. The sequence and locus information for these markers is available at <http://www.gramene.org>.

In view of these facts, it was felt necessary to study the genetic variability at field condition and at molecular level by using ISSR and SSR markers in different genotypes and in segregating population of rice. The present investigation was, therefore, undertaken with following objectives :

1. To study molecular diversity in the genotypes using molecular markers.
2. To study genetic variability and divergence in the genotype for different traits in rice.
3. To identify the QTLs and the linked molecular marker(s) for blast resistance in rice.

CHAPTER II

REVIEW OF LITERATURE

Improvement of both qualitative and quantitative characters of a crop is the main aim of any breeding programme. The information on genetic architecture of various quantitative traits contributing to yield and quality is useful in planning the breeding strategy. Various biometrical techniques have been developed for studying the genetics of quantitative traits. This information can be used for developing more refined and efficient approach to select the parents.

The present investigation is aimed at the assessment of genetic variability, genetic diversity and molecular diversity in rice genotypes and identification of molecular markers associated to blast resistance in rice. Hence, the literature on these aspects has been reviewed and presented in this chapter.

2.1 Genetic variability

Variability refers to the presence of differences among individuals of plant population. It results due to differences either in the genetic constitution of the individuals of the population or in the environments in which they are grown. Most of the quantitative plant characters are highly influenced by environmental conditions. The progress of breeding in such a population is primarily conditioned by the magnitude, nature and interaction of genotypic and environmental variability in various plant characters. Hence, an insight into the magnitude of genetic variability present in a population is of paramount importance to plant breeder for initiating a breeding programme in any crop including rice.

Johannsen (1909) in his famous 'pure line theory' suggested that both heritable and non heritable changes affect the somatic variation and that the variation in the pure line is entirely due to environment. The need to partition the phenotypic variability into genetic and non-genetic components was proposed by Nilson Ehle (1909) and East (1916). The observed variation in the population is the result of interaction of genetic and the environmental factors. The effects due to these two factors are found to be blended together and hence, cannot be resolved directly. Fisher (1930) first presented the method to separate these effects due to

the above two factors. The extent of genotypic variability was presented by him with appropriate statistical method and the value was expressed as a genotypic coefficient of variation. He also considered it as very useful to know how much genetic variation is heritable.

Genetic variance was separated from total variance by use of estimates of environmental variance in the non-segregating population by Charles and Smith (1939). Genotypic coefficient of variation indicates the relative magnitude of genetic diversity present in the material and helps to compare the genetic variability present in different characters.

Burton (1952) suggested genetic coefficient of variation as a measure of the extent of genetic variability present in a population for a particular character whereas, the phenotypic coefficient of variation measures the extent of total or phenotypic variability. He emphasized the utility of estimates of genetic variance components as a basis for prediction of the response of quantitative characters for selection in breeding programme. He proposed that heritability in narrow sense is more valuable in crop improvement programme.

Many research workers have carried out variability studies in rice. A brief review of it is given below.

Chauhan and Tandon (1984) studied thirty hill rice varieties and reported higher genotypic coefficient of variation for grain yield, plant height, tiller bearing ears and number of grains per panicle and low for days to flower, days to maturity, length of panicle and per cent spikelet fertility in rice. Wide variations for different characters were reported by Amirthadevarathinam (1990) among rice genotypes of *indicas* and *japonicas*. The maximum range of variation was observed for grains per panicle followed by culm length, days to 50 per cent flowering, seedling height, plant height, tiller number and panicle length.

Significant variation for yield contributing traits like number of tillers, number of productive tillers, number of grains per panicle, 1000 grain weight and grain yield was reported by Reddy *et al.* (1995). Reddy and De (1996) observed that grain yield per hill, number of grains per panicle and panicle weight exhibited

higher estimates of GCV and PCV, indicating the importance of these traits in selection.

Higher phenotypic and genotypic coefficient of variation for grain length and length : breadth (L:B) ratio was reported by Marekar and Siddique (1997) in the study of 73 rice varieties. Borbora and Hazarika (1998) evaluated 30 genotypes of rice for 11 yield-related traits and reported highly significant variation for different characters. The differences between genotypic and phenotypic coefficient of variation were relatively low for almost all the characters except grain yield per plant. High to moderate genotypic coefficient of variation was recorded for number of filled grains per panicle, 1000 grain weight, grain yield per panicle and number of secondary branches per panicle.

Vange *et al.* (1999) observed significant variation for most of the traits in the study of 10 rice genotypes. High range of variation was reported for grain yield per hectare, 1000 grain weight and number of panicles per square meter.

A relatively low magnitude of differences between GCV and PCV for all the traits, except number of sterile spikelets per panicle was observed by Verma *et al.* (2000). High GCV and PCV for spikelet per panicle, grain yield and harvest index in nine rice cultivars of Chattisgarh was reported by Yadav (2000). Bidhan *et al.* (2001) evaluated 25 medium duration genotypes of rice for eight traits and observed high phenotypic and genotypic variances for grain yield, followed by number of filled grains per panicle.

The PCV was comparatively higher than the corresponding GCV for 10 characters studied in a population of 42 progenies derived from seven crosses in segregating generations by Kumar *et al.* (2001). Roy *et al.* (2001) observed high phenotypic and genotypic variances for grain yield followed by number of filled grains per panicle, whereas Nayak *et al.* (2002) observed high estimates of GCV and PCV for number of spikelets per panicle, number of panicles per plant, number of grains per panicle and grain yield per plant.

In the diversity studies of 200 scented rice genotypes including a non-scented check Ratna, Naik *et al.* (2002) reported that the GCV and PCV values were high

for panicle number, number of filled grains per panicle and grain yield per plant. Medium to low values for GCV and PCV were observed for plant height, panicle length, 1000 grain weight, grain length, Length : breadth ratio and days to 50 per cent flowering. Pandey and Awasthi (2002) observed significant genetic variability among 21 genotypes of aromatic rice for yield contributing traits, viz., plant height, days to 50 per cent flowering, total number of tillers per plant, panicle length, number of grains per panicle, test weight, grain length, grain breadth and grain yield per plant. They concluded that all the yield attributing traits played major role in enhancement of grain yield. Singh *et al.* (2002) evaluated 52 genotypes of lowland rice for 15 characters and reported high genotypic and phenotypic variances for grain yield per plant, panicle weight, number of grains per panicle and number of branches per panicle, medium GCV and PCV for panicle length, 1000 seed weight and low for panicle length and milling percentage.

Evaluation of 55 rice cultivars and their 42 crosses for phenotypic variation in yield and yield components by Kumari *et al.* (2003) revealed that tiller number, number of grains per panicle and boot leaf length exhibited high phenotypic and genotypic coefficients of variation. Vivek *et al.* (2004) estimated genetic variability for 12 characters in 39 “new plant” tropical japonica lines along with three control cultivars and concluded that grain yield per plant, biological yield per plant, number of tillers per plant and panicles per plant had high values of GCV and PCV. Mall *et al.* (2005) recorded a wide range of variation for mean values of plant height, days to panicle initiation, days to 50 per cent flowering, number of tillers per plant, number of panicles per plant, number of spikelets per panicle, panicle length, flag leaf length and flag leaf width.

High GCV and PCV were recorded for number of unfilled grains per panicle, unfilled grain percentage, grain yield per plant, 1000 grain weight, number of ear bearing tillers per plant and number of filled grains per panicle by Patil and Sarawgi (2005).

Assessment of the nature and magnitude of genetic variability for seven characters in 20 rice genotypes including a local check by Singh *et al.* (2005)

revealed high estimates of GCV and PCV for grain yield per hectare, biological yield per hectare, panicle density per square meter and harvest index.

Vaithiyalingan and Nadarajan (2006) observed significant differences between the F_2 populations of different inter sub-specific crosses for all the characters studied, while grain yield showed high genotypic coefficient of variation followed by spikelet fertility, productive tillers per plant and number of grains per panicle.

Evaluation of 56 high yielding rice genotypes from various eco-geological conditions prevailing in Bangladesh, China, India, Indonesia, Malaysia, Pakistan, Philippines and Sri Lanka for yield and quality characters by Vanaja and Babu (2006) indicated the presence of significant variation for grain yield, grain length, grain breadth, L:B ratio and other quality characters. Kumar *et al.* (2007) recorded high GCV and PCV for number of filled grains per panicle, 100 grain weight, biomass per plant and grain yield per plant in the F_2 and F_3 material of six crosses of rice. Similarly, Raut *et al.* (2009b) reported highest GCV and PCV for seed yield per plant followed by 1000 grain weight, grains per panicle and effective tillers per plant among 40 rice genotypes.

2. 2 Heritability and genetic advance

Heritability is a measure of resemblance between relatives. To the plant and animal breeders, heritability estimates are important because they provide information on the transmission of characters from parents to offspring. The concept of heritability is increasingly used in plant breeding to estimate the contribution of genetic variance to the total variance. Such estimates facilitate accurate evaluation of hereditary and environmental effects in phenotypic variation and thus aid in selection. Genetic advance measures the expected gain from the selection applied in the population. Heritability along with genetic advance gives the best picture of the efficiency of selection.

Lush (1949) classified heritability into broad sense and narrow sense heritability. Broad sense heritability means the ratio of genotypic variance to the phenotypic variance and the narrow sense refers to the ratio of additive variance to the phenotypic variance. Robinson *et al.* (1949) defined heritability as the additive

genetic variance in percentage of total variance. According to them, additive genetic variance indicates the degree to which the progenies are likely to resemble the parents. Johnson *et al.* (1955) suggested that heritability in conjunction with genetic advance was more effective and reliable in predicting the resultant effect of selection. Robinson (1966) classified heritability estimates in three categories, viz., low heritability (5 - 10%), medium heritability (10 - 30%) and high heritability (> 30 %).

The heritability values are most important to plant breeders because they indicate that the selection of parents bearing particular measurements will produce offspring of similar phenotypes. Thus, the improvement in valuable traits depends upon a fairly accurate evaluation of the heritability. Further, genotype $\times$ environment interaction is that in which the relative performance of different genotypes vary with environment in which the genotypes are placed. The genotypic variances as a cause for phenotypic differences may arise from a variety of sources, like additive factor, dominant effect, epistasis or interaction effects (Strickburger, 1968).

Reddy and De (1996) reported heritability from 43.60 to 94.96 per cent for 12 agro-morphological characters. They observed high heritability for grain length followed by 1000 grain weight, grain breadth, plant height, panicle weight, grain yield and number of grains per panicle in rice.

High heritability estimates for plant height, 100 seed weight, number of tillers per plant, spikelet density and panicle length were reported by Ali *et al.* (2000). Genetic gain relative to the mean was higher for number of tillers per plant, plant height and spikelet density. Thakur *et al.* (2000) reported high heritability coupled with high genetic advance for biological yield and panicle weight in F_2 progenies of the cross Anupama $\times$ IR 50, whereas Verma *et al.* (2000) recorded high to moderate genotypic coefficient of variation and high heritability coupled with high genetic advance for plant height, days to flowering and 100 grain weight.

Appreciable amount of heritability and genetic advance was observed for total grains per panicle, total grains per plant and grain yield per plant in the study of 15 rice genotypes by Yadav (2000). Bidhan *et al.* (2001) recorded heritability

range from 50 per cent for grain yield per hill to 90 per cent for grain breadth. Number of filled grains per panicle, 1000 grain weight, grain length and grain breadth exhibited less environmental effect and high heritability with moderate to high genetic advance.

Genetic advance as a percentage of mean was higher for number of filled grains per panicle (70.34) followed by grain yield (68.72), while high heritability coupled with moderate to high genetic advance was observed for number of grains per panicle, 1000 grain weight, grain length and breadth indicating less environmental effect (Roy *et al.*, 2001). Higher heritability for days to 50 per cent flowering, plant height and 1000 grain weight was reported by Kumar *et al.* (2001) while studying population of 42 progenies derived from seven rice crosses in segregating generations. Characters like plant height, grain L:B ratio and 1000 grain weight showed moderate genetic advance.

In a study involving 200 scented genotypes and a non-scented check, Naik *et al.* (2002) reported high heritability estimates for all the characters studied with a range of 71.4 per cent (panicle number per plant) to 99.5 per cent (days to 50 per cent flowering). The genetic advance expressed in percentage of mean was high for number of grains per panicle, number of spikelets per panicle and grain yield per plant. Nayak *et al.* (2002) observed high heritability and high genetic advance for number of spikelets per panicle, number of grains per panicle and grain yield per plant indicating less environmental effect in these characters.

Evaluation of 52 lowland rice genotypes from Meghalaya by Singh *et al.* (2002) revealed a broad sense heritability in the range of 3.61 per cent (number of effective tillers per plant) to 99.55 per cent (grain length). High heritability with high genetic advance was recorded for number of grains per panicle followed by panicle length and grain yield per plant. Kumari *et al.* (2003) reported high heritability and genetic advance for tiller number, number of grains per panicle and boot leaf length. Plant height, panicle length and 1000 grain weight showed high heritability coupled with moderate genetic advance, indicating the role of non-additive gene effect in the inheritance of these traits.

High heritability along with high genetic advance was reported by Satish *et al.* (2003) for number of spikelets per panicle, number of grains per panicle and grain yield per plant in a study comprising of 200 scented rice genotypes. They concluded that emphasis should be given on these characters while selecting scented rice varieties for the improvement of grain yield. Mall *et al.* (2005) reported high heritability coupled with high genetic advance for plant height, number of tillers per plant, number of panicles per plant, number of spikelets per panicle and flag leaf length. Similarly, Singh *et al.* (2006) recorded high heritability and high genetic advance for plant height indicating the predominance of additive gene action for this trait.

High heritability estimates coupled with high genetic advance as percentage of mean was reported by Kumar *et al.* (2007) for number of filled grains per panicle, 100 grain weight, biomass per plant and grain yield per plant in F_2 and F_3 segregating population of six crosses. They concluded that these populations could be subjected to simple pure line selection to improve grain yield per plant. Raut *et al.* (2009b) reported high heritability coupled with high genetic advance for grains per panicle, plant height, days to maturity and 1000 grain weight, suggesting the additive genetic control in the inheritance of these characters.

2.3 Genetic divergence

The use of multivariate analysis to measure the degree of divergence among the population helps to understand the pattern of evolution and to assess the contribution of different characters towards the total divergence along with nature of expression at inter and intra-cluster levels. Further, such studies have been carried out extensively in various crops to identify the choice of distinct divergent parents for obtaining recombination in segregating populations. This is possible with a generalized distance between any two pairs of groups as suggested by Mahalanobis (1936). Early workers regarded the geographical isolation as a reasonable index to measure genetic diversity (Vavilov, 1926). The varieties, which come from different localities are usually presumed to be diverse and are utilized in breeding programme. However, several workers in different crop species have emphasized that there is no parallelism in geographical distribution and genetic diversity (Murthy and Anand, 1966 in linseed; Bhatt, 1970 in green gram, Maurya

and Singh, 1977 and De *et al.*, 1992 in rice) advocating that varieties with the same geographical origin could have undergone changes under selection pressure. Thus, for estimation of variation within the germplasm, divergence study in the form of classification into different homogenous groups is an important practice. With the concept realized by many research workers that geographical isolation may not be the only reason for genetic divergence, Mahalanobis D^2 statistic becomes an useful statistical tool to measure it. Analysis of divergence has been used by plant breeders and it has helped them to classify their genotypes into useful groups. Rao (1948) suggested more flexible method which could replace the measurements on a large number of characters, all of which contribute in some degree or other towards discrimination by relatively few measurements.

Murthy and Arunachalam (1966) in the assessment of nature of genetic divergence in relation to breeding systems reported that the geographical distribution and genetic diversity could not be directly related in any of the crop examined. They suggested that genetic drift and selection in different environment could cause greater diversity than geographical distance. They observed that the D^2 analysis, canonical vectors and the size of canonical roots had revealed parallel features in the mechanism of genetic diversity, in all the crops examined in spite of their contrasting breeding systems. They also stated that in general, height, flowering time, tiller number and number of seeds were the largest contributors to genetic diversity in the crops like *Brassica*, linseed, wheat, *Nicotiana* and sorghum. A number of scientist (Griffing and Lindstrom, 1954; Mall *et al.*, 1962; Arunachalam, 1981) have emphasized the importance of genetic diversity in plant breeding for obtaining broad spectrum of desirable variability. Some of the reports on genetic diversity in rice have been reviewed below.

The characters grain length, panicle number, 500 grain weight, flag leaf area and days to flower were reported by Kole (2000) as the major characters contributing towards divergence in four clusters of 20 aromatic rice genotypes. Thirty-eight rice genotypes were classified into four clusters by Shanmugasundaram *et al.* (2000) using Mahalanobis D^2 statistic based on 10 morphological characters. They concluded that the genotypes from a cluster with high degree of divergence would produce more desirable breeding material for achieving maximum genetic advance with regards to yield. Bharadwaj *et al.* (2001) reported that the characters

length : breadth ratio contributed maximum towards the total divergence followed by 100 grain weight and grain yield per plant contributing 87 per cent of total divergence in 50 genotypes of rice.

Geographical origin was not found to be the good parameter for genetic divergence by Rather *et al.* (2001) and the characters 100 grain weight and Length : breadth ratio of grain were identified as important components of divergence. Assessment of genetic diversity for 28 yield and morphological traits in 100 aromatic rice genotypes by Sharma *et al.* (2002) revealed that the distribution of genotypes within various clusters was random and independent of geographical isolation. Similar observation was made by Roy *et al.* (2002) from 10 clusters formed from 50 high yielding varieties and traditional germplasm of rice. Days to 50 per cent flowering, grain length, kernel breadth and grain yield per plant were found to be major components contributing towards genetic divergence.

Forty-nine rice genotypes were grouped into nine clusters based on genetic distance by Jadhav *et al.* (2003). The cultivars from clusters IV, V, VII and IX were found to have potential as parents for the development of hybrids with high yield and quality. Manna *et al.* (2003) recorded genetic divergence for yield and yield components in 60 rice cultivars and concluded that kernel characteristics and 100 embryo weight exhibited the greatest contribution towards genetic divergence. Shiv Datt and Mani (2003) recorded greater genetic variability among Basmati genotypes and the character plant height (52.24%) contributed the maximum genetic divergence followed by days to 50 per cent flowering (22.56%) and grain yield per plant (8.63%).

Nature and magnitude of genetic divergence among 200 scented rice genotypes was studied by Nayak *et al.* (2004) using Mahalanobis D^2 statistic for 10 characters and the results revealed that grain length and days to 50 per cent flowering have major contribution, whereas panicle length had least contribution in total divergence. Roy *et al.* (2004) grouped 35 rice cultivars into five clusters from the divergence analysis for 10 characters over two locations using Mahalanobis D^2 statistic and clustering by Tocher's method. They suggested to use the cultivars from cluster II and IV in hybridization programme to obtain good recombinants since the greatest genetic divergence was observed between them. Awasthi *et al.* (2005) grouped 21 Indian aromatic rice genotypes into six clusters using

Mahalanobis D^2 statistic and reported that number of grains per panicle, grain yield per plant, days to 50 per cent flowering, leaf length and leaf width contributed the maximum towards total divergence.

In the study involving 34 rice genotypes, Sankar *et al.* (2005) grouped the genotypes into seven clusters by diversity analysis using Mahalanobis D^2 statistic for eight characters. The characters days to 50 per cent flowering, single plant yield and grains per panicle were the major characters contributing towards divergence. Based on Mahalanobis D^2 statistic, Senapati and Sarkar (2005) clustered 40 tall *indica* rice genotypes into five clusters and reported that the characters panicle number per hill, panicle length, sterility percentage, yield per plot and 1000 grain weight were the chief contributors towards genetic divergence. Singh *et al.* (2005) clustered 17 induced mutants of Basmati rice into 5 clusters using Mahalanobis D^2 statistic and concluded that, the cultivars included in diverse group can be used as promising parents for hybridization programme for obtaining high heterotic response.

Forty-four rice cultivars from various locations in India were evaluated by Devi *et al.* (2006) for yield and yield components and were grouped into nine clusters using Mahalanobis D^2 statistic. The character plant height contributed most to genetic divergence (22.6%) followed by flag leaf width (20.12%) and yield per plant (7.2%). Similarly, Gahalain (2006) grouped 55 rice genotypes into 12 clusters and the highest contribution towards total divergence was rendered by total grains per panicle (22.6%), followed by fertile grains per panicle (22.6%), panicle weight (10.1%), days to 50 per cent flowering (7.6%) and shoot height (7.2%).

Based on genetic distances, Naik *et al.* (2006) grouped 50 rice genotypes in seven clusters using Mahalanobis D^2 analysis and concluded that high variability was observed between genotypes in different clusters for different characters. Reddy *et al.* (2006) grouped 64 early rice genotypes into 13 clusters based on ten yield contributing characters. No relationship was observed between geographical distribution and genetic diversity. The contribution of 1000 grain weight, plant height, grain yield per plant and number of spikelets per panicle was highest to the total genetic divergence indicating the importance of these characters while selecting parents from distant clusters for hybridization. Shukla *et al.* (2006)

classified 39 “new plant type” tropical japonica lines and three improved cultivars into 14 clusters using Mahalanobis D^2 statistic and found that harvest index contributed maximum (45.99%) to the total divergence.

Fifty-seven upland rice genotypes including 32 local rice germplasm were grouped into five clusters based on 14 agro-morphological traits by Chandra *et al.* (2007). The clustering pattern of the genotypes was quite random indicating that the geographical origin and genetic diversity were not related. The characters 1000 grain weight, grain yield and biological yield contributed more towards total divergence. Sarawgi and Bisne (2007) grouped 81 scented rice genotypes into nine clusters based on D^2 statistic. The genotypes from cluster II had higher mean performance for hulling percentage, milling percentage, head rice recovery and panicle length, whereas cluster VII had higher mean performance for kernel length and L:B ratio and cluster V had the highest means for grain yield. The genotypic combinations from these clusters will be suitable for superior transgressive segregants for the respective characters.

Genetic divergence among 30 genotypes of rice was estimated by Kumar *et al.* (2008) using Mahalanobis D^2 statistic for 12 characters and the genotypes were grouped into eight clusters. The clustering pattern of genotypes did not follow the geographical origin. The inter-cluster distance was highest between the clusters V and VI indicating that the genotypes in these clusters are likely to exhibit high heterosis and have highest possibility of giving transgressive segregants in subsequent generations. Telebanco-Yanoria *et al.* (2008) reported wide variation for resistance to blast disease caused by *Magnaporthe grisea* using 922 rice varieties collected mainly from Asia. The genotypes were classified into six clusters A-F, according to Ward's hierarchical cluster analysis. The two clusters, B and C, represented susceptible genotypes, whereas the clusters E and F represented most resistant varieties.

Forty rice genotypes were grouped into three clusters by Raut *et al.* (2009a) by following Mahalanobis D^2 statistic. Cluster I was the largest cluster with 36 genotypes found most diverse with highest intra-cluster distance, whereas the highest inter-cluster distance was reported in cluster II and cluster III. Length to

breadth ratio of grain exhibited highest contribution in divergence followed by number of grains per panicle, length of grain and seed yield per plant.

Genetic diversity in 29 landraces of rice was assessed by Rajesh *et al.* (2010) using Mahalanobis D^2 statistic for eight quantitative characters and grouped the genotypes into five clusters. The mode of distribution of genotypes from different geographical regions into various clusters was at random indicating that geographical diversity and genetic diversity were not related. The characters days to first flowering and single plant yield contributed maximum towards genetic divergence. The maximum inter-cluster distance was recorded between cluster IV and V, indicating the genotypes in these clusters may serve as potential donors for future breeding programme.

Assessment of the genetic diversity in 50 rice genotypes for five morphological, eight physiochemical and three grain quality characteristics by Singh *et al.* (2011) revealed highly significant differences for all the characters. Cluster analysis based on the genetic similarity grouped the genotypes into three clusters with all the Basmati accessions grouping in one cluster. Most of the germplasm collected from same locality grouped into same cluster.

2.4 Molecular studies

New genetic tools, such as molecular markers provide a new alternative selection approach to enhance genetic improvements in crops. Identification of molecular markers linked to genomic regions or Quantitative trait loci (QTLs) controlling grain yield, quality and biotic and abiotic stress and their proper utilization might enable breeders to develop improved cultivars through marker assisted selection (MAS). One of the practical benefit from the genomics research was the development of DNA markers (or molecular markers) in the late 1980's and 1990's (Bernando, 2008). Molecular markers for many agronomically important traits in rice have been identified and mapped. Marker development is based on the genetic polymorphism between the individuals. Genetic markers represents the 'landmarks' or 'signposts' linked to the trait of interest and is co-inherited along with the trait.

Several genes and QTLs have been identified for several biotic and abiotic stresses using DNA markers in addition to other agronomic traits in rice (Jena and Mackill, 2008). Marker Assisted Selection (MAS) refers to the use of identified DNA markers that are tightly linked to target loci as a substitute for or to assist phenotypic screening (Francia *et al.*, 2005). By determining the alleles of the DNA marker, plants that possess particular gene(s) or QTLs are identified based on their genotype rather than their phenotype. MAS is widely accepted to have great potential to improve the efficiency and precision of conventional plant breeding, which may ultimately lead to the crop improvement (Collard *et al.*, 2008).

In case of disease resistance, the polymorphism for disease resistance is existing as 'tolerant' and 'susceptible', which can be detected through phenotyping (*viz.* screening for the disease reaction). Since phenotyping is not always feasible, MAS can be applied for discriminating the 'tolerant' and susceptible', provided adequate and reliable marker information is available for the trait. The genotypes can be characterized for the presence of the gene of interest without screening for the trait of interest using molecular markers. Thousands of lines can be screened in the laboratory using molecular markers even when there is no pest/disease incidence. For this purpose, ideally a marker should be located very close to the gene or within the gene. In rice, a wide variety of markers have been utilized for the purpose of mapping and selection of the trait of interest. Markers can be broadly classified into i) Morphological, ii) Biochemical and iii) DNA-based or molecular markers.

Morphological markers

Morphological markers are the morphological traits that segregate with the trait(s) of interest. In rice, numerous morphological traits have been characterized and successfully employed to aid in selection (e.g. presence or absence of awns). Although useful in genetic studies, these markers are mostly influenced by the environment, limited in number and controlled by many genes.

Biochemical markers

Biochemical markers include soluble proteins of various plant organs and isozymes, which are variant forms of same enzyme that have identical functions. In rice, around 50 isozyme markers are known and being co-dominant, these markers were applied for selecting various traits e.g. esterase isozyme for sd1 (Cho *et al.*,

1994). Owing to their tissue and stage specific expression, environmental influence and effect of post-translation modifications, protein based markers have limited preference.

DNA-based markers

DNA-based markers revolutionized the marker system as they are ubiquitous, innumerable, discrete and non-deleterious beside being environmentally neutral. Techniques for generating DNA markers have undergone rapid changes in last three decades. Markers based on differences in DNA sequence between individuals generally detect more polymorphism than morphological and protein-based markers and constitutes a new generation of genetic markers (Botstein *et al.*, 1980; Tanksley *et al.*, 1989). Jeffreys *et al.* (1985) developed the technique of DNA fingerprinting for the first time. DNA markers can be broadly classified into two types (A) Hybridization-based markers and (B) PCR-based markers.

(A) Hybridization-based markers

These markers depend on the base pairing between the nucleotides. DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA, to a labelled probe, which is a DNA fragment of known origin or sequence e.g. Restriction Fragment Length Polymorphism (RFLP). Though these markers are highly informative and reliable, their utility is limited due to the need for specialized laboratory requirement for radioisotopes and laborious procedures. About 2600 RFLP markers are available for rice (Yoshimura *et al.*, 1997).

(ii) PCR-based markers

Polymerase Chain Reaction (PCR) is an *in-vitro* method for synthesis of specific DNA sequence with thermostable *Taq* DNA polymerase enzyme. It involves amplification of specific DNA segment using short stretch of single stranded DNA sequence called 'primers'. A primer is about 10-20 bp in length that specifically anneals to opposite strands flanking the region to be synthesized / amplified. Depending upon the type of primers used, two kinds of DNA-based markers can be generated: (i) DNA markers generated through PCR using arbitrary primers and (ii) DNA markers generated through PCR using specific primers.

i) PCR-based markers using arbitrary primers

These markers target multiple genomic loci, as the primer used is arbitrarily primed. Some of the popular markers of this kind include Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP) etc. These are the markers of choice when little or no information is available regarding the DNA template to be amplified. Reproducibility and reliability are major constraints with this type of markers.

Randomly Amplified Polymorphic DNA (RAPD)

This technique detects nucleotide sequence polymorphism by using single arbitrary decamer primer. A single primer anneals to the genomic DNA at two different sites on complementary strands of DNA template and if these priming sites are within the amplifiable range of each other, a discrete DNA product is synthesized. Their dominant nature and lack of reproducibility limit their use.

Inter Simple Sequence Repeats (ISSR)

ISSR-PCR involves the use of single primer that consists of repeat sequence. The repeats can be di-, tri-, tetra- or penta- nucleotide repeats. The primers are anchored at 5' or 3' or at both the ends with 1 to 4 bases extended in the flanking sequences. When two such sequences are present within an amplifiable distance and in an inverted orientation, the intervening DNA segment within these two repeat sequences will be amplified. A characteristic pattern of the multiplied PCR products thus obtained can be considered as a fingerprint of the analyzed DNA template. ISSR technique amplifies a large number of DNA fragments per reaction, representing multiple loci across the genome.

Amplified Fragment Length Polymorphism (AFLP)

AFLP is the combination of RFLP and PCR and it involves three major steps viz., (i) cutting genomic DNA with restriction enzyme(s) (ii) ligating double stranded adaptor to the restriction fragment, and (iii) amplifying selected restriction fragment using universal primers (Vos *et al.*, 1995). This PCR-based technique permits detection of polymorphism at a large number of loci within a

very short period of time and requires very small amount of DNA. Information of several AFLP maps constructed in rice is available for rice in www.gramene.org.

ii) PCR-based markers using specific primers

These markers use specific primers targeting a single locus and include Sequence Tagged Sites (STS), Sequence Characterized Amplified Regions (SCAR), Simple Sequence Repeats (SSR), Cleaved Amplified Polymorphic Sequences (CAPS) etc. Though the nomenclature is different, all these types of markers target a specific locus.

Simple Sequence Repeats (SSR)/ Microsatellites Markers

Microsatellites are repeat sequences of 2-6 nucleotides (motif), which are abundant, dispersed throughout the genome and show higher level of polymorphism. Primer pairs are designed based on the unique flanking sequence for amplification of a particular microsatellite repeat. SSR markers are co-dominant, multi-allelic and are being routinely used in mapping and tagging, genome analysis and diversity studies. Due to their easy detection and co-dominant nature they have become the marker of choice for genetic mapping and genome analysis (McCouch *et al.*, 1997). The first report of microsatellites in plants was made by Condit and Hubbel (1991) and suggested that they are abundant in plant system. Akkaya *et al.* (1992) reported the length polymorphism of SSR marker in soybean which opened up a new source of PCR-based molecular markers for other plant genomes. More than 20,000 microsatellite markers have been identified and mapped in rice. The sequence and locus information for these markers is available at <http://www.gramene.org>. Rice microsatellites have been demonstrated to be polymorphic between (Wu and Tanksley, 1993; Yang *et al.*, 1994; Panaud *et al.*, 1996) and within rice varieties (Olufowote *et al.*, 1997).

Sequence-Tagged Sites (STS)

Based on the nucleotide sequence of the polymorphic probe linked to the trait of interest from RFLP, primers are designed for PCR amplification of specific allele and thus PCR-based STS markers are developed.

Sequence Characterised Amplified Regions (SCAR)

The polymorphic informative fragment from the RAPD/ ISSR/AFLP are sequenced and longer 20-25 base primers are designed for specific amplification of a particular locus. SCARs are well suited for mapping studies, map-based cloning, physical mapping, etc.

Cleaved Amplified Polymorphic Sequences (CAPS)

Based on the sequence information available in databank of genomic or cDNA sequences or cloned RAPD fragments, primers are designed in such a way that the polymorphic patterns are generated by restriction enzyme digestion of PCR products.

Advantages of molecular markers.

- 1) Their use for indirect selection increases the effectiveness of selection as it avoids the confusing effects of the environment.
- 2) They permit pyramiding of disease resistance genes, which is very difficult through conventional breeding.
- 3) They permit screening of the traits linked to them even during seedling stage, which may be extremely desirable in many cases.
- 4) Characters like disease resistance may be confidently assayed without the need of pathogen inoculation and disease development.
- 5) Molecular markers allow mapping of QTLs, which is not possible through conventional methods.
- 6) Indirect selection for QTLs is expected to require much less time, space and resources.
- 7) They can be used for negative selection i.e. for the elimination of undesirable traits.

2.4.1 Molecular Diversity

Genetic diversity and phylogenetic relationship in 42 genotypes of *Oryza* was determined by Joshi *et al.* (2000) used 30 ISSR primers. The consensus tree constructed using binary data from banding patterns generated by 11 polymorphic primers, grouped genotypes into six clusters. The grouping was in accordance with their genome, indicating the usefulness of ISSR markers in the fingerprinting of

cultivated and wild species germplasm and in understanding the evolutionary relationships of *Oryza*.

Nagaraju *et al.* (2002) analyzed 24 traditional and evolved Basmati rice varieties using 19 SSR and 12 ISSR primers. The traditional Basmati varieties grouped separately in both the assessments and reported lowest diversity, whereas the evolved Basmati lines reported high genetic diversity for both type of markers. They reported identification of several markers separating traditional Basmati lines from evolved Basmati varieties.

Genetic relationship among Indian aromatic and quality rice germplasm was assessed by Jain *et al.* (2004) using 30 rice microsatellite markers. The sixty-nine genotypes used in the study reported 235 alleles. The number of alleles per locus ranged from 3 to 22, with an average of 7.8. The diversity analysis based on genetic similarity values (dendrogram) and a two dimensional scale diagram (PCoA analysis) distinguished high quality Indian aromatic and quality rice genotypes from both *indica* and *japonica* cultivars indicating that Indian aromatic and quality germplasm is genetically distinct from other groups within *O. sativa* and is the product of a long, independent pattern of evolution.

Genetic diversity and pattern of relationship between 18 rice genotypes representing traditional Basmati, cross-bred Basmati and non-Basmati rice varieties were evaluated by Saini *et al.* (2004) using AFLP, ISSR and SSR markers. All the three marker systems generated higher level of polymorphism and could distinguish all the 18 rice cultivars. A total of 171 (110 polymorphic), 240 (188 polymorphic) and 160 (159 polymorphic) amplicons were detected using five AFLP, 25 ISSR and 30 SSR markers, respectively. The clustering algorithms and principal component analysis indicated genetical distinction of traditional Basmati type varieties from cross-Basmati and non-Basmati rice varieties.

Shivapriya and Hittalmani (2006) screened 65 Indian local cultivars and landraces of rice and three improved varieties to identify the extent of genetic diversity using RAPD primers. Sixteen primers generated 112 amplicons of which 84 (74.1%) were polymorphic across the genotypes. The total number of amplicons generated varies from 5 to 12 with the average of 7 per primer. The dendrogram

based on RAPD data unveiled considerable amount of variability among the genotypes.

Lapitan *et al.* (2007) assessed 24 rice cultivars carrying good quality traits for genetic diversity using 164 SSR markers. A total of 890 alleles were detected by 151 polymorphic markers with an average of 5.89 per locus, whereas 89 markers generated a total of 147 rare alleles. Based on Shannon's diversity index, an overall genetic diversity of 0.71 was revealed indicating a high level of genetic variation among these cultivars. Cluster analysis of these cultivars grouped these genotypes in three groups at 40% level of similarity. The clusters separated *japonica* and *indica* groups.

Rabbani *et al.* (2008) evaluated genetic diversity and relationship among 30 traditional and improved rice cultivars of Pakistan using RAPD primers. Twenty-five decamer primer generated a total of 208 RAPD fragments, of which 186 (89.4%) were polymorphic. The number of amplified products produced by each primer varied from 4 to 16 with an average of 8.3 per primer. Pair-wise Nei and Li's similarity had estimated 0.50 to 0.96 between rice cultivars. Based on the analysis performed on a similarity index using UPGMA, 40 cultivars were grouped into three main clusters corresponding to aromatic, non-aromatic and japonica group. The improved and traditional cultivars originated from diverse sources did not form well defined groups and were interspersed, indicating no association between RAPD pattern and the geographical origin of the cultivar.

Pervaiz *et al.* (2010) measured genetic diversity and its effect on agronomic traits among 75 Pakistani landraces with 25 microsatellite markers. A total of 142 alleles were detected at 32 polymorphic loci, while three loci were monomorphic. The number of alleles identified by each marker ranged between 2 to 13 with a mean of 4.4. At nine microsatellite loci, Basmati-type landraces amplified more different alleles than those in coarse-type. Dendrogram based on total microsatellite polymorphism grouped 75 genotypes into four major clusters at 0.40 similarity coefficient, differentiating tall, late maturing and slender aromatic type from the short, early and bold non-basmati ones.

Kiani (2011) evaluated genetic diversity of 16 Iranian improved rice varieties with RAPD primers. Among the 15 primers used, six primers producing amplified products were selected for evaluating genetic relationship of rice cultivars. The number of amplified products generated by each primer ranged from 4 to 11 with an average of 8.2 per primer. Pair-wise Nei and Li's similarity estimates ranged from 0.59 to 0.98. UPGMA-based cluster analysis grouped 16 varieties into five main clusters at the similarity coefficient of 0.82.

Rekha *et al.* (2011) performed RAPD fingerprinting to assess the genetic diversity among 10 rarely cultivated traditional *indica* rice varieties collected from a tribal hamlet of Kerala. A total of 664 DNA fragments were amplified with 15 primers exhibiting 72.9% polymorphism with an average of 32.3 polymorphic fragments per primer. The variety Adukkann (78 fragments) and Jeerakasala (56 fragments) yielded the highest and lowest number of amplicons, respectively. UPGMA analysis using Dice similarity coefficient showed grouping of the varieties in three clusters.

Singh *et al.* (2011) utilized 67 SSR markers to assess the genetic diversity among 50 aromatic rice accessions. About 28 markers showed polymorphism generating 137 alleles among which 77 were polymorphic. The number of amplified alleles varied from 1 to 4 with an average of 1.9 per marker. Cluster analysis performed using Dice similarity coefficient with UPGMA algorithm grouped these genotypes into eight clusters. The dendrogram revealed that the genotypes that were derivatives of genetically similar types clustered more or less together.

Skaria *et al.* (2011) analyzed genetic diversity and phylogenetic relationship in eight rice varieties from Kerala using RAPD primers. Out of 20 RAPD primers used, 11 produced amplicons and were used in comparative study. Eleven primers produced 101 fragments, of which, 73 were polymorphic (72.27%). UPGMA dendrogram based on genetic distance grouped eight varieties into two clusters following geographical proximity.

Ghaley *et al.* (2012) studied genetic diversity for blast resistance in 402 accessions of Bhutan landraces at three hot spot locations followed by genetic analysis of 352 landraces by 27 microsatellite markers. All microsatellites were

polymorphic with 2 to 21 different alleles per marker. Cluster analysis of the landraces revealed two distinct rice cultivar groups according to origin of cultivars.

2.4.2 Genetics of Blast resistance in Rice

Two susceptible (Bluebella and IAC47) parents were crossed with seven resistant parents (Ramtulasi, Arguaia, Carren, Huan-Sen-Goo, Dawn, Tres Marias and Basmati 370) by Filippi and Prabhu (1996) to study inheritance of blast resistance in rice. The F_2 , BC_1 and BC_2 data of 12 crosses showed that the resistance was controlled by one to three genes that segregate independently in most donors. They also reported the presence of non-allelic interaction among resistance genes, including dominant epistasis. Chen *et al.* (1999) studied 276 RILs derived from a cross between CO39 and Moroberekan for mapping blast resistance gene *Pi44(t)*. About 100 F_2 progenies derived from a cross between CO39 and RIL276 reported segregation of resistance in 3:1 ratio, with Chi-square values of 0.2, whereas F_3 families fitted a 1:2:1 segregation ratio indicating that the resistance was governed by single dominant gene. Among the 314 AFLP primer combinations used for marker polymorphism among bulks and parents, 93 primer combinations reported polymorphism and two dominant RFLP markers, AF348 and AF349, were found linked to *Pi44(t)*

Hittalmani *et al.* (2000) fine mapped three major genes *Pi1*, *Piz-5* and *Pita* for blast resistance on chromosome 11, 6 and 12, respectively from three F_2 populations developed from the crossing of susceptible parent, CO39 with three NILs, C101LAC, C101A51 and C101PKT, each carrying the major gene *Pi1*, *Piz-5* and *Pita*, respectively. The RFLP markers Npb181 and RZ536 were found linked to *Pi1*, whereas RZ64, RZ612, RG456 and RG64-SAP were linked to the gene *Piz-5*. The markers RG869, RZ397 and RG241 were associated with the blast resistant gene *Pita*. F_2 segregating populations of two crosses OM1308 $\times$ Tetep and Socnau $\times$ Om997 were studied by Lang *et al.* (2001) for fine mapping blast resistance genes. Genetic analysis revealed a single dominant gene controlling blast resistance in Tetep. The molecular studies conducted with 15 RFLP markers showed parental

polymorphism with eight markers and one STS marker, RG64 was detected associated with blast resistance.

About 141 RILs were screened by Sirithunya *et al.* (2002) using 90 RFLP and 31 SSR primers to identify QTLs associated with leaf and neck blast resistance in rice. They reported QTL for broad resistance spectrum for leaf blast on chromosome 7 and 9, in which the QTL on chromosome 9 was mapped near the *Pi5(t)* locus. Two neck blast QTLs were mapped on chromosome 5 and 6. Moumeni and Leung (2003) used a set of 161 RILs to identify defence-related candidate gene, RFLP and SSR markers linked to components of resistance to blast, i.e. infection type, lesion density, per cent of diseased leaf area and lesion size in rice. The RFLP markers RZ536, RG351, RZ76 and RZ397 on chromosome 7, 11 and 12 and SSR markers RM224, RM179 and RM277 on chromosome 11 and 12 were found linked to components of resistance to blast.

A Mendelian segregating ratio of 3:1 for resistance to susceptibility in 175 F_2 plants derived from a cross between the rice cultivar CO39 (susceptible) and Tetep (resistant) was reported by Barman *et al.* (2004) confirming the presence of a major dominant locus in Tetep. The simple sequence length polymorphism (SSLP) marker RM246 was found linked to novel blast resistance gene *Pi-tp(t)* in Tetep. Jian-li *et al.* (2004) analysed F_3 population derived from the cross between a blast resistant line C101LAC and susceptible variety CO39 for blast resistance with 48 candidate genes. Genetic analysis confirmed that blast resistance of the population was controlled by a single gene *Pi1* and one of the candidate gene R10 was identified to be associated with blast resistance gene on the long arm of chromosome 11.

An advance backcross population consisting of 80 BC_3F_3 lines derived from a cross between Vandana (susceptible) and Moroberekan (resistant) was analysed by Wu *et al.* (2004) using 50 candidate genes and 23 SSR markers. Four candidate defence genes (oxalate oxidase, 14-3-3 protein and two RGA markers) and four SSR markers (RM21, RM168, RM215 and RM250) were significantly associated with resistance in rice. Zhou *et al.* (2004) identified a single dominant gene *Pi-g(t)* in the blast resistant Chinese *indica* rice cultivar Guangchangzhan. The F_2 population of the cross Lijiangxintuanheigu $\times$ Guangchangzhan confirmed the ratio

of 3R:1S and the backcross population fitted to the ratio of 1R:1S, which confirms the presence of single dominant gene for blast resistance in Guangchangzhan. This gene was mapped on long arm of chromosome 2 and flanked by SSR markers RM166 and RM208.

Blast resistance in the *indica* cultivar Q61 was reported to be governed by single dominant gene in two F₂ populations derived from its cross with Aichi Asahi and Lijiangxintuanheigu (LTH) by Li *et al.* (2005). One of the three linked markers, BA1126₅₅₀, was cloned and sequenced. The locus was further mapped to a 5.8cM interval bound by RM5647 and RM8018 on short arm of chromosome 8. This novel R gene is tentatively designated as *Pi36(t)*, which was then fine mapped at the interval of 0.6 cM flanked by the markers RM5647 and CRG2 and co-segregated with the markers CRG3 and CRG4. Padmavati *et al.* (2005) studied inheritance pattern of blast resistance gene in rice which fit in 3R : 1S ratio in F₂, indicating the presence of monogenic dominant gene. However, CNM 4140 when crossed with Dular, Tetep, Zenith and Tadukan segregated in 15R:1S in F₂ generation, indicating the involvement of two independent dominant genes for blast resistance.

Molecular mapping and cloning of dominant gene (*Pi-k^h*) present in rice line Tetep, associated with rice blast disease caused by *Magnaporthe grisea* was reported by Sharma *et al.* (2005). They evaluated F₂ mapping population consisting of 208 individuals developed from the cross between a highly susceptible cultivar HP2216 with a resistant line Tetep with 178 markers and gene *Pi-k^h* was mapped between two SSR markers (TRS26 and TRS33) which are 0.7 and 0.5 cM away, respectively. Fjellstorm *et al.* (2006) evaluated 977 progenies from three mapping populations derived by crossing susceptible and resistant rice genotypes reported that three SSR markers AP5659-1, AP5659-3 and AP5659-5 were tightly linked to the *Pi-z* locus responsible for blast resistance in rice and one marker AP5659-3 co-segregated with *Pi-z* resistance reaction.

A set of allele-specific PCR and insertion / deletion (InDel) markers for nine rice blast resistance genes viz., *Piz*, *Piz-t*, *Pit*, *Pik*, *Pik-m*, *Pik-p*, *Pita*, *Pita-2* and *Pib* was developed by Hayashi *et al.* (2006) that are commonly used in blast resistance rice breeding programme to facilitate the development of multiline cultivars.

Inheritance of blast resistance in the rice cultivar 'Laxmi' was studied by Sharma *et al.* (2007) by evaluating F₁, F₂, backcross, F₃ and F₄ progeny with parents of the cross between resistant (R) 'Laxmi' and susceptible (S) 'Mahsuri' cultivars. The F₁ plants were blast resistant. The test cross progeny segregated in the ratio of 1R:1S. The F₂ population segregated into 3R:1S ratio. The F₃ and F₄ progeny showed a segregation ratio of 1R : 2Sg (segregating) : 1S indicating that blast resistance in Laxmi is governed by a single dominant gene. Ten homozygous resistant and 10 homozygous susceptible F₄ progenies based on their blast reaction in F₃ were subjected to microsatellite analysis with 166 primers. The microsatellite markers RM25 and RM310 showed association with resistant and susceptible progenies.

Genetic analysis of blast resistance in rice was conducted by Persaud *et al.* (2007) in five resistant parents and revealed that the resistant line RR 166-645 and Bala possessed single dominant gene, whereas the resistant line RR 345-2 and moderately resistant lines IAC 25 and IR 42221-2-3-2 possessed two independent dominant genes.

Two flanking and three co-segregating SSR markers for the blast resistant gene *Pi20(t)* were reported by Li *et al.* (2008) in F₂ generation derived from a cross of Asominori, an extremely susceptible cultivar, with resistant cultivar IR 24. The SSR OSR32 and RM28050 were mapped on either side of *Pi20(t)* at a distance of 0.2 cM and 0.4 cM, respectively, whereas the markers RM1337, RM5364 and RM7102, were found to be co-segregated with *Pi20(t)*.

A ratio of 3 resistant : 1 susceptible was reported by Lee *et al.* (2009) in 162 RILs of F₁₀₋₁₁ from a cross between susceptible cultivar KBNT/pa1-1 and resistant variety Zhe733, indicating two major R genes in Zhe733 confers resistance. The SSR markers RM1233 and RM224 on chromosome 11 were found to be associated with blast resistance. Similarly, Srinivas Prasad *et al.* (2009) recorded a Mendelian segregation ratio of 3:1 for resistant to susceptible in F₂ plants developed from a cross between BPT 5204, a blast susceptible cultivar and C101LAC, a cultivar highly resistant to rice blast. SSR marker RM224 was found to be linked with blast

showing resistant fragment of ~146 bp amplified from resistant plants, while was absent in susceptible plants.

About 320 individuals of an F_2 population derived from Pongsu Seribu 2, a resistant variety, and Mahsuri, a susceptible rice cultivar was analyzed by Ashkani *et al.* (2011) using 23 polymorphic SSR markers. In the phenotypic data analysis, the F_2 population segregates in a 3:1 ratio for resistant to susceptible plants, respectively indicating the resistance in Pongsu Seribu 2 is most likely controlled by single dominant gene. Among the 23 polymorphic SSR markers used to study inheritance 11 markers showed good goodness of fit to the expected segregation ratio (1:2:1) for the single gene model in Chi-square analysis, whereas six SSR markers (RM168, RM8225, RM1233, RM6836, RM5961 and RM413) were found to be linked to the resistant gene. These diagnostic markers could be used in MAS programme to develop a durable blast resistant variety. Selvaraj *et al.* (2011) studied 220 F_2 individuals developed from the cross between White Ponni and Moroberekan for blast resistance under artificial conditions. They reported resistance in Moroberekan was governed by single dominant gene. Among the 12 RAPD and 400 SSR primers used in the study, 11 RAPD and 52 SSR markers were polymorphic. Three SSR markers (RM5757, RM451 and RM492) and four RAPD markers (OPBB5259, OPBB5194, OPAL16940 and OPBD12680) were found to be linked to leaf blast resistance based on single marker analysis.

CHAPTER III

MATERIAL AND METHODS

The present investigation was conducted at the Agricultural Research Station, Radhanagari, Dist. Kolhapur and Agricultural Research Station, Lonavala, Dist. Pune during *kharif* 2010 and *kharif* 2011. The molecular studies were conducted at State Level Biotechnology Centre, MPKV, Rahuri. The investigation was carried out in three experiments viz., Experiment I : Genetic diversity in rice germplasm for agro-morphological and pathological traits, Experiment II : Genetic diversity in rice germplasm by using molecular markers and Experiment III : Genetics of blast resistance in rice. The details of the material used and procedures followed during the course of this investigation are given below.

3.1. Experiment I : Genetic diversity in rice germplasm for agro-morphological and pathological traits

3.1.1 Material

Sixty-five genotypes of rice (*Oryza sativa* L.) maintained at Agricultural Research Station, Radhanagari, District Kolhapur under Mahatma Phule Krishi Vidyapeeth, Rahuri were used for present investigation. The details of the genotypes used with their pedigree are presented in Table 3.1.

3.1.2 Methods

3.1.2.1 Raising of the experimental material for agro-morphological evaluation

Sixty-five genotypes of rice were sown on 10th June, 2010 at ARS, Radhanagari, Dist. Kolhapur and on 14th June 2010 at ARS, Lonavala, Dist. Pune on raised beds in rows with 10 cm spacing between rows. The genotypes were transplanted on 09th and 13th July 2010 at Radhanagari and Lonavala, respectively in randomised block design with two replications. Each entry was represented by four rows of 3 m length with a spacing of 20 cm between rows and 15 cm between hills. Two seedlings were transplanted per hill.

3.1.2.1.1 Cultural practices

All recommended agronomic practices were followed as and when required. Total dose of fertilizers applied during crop duration was 100 kg N, 50 kg P₂O₅ and 50 kg K₂O per hectare. Nitrogen was applied in three split doses of 50, 25 and 25 kg N/ha at the time of transplanting, 25-30 days and 55-60 days after transplanting, respectively. Total P₂O₅ and K₂O were applied at transplanting.

Table 3.1 Rice genotypes with their pedigree

S. No.	Genotype	Pedigree
1	Heera	CR 404-48 × CR 289-1208
2	Halvi Sal 17	Local selection from Kolhapur (M.S.)
3	RDN 185-2	Halvi Sal 17 × TN 1
4	Phule Radha	TN 1 × Kolamba 540
5	RTN 1	IR 8 × RTN 24
6	Ratna	TKM 6 × IR 8
7	Phule Samruddhi	Indrayani x Sonsali
8	Jaya	TN 1 × T 141
9	Phule Maval	Pawana × Indrayani
10	Pawana	Pusa 33 × IR 28
11	Indrayani	Ambemohar 157 × IR 8
12	Bhogavati	Selection from Basmati Composite
13	Basmati 370	Pureline selection from Basmati landraces
14	Pusa Basmati 1	Pusa 150 × Karnal Local
15	Ambemohar 157	Local selection from Pune (M.S.)
16	Ghansal	Local selection from Kolhapur (M.S.)
17	Kalajirga	Local selection from Kolhapur (M.S.)
18	Vivek Dhan 82	VL 221 × UPR 82-1-7
19	Badshabhog	Local type from Orissa
20	Kothimbire	Landrace from Kolhapur (M.S.)
21	BPT 5204	(GEB 24 × TN 1) × Mahsuri
22	RDN 98-2-3-5-14	Halvi Sal 17 × TN 1
23	RDN 01-2-10-9	Ambemohar 157 × IR 8
24	KJT 2	R.P. 6-17 × R.P. 4-14
25	EK 70	Landrace from Igatpuri, Nasik (M.S.)
26	Patni	Landrace from Sindhudurg (M.S.)
27	Diwani	Landrace from Uttar Pradesh
28	Shyamjeer	Landrace from Bihar
29	Juhibengal	Landrace from West Bengal
30	Improved Pusa Basmati 1	Pusa Basmati 1 × (Pusa Basmati 1 × IRBB 55)
31	Kasturi	Basmati 370 × CRR 88-17-1-5
32	Karnal Local	Selection from Basmati landraces

Table 3.1 cont...

S. No.	Genotype	Pedigree
33	SD 7	PR 109 × Pak Basmati
34	SD 17	Pusa Basmati 1 × IET 12603
35	Sugandhamati	Pusa Basmati 1 × IET 12603
36	Basmati 386	Selection from Pak Basmati
37	MC 4	Selection from Karnal Local
38	Haryana Basmati	Local selection from Haryana
39	Mahisugandha	BK 79 × Basmati 370
40	Khalibagh	Landrace from Sindhudurg (M.S.)
41	Jagatpuri	Landrace from Sindhudurg (M.S.)
42	Vikram	Landrace from Sindhudurg (M.S.)
43	Velkat	Landrace from Sindhudurg (M.S.)
44	Tulshi tall	Landrace from Kolhapur (M.S.)
45	Champakali	Landrace from Kolhapur (M.S.)
46	Siddhagiri	Landrace from Kolhapur (M.S.)
47	RDN 97-2	TN 1 × IR 64
48	RDN 99-12	Selection from Phule Maval
49	RDN 99-14	Bhogavati × RTN 24
50	RTN purple	Local selection from Ratnagiri (M.S.)
51	Taroari Basmati	Pureline selection from NBC 19
52	RDN 99-18	Kalagirga × IR 36
53	RDN 02-80	Indrayani × Pusa Basmati 1
54	Ajara Local 1	Landrace from Kolhapur (M.S.)
55	Ajara Local 3	Landrace from Kolhapur (M.S.)
56	IGT 13857	Landrace from Nasik (M.S.)
57	Pavsal	Landrace from Pune (M.S.)
58	Kunchi	Landrace from Pune (M.S.)
59	Ambemohar 102	Landrace from Pune (M.S.)
60	Antersal	Landrace from Pune (M.S.)
61	Nalabhat	Landrace from Pune (M.S.)
62	Sonsali	Landrace from Pune (M.S.)
63	Pinjarwadi Local	Landrace from Pune (M.S.)
64	L K 248	Local selection from Nasik (M.S.)
65	Pomendi Local	Landrace from Ratnagiri (M.S.)

3.1.2.1.2 Recording of observations

At both the locations five plants were selected at random in each genotype per replication. The randomly selected plants were tagged for recording observations on various agro-morphological characters. The selected plants were harvested separately.

3.1.2.1.3 Agro-morphological characters

1) Days to 50 per cent flowering

The total number of days required from sowing to opening of first flower in 50 per cent of the plants in each entry were recorded.

2) Days to maturity

The number of days required from sowing to 50 per cent plants showing physiological maturity on plot basis were recorded.

3) Plant height (cm)

Height at maturity from base of the plant to tip of the main panicle (excluding awns if any) / leaf tip (whichever longer).

4) Panicle length (cm)

The total length of the panicle from its base to the tip excluding awns, if any.

5) Spikelets per panicle

The total number of spikelets for all the panicles per plant were counted and averaged to obtain the number of spikelets per panicle.

6) Spikelet fertility (%)

The spikelet fertility was estimated by dividing total number of filled spikelets per panicle by total number of spikelets per panicle and expressed in per cent.

7) Tillers per plant (number)

The numbers of tillers bearing the panicle per plant were recorded.

8) Test weight (g)

The 1000 grains were counted at random for each genotype in each replication and their weight was recorded as test weight.

9) Grain length (mm)

The average length of ten dehulled grains from each entry in each replication was measured and averaged.

10) Grain breadth (mm)

Ten dehulled grains (used for measuring length) of each accession were arranged breadth-wise, for cumulative measurement of breadth. The average breadth of these dehulled grains from each entry in each replication was measured and averaged.

11) Length : Breadth (L:B) ratio

Average of length to the breadth ratio of ten dehulled grains was calculated.

12) Seed yield per plant (g)

The total weight of all the filled grains from each of the selected plant was measured in grams.

3.1.2.2 Raising of the experimental material for pathological evaluation

Sixty-five genotypes of rice were sown on 07th August, 2010 at ARS, Lonavala in Uniform Blast Nursery in two replications. Each entry was represented by a single row of 0.50 m length with a spacing of 10 cm between rows. A bed of the susceptible check, EK 70 was planted after every bed of test entries. Similarly, two rows of susceptible check were also planted after every ten entries for providing uniform inoculum load of the pathogen.

3.1.2.2.1 Recording of observations

Five randomly selected plants of each genotype in each replication were tagged for recording observations on various pathological characters. The final observations were recorded at maximum disease incidence *i.e.* 45 days after sowing.

3.1.2.2.2 Pathological characters

1) Infection type

The blast reaction of each entry was recorded as per Standard Evaluation System (SES) scale (Anonymous, 2002). Observations were recorded on each entry at eight days interval starting from 15th day after sowing and continued up to 45 days after sowing. The maximum score was considered for judging the reaction and further analysis.

2) Lesion density

Number of sporulating (having ashy-white centres) lesions per cm² were measured on five leaves per plant from top, middle and basal part of leaf. The lesion density was expressed by averaging three observations per leaf from five plants.

3) Lesion size (mm²)

Lesion size of five randomly selected lesions per leaf was estimated in mm².

4) Diseased leaf area (%)

Diseased leaf area was calculated based on lesion density and lesion size and is expressed in per cent leaf area infected.

Table 3.2 Blast disease score card as per SES

Score	Description
0	No lesion
1	Small brown specks of pin head size without sporulating centre
2	Small, roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter, with distinct brown margin, lesions are mostly found on lower leaves
3	Lesion type is same as in scale 2, but significant number lesions are on the upper leaves
4	Typical sporulating lesions, 3 mm or longer, infecting < 2 per cent of the leaf area
5	Typical blast lesions infecting 2 - 10 per cent of leaf area
6	Typical blast lesions infecting 11 - 25 per cent of leaf area
7	Typical blast lesions infecting 26 - 50 per cent of leaf area
8	Typical blast lesions infecting 51 - 75 per cent of leaf area
9	Typical blast lesions infecting more than 75 per cent of leaf area

5) Per cent Disease Intensity (PDI)

Per cent disease intensity (PDI) was calculated as per the following formula (Wheeler, 1969).

$$\text{PDI} = \frac{\text{Sum of individual observations i.e. total numerical rating}}{(\text{Maximum disease score i.e. } 9 \times \text{No. of observations i.e. } 10)} \times 100$$

3.1.3 Statistical analysis

3.1.3.1 Analysis of the design of experiment

The data available on individual characters were subjected to the method of analysis of variance commonly applicable to the randomised block design to test the null hypothesis that there are no genotypic differences for the characters studied. The analysis of variance was done according to Panse and Sukhatme (1985) in the following form.

Table 3.3 Analysis of variance

S. No.	Sources of variation	Degrees of freedom	Mean sum of squares	Expected mean sum of squares
1	Replication	$r - 1$	RMS	$\sigma^2 e + g\sigma^2 r$
2	Genotypes	$g - 1$	GMS	$\sigma^2 e + r\sigma^2 g$
3	Error	$(r - 1)(g - 1)$	EMS	$\sigma^2 e$
4	Total	$(rg - 1)$		

where,

r = number of replications

g = number of genotypes

The genotype mean square (GMS) was tested against error mean square (EMS) by 'F' test at $n_1 = (g - 1)$ and $n_2 = (r - 1)(g - 1)$ degrees of freedom.

3.1.3.2 Estimation of mean and range

The mean value for each character was worked out by using the following formula.

$$\bar{X} = \frac{1}{n} \left(\sum_{i=1}^n X_i \right)$$

where,

$\sum X_i$ = sum of the character

n = number of observations

The lowest and highest values from mean of each character were recorded as range, whereas the arithmetic average of all the observations as mean of the character.

3.1.3.3 Estimation of standard error of mean, standard error of difference and critical difference

i. The S.E. of mean difference was calculated as

$$\text{S. E. of mean} = \sqrt{\sigma^2 e / r}$$

ii. The standard error of difference between two means was calculated as

$$\text{S. E. of difference [SE(d)]} = \text{S.E.(m)} \times \sqrt{2}$$

iii. The critical difference between any two means was calculated as

$$\text{C. D.} = \text{S.E.(d)} \times 't' \text{ at error d.f.}$$

3.1.3.4 Estimation of components of variation

The phenotypic and genotypic variances were calculated by utilizing the respective mean square values from the variance table (Johnson *et al.*, 1955).

- i. Environmental variance $= \sigma^2_e = \text{EMS}$
- ii. Genotypic variance $= \sigma^2_g = (\text{GMS} - \text{EMS})/r$
- iii. Phenotypic variance $= \sigma^2_p = \sigma^2_g + \sigma^2_e$

where,

GMS = genotypic mean sum of squares

EMS = error mean sum of squares

r = number of replications

3.1.3.5 Estimation of coefficients of variation

The genotypic and phenotypic coefficients of variation were calculated by the formula suggested by Burton (1952).

- i. Genotypic coefficient of variation (GCV)

$$\text{GCV (\%)} = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

where,

$$\sigma^2_g = \frac{\text{Mean sum of square due to genotypes} - \text{Mean sum of square due to error}}{r}$$

Here,

$\sigma^2_g = Vg$ = genotypic variance

$\bar{X}$ = general mean of the character

r = number of replications

- ii. Phenotypic coefficient of variation (PCV)

$$\text{PCV (\%)} = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$$

where,

σ^2_p = Genotypic variance + mean sum of square due to error

Here,

$\sigma^2_p = Vp$ = phenotypic variance

3.1.3.6 Estimation of heritability (broad sense)

Heritability in broad sense was estimated for various characters as per the formula suggested by Hanson *et al.* (1956).

$$h^2 \text{ (b.s.)} = (\sigma^2 g / \sigma^2 p) \times 100$$

where,

h^2 = heritability percentage in broad sense

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

3.1.3.7 Estimation of genetic advance (G.A.)

The extent of genetic advance expected through selection for each character was calculated as per the formula suggested by Johnson *et al.* (1955).

$$\text{G.A.} = (\sigma^2 g / \sigma^2 p) \times \sigma p \times K$$

OR

$$\text{G.A.} = h^2 \times \sigma p \times K$$

where,

h^2 = heritability percentage in broad sense

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

K = selection differential (2.06 at 5 % selection intensity, Lush, 1949)

σp = phenotypic standard deviation

Genetic advance as per cent of mean

$$\text{G.A. as per cent mean} = (\text{G.A.} / \bar{X}) \times 100$$

where,

G.A. = genetic advance

$\bar{X}$ = mean of the character

3.1.3.8 Genetic diversity

The generalized distance between two populations is defined by Mahalanobis (1936) as,

$$D^2 = \sum \sum \lambda_{ij} \cdot \delta_i \cdot \delta_j$$

where,

λ_{ij} = reciprocal matrix of the common dispersion matrix

δ_i = difference between the mean values of two populations for i^{th} character

δ_j = difference between the mean values of two populations for j^{th} character

Estimation of D^2 values from the above formula is very complicated in the present study. Since, it requires the inversion of a seventeenth order determinant and then the evaluation of $17(17+1)/2$, term whose sum is D^2 . It was found convenient to work with a set of uncorrelated characters constructed from the original measurements. D^2 with such transformable variables reduced to the evaluation of simple sum of squares. Transformation was done by using pivotal condensation method (Singh and Chaudhary, 1977). The coefficient for the transformation was obtained by dividing the first row of the reduced matrix by the square root of the corresponding pivotal condensation elements.

3.1.3.9 Testing the significance of D^2 values

The D^2 value obtained for a pair of population is taken as the calculated value of χ^2 and was tested against the tabulated values of χ^2 for 'p' degrees of freedom where p is the number of characters considered (Singh and Chaudhary, 1977).

3.1.3.10 Determination of group constellations

Tocher's method as described by Rao (1952) was followed for cluster formation. No formal rules can be laid down for finding the clusters because a cluster is not a well defined term. The only criterion appeared to be that any two groups belonging to the same cluster should at least on the average show a smaller D^2 value than those belonging to two different clusters. A simple method suggested by K. D. Tocher (Rao, 1952) was to start with two closely associated groups and find a third group which has the smallest D^2 value from the two. Similarly, fourth is chosen to have the smallest D^2 from the first three and so on. If at any stage the average D^2 of the group from those already listed appears to be high, then this group does not fit in the former groups and therefore, taken outside the former cluster. The group of the first cluster are then omitted and the rest are treated similarly. It was

also helpful to calculate the change in average D^2 value within a cluster due to inclusion of an additional group. If the change was appreciable, then the newly added group had to be considered as outside the cluster. The computational scheme was as given below.

Population added to a cluster	D^2	Number of terms (n)	Increase in D^2 / increase in n	Average $D^2 = \sum D^2 / n$	Cluster
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3.1.3.11 Average intra and inter-cluster distance

- i) Average intra-cluster $D^2 = \sum D_i^2 / n$

where,

$\sum D_i^2$ is sum of distances between all possible combinations (n) of the population included in the cluster.

- ii) Average inter-cluster D^2

$$D^2 = (\sum \text{of distances between the population of cluster i and j}) / n_i \cdot n_j$$

where,

n_i = number of populations in the cluster i

n_j = number of populations in the cluster j

- iii) Average intra and inter cluster distance

$$D = \sqrt{D^2}$$

3.1.3.12 Cluster means

Cluster means were calculated for individual character on the basis of mean performance of the genotypes included in that cluster.

3.2. Experiment II : Genetic diversity in rice germplasm by using molecular markers

3.2.1 Material

Out of 65 genotypes of rice used in Experiment I, 50 were used for present investigation. The genotypes for the molecular evaluation were selected by discarding fifteen most similar genotypes based on the diversity analysis using agro-morphological and pathological traits.

3.2.2 Methods

3.2.2.1 Isolation of genomic DNA from young leaves

Total DNA was extracted from the leaves of fifty genotypes by CTAB (Cetyl Tri-methyl Ammonium Bromide) method as described by Doyle and Doyle (1990) with some modifications.

Reagents

- 1) Extraction buffer (2X CTAB DNA extraction buffer) : 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2 % CTAB (w/v) and 0.2 % β -mercaptoethanol
- 2) Chloroform : isoamyl alcohol (24:1)
- 3) Ethanol : 70 % (v/v)
- 4) TE (10/1) buffer : 10 mM Tris-HCl, 1mM EDTA (pH 8.0)

Procedure

- 1) About 1 gm fresh young leaves of rice were taken and cut into small pieces (about 10 mm²) with sterilized scissors, ground using liquid nitrogen (N₂) with pestle and mortar to obtain fine powder.
- 2) The powder was immediately transferred to 50 ml polypropylene centrifuge tube containing 18-20 ml pre-warmed CTAB DNA extraction buffer.
- 3) The tubes were incubated for 60 min. at 65°C in a thermostatic water bath.
- 4) The contents in tubes were mixed after every 10 min. by inversion during incubation.
- 5) After incubation, the tubes were allowed to cool at room temperature.
- 6) An equal volume (20 ml) of Chloroform : Isoamyl alcohol (24:1) was added and mixed gently by inversion. The tubes were then centrifuged at 7000 rpm for 8 min. at 4°C in high speed refrigerated centrifuge (Kubota 6500, Japan).
- 7) Then upper aqueous phase was carefully transferred to a fresh tube and extracted again with equal volume of Chloroform : Isoamyl alcohol (24:1 v/v) by centrifugation at 7000 rpm for 10 min.
- 8) Ice-cold isopropanol (0.6 volume) was added to the tube and mixed by gentle inversion. The tubes were incubated at -20°C for 20 min. for precipitation of DNA.

- 9) The tubes were centrifuged at 10,000 rpm for 10 min. The aqueous layer was carefully discarded and the diffused DNA/ DNA pellet was retained.
- 10) About 5 ml ice cold 70 per cent ethanol was added to the tubes and centrifuged at 5000 rpm for 5 min. The pelleted DNA was once more rinsed with ice-cold 70 per cent ethanol. The DNA pellet was air dried till the last trace of ethanol was evaporated.
- 11) The pellet was dissolved completely by adding 1 ml of TE (10/1) buffer.

3.2.2.2 Purification of genomic DNA

Reagents

- 1) RNase A (10 mg/ml)
- 2) Phenol : Chloroform (1:1)
- 3) Chloroform : isoamyl alcohol (24:1)
- 4) Sodium acetate 3M (pH 5.5)
- 5) Ethanol : 70 % (v/v)
- 6) Ethanol (aqueous)
- 7) TE (10/1) buffer : 10 mM Tris-HCl, 1mM EDTA (pH 8.0)

Procedure

- 1) To the above isolated DNA, 30 μ l of RNase A (10 mg/ml) was added and the tubes were incubated at 37°C for 60 min.
- 2) About 1 ml Phenol : Chloroform (1:1) was added and mixed gently by inversion. The tubes were then centrifuged at 3000 rpm for 5 min. at 4°C in high speed refrigerated centrifuge. The aqueous phase was transferred to a new 2 ml centrifuge tube.
- 3) One ml Chloroform : Isoamyl alcohol (24:1) was added and mixed gently by inversion. The tubes were then centrifuged at 3000 rpm for 5 min. at 4°C and the aqueous phase was transferred to a new tube.
- 4) About 500 μ l 3M Sodium acetate and 250 μ l absolute ethanol was added, the tubes were inverted 10-15 times and incubated at -20°C for overnight for precipitation of DNA.

- 5) The tubes were centrifuged at 10,000 rpm for 10 min. and the aqueous solution was carefully discarded to get DNA pellet.
- 6) The pellet was then washed with 70 % ethanol and air dried till the last traces of ethanol was evaporated.
- 7) Purified DNA was dissolved in 200 µl of TE (10/1) buffer.

3.2.2.3 DNA quantification and purity analysis

Reagents

- 1) TBE buffer (10X) : 1M Tris base, 830 mM boric acid, 10 mM EDTA, pH 8.0.
- 2) TBE buffer (1X) : 10 ml of 10X buffer was diluted to 100 ml with ddwater.
- 3) Ethidium bromide : 10 mg/ml
- 4) Tracking dye : 1 % (w/v) bromophenol blue + 20 % (w/v) Ficoll + 10 mM EDTA

Procedure

Quantification of purified DNA was performed using UV visible spectrophotometer (Nanodrop, ND-1000 USA) at 260 and 280 nm wavelength. The ratio of absorbance 260/280 was calculated. Two µl of all DNA extracts were electrophoresed (Bio Rad sub cell model 96 USA) in 0.8 % (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in 1X TBE buffer. After electrophoresis the band intensity of genomic DNA was visualized on gel documentation unit (Flour Chem. TM Alpha innotech, USA) and compared to that of Calf thymus standard DNA. These gels also provided a visual measure of purity and integrity of DNA. The samples were diluted to a final concentration of 10 ng/µl.

3.2.2.4 DNA amplification by ISSR primers

A set of 25 ISSR primers from 100 ISSR primers obtained from Biotechnology Laboratory, University of British Columbia, Canada (UBC set/ ISSR no. 801 to 900) were used in PCR analysis. The details of the ISSR primers used in present investigation are given in Table 3.4.

Amplification was performed in a 0.2 ml PCR tube having 20 µl PCR reaction mixture volume as described by Joshi *et al.* (2000) with some modifications. The composition of PCR mixture is presented in (Table 3.5).

Table 3.4 Sequences details of ISSR primers used for diversity study in rice

S. No.	Primer name	Primer sequence (5'-3')
1	ISSR807	AGAGAGAGAGAGAGAGT
2	ISSR808	AGAGAGAGAGAGAGAGC
3	ISSR809	AGAGAGAGAGAGAGAGG
4	ISSR810	GAGAGAGAGAGAGAGAT
5	ISSR811	GAGAGAGAGAGAGAGAC
6	ISSR812	GAGAGAGAGAGAGAGAG
7	ISSR813	CTCTCTCTCTCTCTT
8	ISSR814	CTCTCTCTCTCTCTTA
9	ISSR815	CTCTCTCTCTCTCTTG
10	ISSR817	CACACACACACACAA
11	ISSR818	CACACACACACACAG
12	ISSR821	GTGTGTGTGTGTGTGTT
13	ISSR822	TCTCTCTCTCTCTCTCA
14	ISSR823	TCTCTCTCTCTCTCTCC
15	ISSR824	TCTCTCTCTCTCTCTCG
16	ISSR825	ACACACACACACACT
17	ISSR826	ACACACACACACACC
18	ISSR827	ACACACACACACACG
19	ISSR835	AGAGAGAGAGAGAGAGYC
20	ISSR841	GAGAGAGAGAGAGAGAYC
21	ISSR844	CTCTCTCTCTCTCTRC
22	ISSR846	CACACACACACACART
23	ISSR868	GAAGAAGAAGAAGAAGAA
24	ISSR875	CTAGCTAGCTAGCTAG
25	ISSR895	AGAGTTGGTAGCTCTTGATC

Table 3.5 Composition of PCR mixture for ISSR analysis

S. No.	Components	Stock concentration	Volume of PCR reaction mixture per tube (20 μ l)
1	<i>Taq</i> DNA polymerase buffer 100 mM Tris (pH 9.0) 500 mM KCl and 0.1 % gelatin	10X	2.0 μ l
2	MgCl ₂	25 mM	1.6 μ l (1.6 mM)
3	dNTPs	10 mM (2.5 mM each)	2.0 μ l (200 μ M each)
4	Primer	1 μ M	2.0 μ l (0.200 μ M)
5	<i>Taq</i> DNA polymerase	3 U/ μ l	0.3 μ l (1 U)
6	Template DNA	10 ng/ μ l	2.0 μ l (20 ng)
7	Sterile distilled water	-	10.1 μ l
8	Total volume	-	20.0 μ l

3.2.2.4.1 PCR amplification of DNA

The 20 μ l reaction mixture was gently vortexed and spinned down. The DNA amplification was carried out on a thermal cycler (Corbett thermocycler). The thermal profile used for amplification is given below (Table 3.6).

Table 3.6 Thermal profile for ISSR analysis

Step no.	Name of the step	Temp. (°C)	Time (min.)	Number of cycles
First step	Initial denaturation	95	5	1
Second step	Denaturation	94	1	} 45
	Annealing	++	1	
	Extension/Elongation	72	1	
Third step	Final extension	72	10	1
Fourth step	Final hold	4	Till retrieval	--

++ primer specific annealing temperature

3.2.2.4.2 Agarose gel electrophoresis

Requirements

Electrophoresis unit (gel casting unit, gel combs, power pack)

UV transilluminater

Solutions

- i. Ethidium bromide: 10 mg/ml
 - ii. Bromophenol blue (Loading dye) 6X
 - iii. Agarose
 - iv. Stock solution of 10X TBE: 121 g Tris (1 M), 51.3 g Boric acid (830 mM) and 7.6 g EDTA (10 mM) to make final volume 1000 ml and adjust pH 8.0
4. Working solution of 1X TBE: Ten ml of 10X TBE was diluted to 100 ml using ddwater

Procedure

- 1) Agarose (1.2 g) was added to 100 ml of 1X TBE buffer and was melted by heating the solution in microwave oven.
- 2) Solution was cooled to about 55-60 °C and 5 µl of ethidium bromide (10 mg/ml) was added to it.
- 3) The gel solution was poured into the gel casting unit after keeping the comb in the assigned slots.
- 4) The gel was allowed to solidify at room temperature.
- 5) Gel was placed in the electrophoresis apparatus in such a way that the end with wells is in line with the cathode.
- 6) The apparatus was filled with 1X TBE buffer in order to submerge the gel in the buffer to prevent the entry of air bubbles while removing the gel combs.
- 7) The 20 µl PCR products to be analyzed were mixed with 3.5 µl tracking dye (6X) and loaded carefully in the wells of the gel.
- 8) The unit was connected to a power pack and electrophoresis was carried out at 80 volts.
- 9) The power supply was switched off when the front dye was about 2 cm away from positive end (anode).
- 10) The amplified PCR products were observed under UV transilluminater in gel documentation system (Flour Chem. TM Alpha innotech, USA) and image was captured.

3.2.2.5 DNA amplification by SSR primers

A set of 17 SSR primers were used for the present investigation. The sequence information for these primers was collected from the website www.gramene.org and the synthesis was done by Custom Oligo Synthesis Division, Merck Specialities Pvt. Ltd., Bangalore. The details of the primers used are presented in Table 3.7.

Table 3.7 Sequences details of SSR primers used for diversity study in rice

S. No.	Primer name	Primer sequence (5'-3')	
1	RM11	F	TCTCCTCTTCCCCCGATC
		R	ATAGCGGGCGAGGCTTAG
2	RM21	F	ACAGTATTCCGTAGGCACGG
		R	GCTCCATGAGGGTGGTAGAG
3	RM25	F	GGAAAGAATGATCTTTTCATGG
		R	CTACCATCAAAACCAATGTTC
4	RM44	F	ACGGGCAATCCGAACAACC
		R	TCGGGAAAACCTACCCTACC
5	RM72	F	CCGGCGATAAAACAATGAG
		R	GCATCGGTCCTAACTAAGGG
6	RM162	F	GCCAGCAAAACCAGGGATCCGG
		R	CAAGGTCTTGTGCGGCTTGCGG
7	RM204	F	GTGACTGACTTGGTCATAGGG
		R	GCTAGCCATGCTCTCGTACC
8	RM209	F	ATATGAGTTGCTGTCTGTGCG
		R	CAACTTGCATCCTCCCCTCC
9	RM215	F	CAAAATGGAGCAGCAAGAGC
		R	TGAGCACCTCCTTCTCTGTAG
10	RM224	F	ATCGATCGATCTTCACGAGG
		R	TGCTATAAAAGGCATTCTGGG
11	RM241	F	GAGCCAAATAAGATCGCTGA
		R	TGCAAGCAGCAGATTTAGTG

Table 3.7 cont...

S. No.	Primer name		Primer sequence (5'-3')
12	RM246	F	GAGCTCCATCAGCCATTCAG
		R	CTGAGTGCTGCTGCGACT
13	RM250	F	GGTTCAAACCAAGCTGATCA
		R	GATGAAGGCCTTCCACGCAG
14	RM259	F	TGGAGTTTGAGAGGAGGG
		R	CTTGTTGCATGGTGCCATGT
15	RM307	F	GTACTACCGACCTACCGTTCAC
		R	CTGCTATGCATGAACTGCTC
16	TRS26	F	GGAGAGCCAATCTGATAAGCA
		R	CAACAAGAGAGGCAAATTCTCA
17	TRS33	F	AAGAAGAAGCGTACGCATGAAT
		R	GTCCTGGAGGGGAGGAGA

Amplification was performed in a 0.2 ml PCR tubes having 20 µl reaction volume as described by Joshi *et al.* (2000) with some modifications. The composition of PCR mixture is presented in Table 3.8.

Table 3.8 Composition of PCR mixture for SSR analysis

S. No.	Components	Stock concentration	Volume of PCR reaction mixture per tube (20 µl)
1	<i>Taq</i> DNA polymerase buffer 100 mM Tris (pH 9.0) 500 mM KCl and 0.1 % gelatin	10X	2.0 µl
2	MgCl ₂	25 mM	1.6 µl (1.6 mM)
3	dNTPs	10 mM (2.5 mM each)	2.0 µl (200 µM each)
4	Primer - F	1 µM	1.0 µl (0.100 µM)
5	Primer - R	1 µM	1.0 µl (0.100 µM)
6	<i>Taq</i> DNA polymerase	3 U/ µl	0.3 µl (1 U)
7	Template DNA	10 ng/ µl	2.0 µl (20 ng)
8	Sterile distilled water	-	10.1 µl
9	Total volume	-	20.0 µl

3.2.2.5.1 PCR amplification of DNA

The 20 µl reaction mixture was gently vortexed and spun down. The DNA amplification was carried out on a thermal cycler (Corbett thermocycler). The thermal profile used for amplification is given in Table 3.9.

Table 3.9 Thermal profile for SSR analysis

Step no.	Name of the step	Temp. (°C)	Time	Number of cycles
First step	Initial denaturation	95	5 min.	1
Second step	Denaturation	94	0.30 sec.	} 40
	Annealing	55/60	0.30 sec.	
	Extension / Elongation	72	0.30 sec.	
Third step	Final extension	72	10 min.	1
Fourth step	Final hold	04	Till retrieval	--

3.2.2.5.2 Separation of amplified products

The amplified products were resolved on 2.5 % agarose / 10 % denaturing poly-acrylamide gel electrophoresis.

3.2.2.5.2.1 Agarose gel electrophoresis of amplified PCR products

The procedure for agarose gel electrophoresis described previously was followed, except the agarose concentration of 2.5 per cent.

3.2.2.5.2.2 Denaturing polyacrylamide gel electrophoresis

Requirements

- 1) Electrophoresis unit (gel casting unit, glass plates, spacer, clamps, combs, power pack)
- 2) Solutions
 - a) 10 % Polyacrylamide Gel (PAGE): Urea 100 g, Acrylamide 95 g, Bis acrylamide 5 g, 10X TBE 100 ml, distilled water 700 ml (Total volume made to 1 lit.)
 - b) 10 % APS: 0.1 g Ammonium per sulphate dissolved in 1 ml distilled water
- 3) Stock solution of 10X TBE
- 4) Working solution of 1X TBE
- 5) TEMED

Procedure

- 1) The glass plates were first washed with liquid detergent, then rinsed thoroughly with tap water, washed with distilled water and air-dried. The dried plates were rinsed with methanol and again air-dried.
- 2) The plates with spacers were assembled together with the help of clamps and the bottom of the plates was sealed using a small volume of PAGE solution with APS and TEMED.
- 3) Required volume of APS (0.075 %) and TEMED (0.0875 %) was added to the PAGE solution and it was quickly poured between the glass plates (taking precaution not to introduce any air bubble). Immediately the comb was inserted and the gel was allowed to polymerize for about 1/2 an hour.
- 4) After polymerization, the comb was removed carefully and the wells were carefully cleaned with the distilled water.
- 5) The gel plates were mounted on the vertical gel electrophoresis system with the help of clamps and the upper and lower buffer chambers were filled to the required volume with 1X TBE.
- 6) After completing the assembly, gel was pre-run for 30 min. at constant voltage.
- 7) After pre-run, 20 µl of amplified PCR product with the loading buffer was loaded with the help of loading tip in each well and the gel was electrophoresed at constant voltage of 90 to 100 V for 15 min.
- 8) Loading the gel in second plate was done after period of 15 min. Finally, both the gels were run at constant voltage of 90 to 100 V for 12 – 14 hours.
- 9) After electrophoresis the amplicons were visualized by staining the gel using silver staining.

Silver staining

To visualize the PCR products, silver staining was carried out following the procedure of Tagelstrom (1992) with some modification. Following steps were involved :

- a) After electrophoresis, the gels were carefully removed from the glass plates and transferred to a tray containing double distilled water and kept for 5 min. with gentle shaking.
- b) The distilled water was replaced with fixing solution containing 135 ml ddH₂O + 15 ml Methanol + 750 µl glacial acetic acid and kept for another five min. with gentle shaking.
- c) The above fixing solution was removed from the tray and retained for further use.
- d) Silver solution i.e. staining solution was now poured in the tray. The silver solution was prepared by dissolving 0.3 g AgNO₃, 15 ml of methanol and 750 µl glacial acetic acid in 150 ml of distilled water.
- e) The staining was done for 5 min. with gentle shaking.
- f) The silver solution was removed from the tray and gel was rinsed for a while in distilled water.
- g) The gel was transferred to a developing solution (prepared by dissolving 4.5g NaOH and 450 µl Formaldehyde in 150ml distilled water).
- h) The solution in the tray was shaken gently for 5 to 10 min. till the amplicons appear.
- i) The staining was stopped by rinsing the gel for 5 min. in the fixing solution retained after step c.
- j) The gel was placed on the platform of gel documentation system and photographed under EPI white light.

3.2.3 Data analysis

The clearly resolved amplicons of rice genotypes were scored manually for their presence (1) and absence (0) in the data sheet. Data were analyzed and similarity matrix was constructed from binary data with Dice similarity coefficients, which were calculated as per model suggested by Nei and Li (1979). Unweighted Pair Group Method using Arithmetic Averages (UPGMA) was employed for cluster analysis. The analysis was carried out using the software package NTSYSpc 2.02i (Rohlf, 1998).

3.3 Experiment III : Genetics of blast resistance in rice

3.3.1 Material

The material for generating mapping population comprised of four rice genotypes selected from the germplasm collection maintained at ARS, Radhanagari. The selection of parents was made on the basis of the blast evaluation studies carried out at ARS, Lonavala during *kharif* 2007-2008 (Anonymous, 2008, 2009). The selected genotypes comprised of two susceptible genotypes (EK 70 and Phule Radha) and two resistant (RDN 98-2-3-5-14 and KJT 2) genotypes. The salient features of the parental lines are presented in Table 3.10.

Table 3.10 Salient features of parental lines

S. No.	Genotype	Pedigree	Salient features
1	Early Kolpi 70 (EK 70)	Local selection from Igatpuri, Dist. Nasik (M.S.)	Early, tall, long-slender grain, highly susceptible to blast.
2	Phule Radha	TN 1 × Kolamba 540	Early, dwarf, short-slender grain, susceptible to blast.
3	RDN 98-2-3-5-14	Halvi Sal 17 × TN 1	Mid-late, tall, medium slender grain, resistant to blast.
4	KJT 2	RP 6-17 × RP 4-14	Late, dwarf, long slender grain, resistant to blast.

3.2.2 Methods

3.3.2.1 Production of hybrid Seeds

The susceptible parents (EK 70 and Phule Radha) were crossed with resistant parents (RDN 98-2-3-5-14 and KJT-2) by making all possible straight single crosses. The crossing programme was undertaken during April - May 2010 at Agricultural Research Station, Radhanagari.

To overcome the considerable variability for the initiation of flowering, care was taken to make staggered sowing of parents at three different dates at seven days interval (*i.e.* on 10th, 16th and 24th January, 2010) to achieve longer and successful crossing period among the parents.

3.3.2.2 Emasculation

The emasculation was done on previous evening of the flower opening. The spikelets which will open on next day were selected. All other spikelets from the selected panicles were removed. From the selected spikelets, anthers were removed carefully, by opening the lemma and palea with the help of forceps. Care was taken to avoid any damage to female reproductive organ *i.e.* stigma, style or ovary. After emasculation, the panicle was covered with paper bag and labelled immediately.

3.3.2.3 Pollination

The pollination of emasculated spikelets was done on the next morning of emasculation. The pollen grains from the desired parent were collected and shaded over the stigma of emasculated flower by brush. The pollination was done in the morning between 8.30 and 10.00 a.m. All the emasculated spikelets from single panicle were pollinated by same male parent. The panicle was immediately covered with perforated paper bag. The panicle was labelled indicating male and female parents, date of emasculation and date of pollination.

3.3.2.4 Harvesting and storage of crossed seed

Out of 1134 crosses made, only 143 seeds were developed indicating 12.61 per cent success. The successful hybrids were harvested at full maturity. The seeds of single hybrid were kept separately. The seeds were dried for three days. Methyl parathion (2 % dust) was added in the seed bag to avoid any insect damage. The seed bags were kept in closed container and were examined frequently for any infection.

3.3.2.5 Raising of F₁ generation

The hybrid seeds of all four single crosses and parents were sown on raised beds at ARS, Radhanagari on 10th June 2010. Single seedling was transplanted at a spacing of 20 cm between rows and 15 cm between plants in breeding nursery at ARS, Radhanagari on 09th July 2010. The recommended agronomic practices were followed.

3.3.2.6 Harvesting and storage of seeds from F₁ hybrid

The hybrids EK 70 × KJT 2 and Phule Radha × KJT 2 were found sterile. The seed of single plants of two crosses (EK 70 × RDN 98-2-3-5-14 and Phule

Radha × RDN 98-2-3-5-14) was harvested separately at full maturity. The seeds of single hybrid were kept separately. The seeds were dried for three days. Methyl parathion (2 % dust) was added in the seed bag to avoid any insect damage. The seed bags were kept in closed container and were examined frequently for any infection.

3.3.2.7 Raising of F₂ generation (mapping population)

The seeds harvested from two single crosses and parents were sown on raised beds at ARS, Lonavala on 11th June 2011. Single seedling was transplanted at a spacing of 20 cm between rows and 15 cm between plants in disease screening nursery on 08th July 2011. Four border rows of the susceptible check, EK 70 were planted on all sides of block. Similarly, two rows of susceptible check were also planted after every five test rows of the F₂ population to provide adequate blast inoculum for uniform occurrence of the disease.

3.3.2.8 Cultural practices

All recommended agronomic practices were carried out as and when required. Total dose of fertilizers applied during crop duration was 100 kg N per hectare in three split doses of 50, 25 and 25 kg at time of transplanting, on 02nd August 2011 and 29th August 2011, respectively. To have good blast incidence P₂O₅ and K₂O were not applied. The seedlings were inoculated three times at two days interval with the suspension of *Magnaporthe grisea* spores at tillering stage.

3.3.2.9 Recording observation

One hundred fifty plants were tagged at random in each cross for recording blast observations. The disease observations were recorded by adopting SES scale (Anonymous, 2002). Among the two crosses studied, the F₂ segregating population of the cross Phule Radha × RDN 98-2-3-5-14 showed the variation for infection type ranged from 1 to 5, whereas it was from 0 and 9 for the cross EK 70 × RDN 98-2-3-5-14. As per SES 2002, the cross EK 70 × RDN 98-2-3-5-14 showed complete range for blast indicating best segregation for the character. Therefore, the cross EK 70 × RDN 98-2-3-5-14 was selected for further analysis.

3.3.3 Molecular analysis

3.3.3.1 DNA extraction, purification and quantification

Total DNA was extracted from the leaves of 150 individual F_2 plants of the best segregating population (EK 70 $\times$ RDN 98-2-3-5-14) by CTAB (Cetyl Trimethyl Ammonium Bromide) as per the protocol used in Experiment I, except the sample size was 200 μ l and quantity of chemicals required there upon.

3.3.3.2 PCR Amplification

Twenty-five SSR primer pairs were used for the present investigation. The primers were selected based on previous studies on blast resistance in rice and the panel of 50 standard SSR markers reported on the website www.gramene.org. The genes for blast resistance were reported on almost all the chromosomes of rice genome and the markers associated / linked to these genes were reported (Moumeni and Leung, 2003; Wu *et al.*, 2004; Sharma *et al.*, 2005; Barman *et al.*, 2004; Fjellstorm *et al.*, 2006; Li *et al.*, 2008 and Lee *et al.*, 2009). The markers reported by these scientists and mapped on different chromosomes (Chen *et al.*, 1997; Temnykh *et al.*, 2001) were selected for present study. The sequences of the primers were collected from the website www.gramene.org and synthesised by Custom Oligo Synthesis Division, Merck Specialities Pvt. Ltd., Bangalore. Details of the primers used are presented in Table 3.11.

3.3.3.3 DNA amplification

PCR amplification of genomic DNA was done as per that of SSR markers in Experiment I. The amplified products were resolved on 2.5 % MetaPhor agarose gel. The protocol for metaphor agarose gel preparation was as per agarose gel followed in Experiment I.

3.3.3.4 Bulk Segregant Analysis (BSA)

Bulk segregant analysis as suggested by Michelmore (1991) was used for rapid identification of markers associated to blast resistance. Based on phenotypic observations, two bulks viz., susceptible bulk (B1) comprising of five susceptible F_2 s and resistant bulk (B2) comprising of five resistant F_2 s were made. A pooled DNA sample was prepared for each bulk by mixing in equal quantity the DNA of five respective component F_2 s. The parents and the bulks were screened for 25 SSR primers to identify the polymorphic markers associated with blast resistance.

Table 3.11 Sequences details of SSR primers

S. No.	Primer name	Chromo. No.	Primer sequence (5'-3')	
1	RM259	1	F	TGGAGTTTGAGAGGAGGG
			R	CTTGTTGCATGGTGCCATGT
2	RM246	1	F	GAGCTCCATCAGCCATTGAG
			R	CTGAGTGCTGCTGCGACT
3	RM250	2	F	GGTTCAAACCAAGCTGATCA
			R	GATGAAGGCCTTCCACGCAG
4	RM213	2	F	ATCTGTTTGCAGGGGACAAG
			R	AGGTCTAGACGATGTCGTGA
5	RM307	4	F	GTACTACCGACCTACCGTTCAC
			R	CTGCTATGCATGAACTGCTC
6	RM241	4	F	GAGCCAAATAAGATCGCTGA
			R	TGCAAGCAGCAGATTTAGTG
7	RM204	6	F	GTGACTGACTTGGTCATAGGG
			R	GCTAGCCATGCTCTCGTACC
8	RM162	6	F	GCCAGCAAAACCAGGGATCCGG
			R	CAAGGTCTTGTGCGGCTTGCGG
9	AP5659-3	6	F	TCTTTCCTAGGGAACCAAAG
			R	AAGTAGTTGCTGAGCCATTG
10	RM11	7	F	TCTCCTCTTCCCCCGATC
			R	ATAGCGGGCGAGGCTTAG
11	RM44	8	F	ACGGGCAATCCGAACAACC
			R	TCGGGAAAACCTACCCTACC
12	RM25	8	F	GGAAAGAATGATCTTTTCATGG
			R	CTACCATCAAAACCAATGTTC
13	RM72	8	F	CCGGCGATAAAACAATGAG
			R	GCATCGGTCCTAATAAGGG
14	RM210	8	F	TCACATTCGGTGGCATTG
			R	CGAGGATGGTTGTTCACTTG

Table 3.11 cont....

S. No.	Primer name	Chromo. No.	Primer sequence (5'-3')	
15	RM316	9	F	CTAGTTGGGCATACGATGGC
			R	ACGCTTATATGTTACGTCAAC
16	RM215	9	F	CAAAATGGAGCAGCAAGAGC
			R	TGAGCACCTCCTTCTCTGTAG
17	RM201	9	F	CTCGTTTATTACCTACAGTACC
			R	CTACCTCCTTTCTAGACCGATA
18	RM224	11	F	ATCGATCGATCTTCACGAGG
			R	TGCTATAAAAGGCATTCGGG
19	RM209	11	F	ATATGAGTTGCTGTCTGTGCG
			R	CAACTTGCATCCTCCCCTCC
20	RM21	11	F	ACAGTATTCCGTAGGCACGG
			R	GCTCCATGAGGGTGGTAGAG
21	TRS26	11	F	GGAGAGCCAATCTGATAAGCA
			R	CAACAAGAGAGGGCAAATTCTCA
22	TRS33	11	F	AAGAAGAAGCGTACGCATGAAT
			R	GTCCTGGAGGGGAGGAGA
23	RM277	12	F	CGGTCAAATCATCACCTGAC
			R	CAAGGCTTGCAAGGGAAG
24	RM4	12	F	TTGACGAGGTCAGCACTGAC
			R	AGGGTGTATCCGACTCATCG
25	RM1337	12	F	GTGCAATGCTGAGGAGTATC
			R	CTGAGAATCTGGAGTGCTTG

3.3.3.5 F₂ progeny analysis

The SSR markers found polymorphic among the parents as well as the resistant and susceptible bulks were used for F₂ progeny analysis. DNA of 150 F₂ progenies and parents were analysed to study co-segregation of these markers.

3.3.3.6 SSR genotyping

The SSR alleles obtained from PCR amplification of the various SSR markers in the F₂s were observed under UV transilluminater in gel documentation system (Flour Chem. TM Alpha innotech, USA). The alleles were scored as follows :

0 = homozygote for the allele for susceptible parent (P₁) (EK 70)

1 = homozygote for the allele for resistant parent (P₂) (RDN 98-2-3-5-14)

2 = heterozygote carrying the alleles from both P₁ and P₂.

- = missing data for the individual

After scoring the individual progeny, a data set was assembled in a Microsoft Excel spreadsheet in a format suitable for statistical analysis.

3.3.4 Statistical analysis

3.3.4.1 Chi-square test

Chi-square (χ^2) test was performed to test the goodness of fit of the F₂ population by comparing an observed frequency distribution with a expected one. The χ^2 value was calculated by the formula

$$\chi^2 = ((O - E)^2 / E)$$

where,

O = observed frequency

E = expected frequency

The calculated χ^2 value was tested against standard χ^2 values at (n-1) degrees of freedom.

3.3.4.2 Simple linear regression

The polymorphic SSR markers identified to be associated with the blast resistance by bulk segregant analysis were utilized further for the molecular data scoring to know the genotype of the randomly selected 150 F₂s and the parents. Allelic pattern revealed by SSR markers was scored as 0 for resistant and 1 for susceptible reaction based on respective product sizes of each SSR primer observed for susceptible (EK 70) and resistant (RDN 98-2-3-5-14) parent. The molecular and phenotypic data obtained were analyzed by simple linear regression method to

know the association between the markers and the blast score. For each of the F_2 s, molecular data (0, 1) was considered as an independent variable (X) and phenotypic data for a particular character under consideration was considered as a dependant variable (Y). The linear regression model (Fisher, 1922) was used to study regression of linked polymorphic markers on blast score.

Regression model

$$Y = \alpha + \beta X + \varepsilon$$

where,

Y = dependent variable

X = independent variable

α = Y-intercept of regression line

β = slope of the line (linear regression coefficient)

It is the amount of change in Y for each unit change in X

ε = error / unexplained variance.

The means (X and Y), corrected sum of squares $\sum(X_i - \bar{X})^2$ and $\sum(Y_i - \bar{Y})^2$ and the corrected sum of products $\sum(X_i - \bar{X})(Y_i - \bar{Y})$ of variables X and Y were computed.

where,

$i = 1$ to n and

$n = F_2$ s (out of 150) and the two parents showing association with the SSR primer under consideration.

Estimates of regression parameters α and β

$$a = Y - b X$$

$$b = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sum(X_i - \bar{X})^2}$$

where,

a is the estimate of α and b , the estimate of β

$$\text{MSE} = \text{error mean sum of squares} = \frac{\text{SSE}}{n-2}$$

Test of significance of β

The following estimates of sum of squares were calculated to test the significance of β .

$$\text{TSS} = \text{total sum of squares} = \sum (Y_i - \bar{Y})^2$$

$$\text{SSR} = \text{sum of squares due to residuals} = b * \sum (X_i - \bar{X})(Y_i - \bar{Y})$$

$$\text{SSE} = \text{error sum of squares} = \text{TSS} - \text{SSR}$$

$$\text{MSE} = \text{error mean sum of squares} = \frac{\text{SSE}}{n - 2}$$

$$\text{SE}(b) = \text{standard Error of } b = \sqrt{\frac{\text{MSE}}{\sum (X_i - \bar{X})^2}}$$

$$t_{cal} = \frac{|b|}{\text{SE}(b)}$$

Calculated t values (t_{cal}) were compared with the tabular t values with $n-2$ degrees of freedom. β was judged to be significantly different from zero if the absolute value of the t_{cal} was greater than the tabular t value at 5 per cent level of significance. The standard errors [SE (b)] and the 't' values were calculated for polymorphic SSR primer. The significance of calculated 't' value indicated the presence of significant association of the marker tested with the blast resistance in rice.

CHAPTER IV

EXPERIMENTAL RESULTS

The present investigation entitled “Genetic and molecular analysis for blast resistance in rice (*Oryza sativa* L.)” was conducted at Agricultural Research Station, Radhanagari, Dist. Kolhapur; Agricultural Research Station, Lonavala, Dist. Pune and State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2009-10 to 2011-12. The research was conducted to study divergence, genetic diversity and molecular diversity in rice genotypes and to tag the genes associated with blast resistance in rice. The investigation was carried out in three experiments. The results obtained in these experiments are presented in this chapter.

4.1 Experiment I : Genetic diversity in rice germplasm for agro-morphological and pathological traits

The present study was conducted to evaluate the pattern and extent of genetic variability and diversity among 65 rice genotypes employing important agro-morphological and pathological traits related to rice blast. The results will enable selection of diverse parents and assist in broadening the germplasm base of future rice breeding programme in order to develop high yielding blast resistant desirable genotypes in rice.

4.1.1 Genetic diversity in rice germplasm for agro-morphological traits

The experimental material was evaluated in randomised block design with two replications at two locations (Location I: ARS, Radhanagari and Location II: ARS, Lonavala) for 12 agro-morphological traits. The data obtained was subjected to statistical analysis. The results obtained are presented below.

4.1.1.1 Mean performance of genotypes for agro-morphological characters

The mean performance of 65 genotypes under study at two locations and pooled mean performance is presented in Table 4.1. The trait wise results of the mean performance are documented below.

4.1.1.1.1 Days to 50 per cent flowering

The substantial variation for days to 50 per cent flowering (Table 4.1) was observed for the material studied. It ranged between 61.00 and 120.50 days at Location I and 63.50 to 129.00 days at Location II, with the average of 104.05 and 106.18 days, respectively. The range for pooled mean performance for the character was 62.25 to 124.75 days with overall population mean of 105.12 days. The genotype Heera showed 61.00 and 63.50 days to 50 per cent flowering at Location I and Location II, respectively that was earliest among all the genotypes at both the locations. This was followed by Halvi Sal 17 (80.50 days) and EK 70 (85.50 days) at Location I and EK 70 (85.00) and Halvi Sal 17 (88.50 days) at Location II. The pooled performance indicated that the genotype Heera (62.25 days) was the earliest in flowering followed by Halvi Sal 17 (84.50 days) and EK 70 (85.20 days). Maximum days of 120.50, 129.50 and 124.75 days for flowering were required for the genotype Antersal at Location I, Location II and pooled, respectively followed by the genotype Sonsali wherein 119.50, 127.50 and 123.50 days were required at Location I, Location II and pooled, respectively. The pooled performance indicated that eight genotypes were early (flowering in < 95 days), 22 genotypes were midlate (flowering in 95 to 105 days) and 35 genotypes were late (flowering in ≥ 105 days) in flowering.

4.1.1.1.2 Days to maturity

The observations for days to maturity (Table 4.1) indicated that the genotypes required on an average 131.97 days for maturity at Location I and 135.11 days for maturity at Location II with pooled mean of 133.54 days over two locations. The genotype Heera (86 days) matured earliest at both locations followed by Halvi Sal 17 (105.50 days) and EK 70 (108.50 days) at Location I and EK 70 (109.00 days) and Halvi Sal 17 (112.00 days) at Location II. Maximum days of 150.50 and 160.00 days for maturity were recorded by the genotype Antersal at Location I and Location II, respectively followed by Sonsali (149.50 and 159.00 days, respectively). Among 65 genotypes studied, 15 genotypes were found significantly earlier than the population mean.

4.1.1.1.3 Plant height

The data in Table 4.1 indicate that the rice genotypes had a wide range of plant height varying from 67.75 to 156.90 cm at Location I, 61.95 cm to 162.20 cm at Location II with pooled mean of 65.03 to 152.48 cm. The average plant height of 65 genotypes was 104.53, 109.95 and 107.24 cm at locations I, II and pooled, respectively. The genotype Kunchi (156.90 cm) was tallest at Location I followed by Nalabhat (152.30 cm) and Sonsali (145.60 cm). While at Location II, the genotype Ajara Local 3 (162.20 cm) was tallest followed by Patni (149.70 cm) and Kunchi (146.80 cm). Pooled performance over two locations indicated that Ajara local 3 (152.48 cm) was tallest followed by Kunchi (151.85 cm) and Nalabhat (148.13 cm), whereas the variety Heera (65.03 cm) was dwarfest followed by RDN 02-80 (69.45 cm) and BPT 5204 (76.83 cm). Among the genotypes, 28 were significantly dwarfer and 21 were significantly taller than population mean of 107.24 cm.

4.1.1.1.4 Panicle length

The variation in the panicle length of 65 genotypes (Table 4.1) ranged from 16.85 to 26.90 cm at Location I and 17.15 to 28.55 cm at Location II, with the average of 22.56 and 23.50 cm, respectively. The genotype Vivek Dhan 82 had longest panicle (26.90 cm) at Location I followed by Pinjarwadi Local (26.75 cm) and Ghansal (26.65 cm), whereas Ajara Local 3 and IGT 13857 showed maximum panicle length at Location II that was followed by Antersal (27.70) and Diwani (27.25 cm). On the contrary, Heera reported smallest panicles (16.85 and 17.15 cm at Location I and II, respectively) followed by Jagatpuri (17.35 cm) at Location I and Halvi Sal 17 (17.70 cm) at Location II.

The pooled data revealed that the mean length of panicle was 23.03 cm that varied from 17.00 to 27.55 cm. The genotype IGT 13857 showed longest panicle (27.55 cm) followed by Ajara Local 3 (27.33 cm) and Pinjarwadi Local (26.50 cm), whereas Heera recorded shortest panicle (17.00 cm) followed by Halvi Sal 17 (17.70 cm) and BPT 5204 (17.70 cm) in pooled performance over two locations.

Table 4.1 Performance of rice genotypes for agro-morphological characters

S. No.	Genotype	Days to 50 % flowering (number)			Days to maturity (number)			Plant height (cm)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
1	Heera	61.00	63.50	62.25	86.00	86.00	86.00	68.10	61.95	65.03
2	Halvi Sal 17	80.50	88.50	84.50	105.50	112.00	108.75	100.20	101.15	100.68
3	RDN 185-2	87.00	89.00	88.00	111.50	112.50	112.00	85.25	88.55	86.90
4	Phule Radha	95.50	94.00	94.75	117.50	118.00	117.75	88.25	92.15	90.20
5	RTN 1	91.00	89.00	90.00	115.50	113.50	114.50	84.80	87.90	86.35
6	Ratna	95.50	100.50	98.00	120.00	129.00	124.50	80.05	93.75	86.90
7	Phule Samruddhi	104.00	108.00	106.00	133.00	137.50	135.25	85.20	88.50	86.85
8	Jaya	103.00	100.00	101.50	131.00	128.00	129.50	94.35	90.15	92.25
9	Phule Maval	98.50	93.50	96.00	127.50	117.50	122.50	79.70	88.95	84.33
10	Pawana	94.50	96.50	95.50	119.00	121.00	120.00	86.10	89.50	87.80
11	Indrayani	106.50	102.00	104.25	135.00	130.00	132.50	84.90	88.15	86.53
12	Bhogavati	106.50	106.00	106.25	136.00	136.00	136.00	86.85	88.95	87.90
13	Basmati 370	102.50	105.50	104.00	130.50	135.00	132.75	118.60	131.30	124.95
14	Pusa Basmati 1	97.50	99.00	98.25	120.50	127.00	123.75	96.20	100.35	98.28
15	Ambemohar 157	112.00	113.50	112.75	142.00	143.50	142.75	143.65	143.60	143.63
16	Ghansal	111.50	117.50	114.50	140.50	148.50	144.50	138.35	136.15	137.25
17	Kalajirga	115.00	117.00	116.00	144.00	147.50	145.75	127.55	139.60	133.58
18	Vivek Dhan 82	90.00	92.00	91.00	113.50	116.00	114.75	128.35	137.55	132.95
19	Badshabhog	114.50	112.50	113.50	145.50	142.50	144.00	134.80	133.75	134.28
20	Kothimbire	97.00	101.50	99.25	121.50	131.00	126.25	114.60	120.40	117.50
21	BPT 5204	105.00	103.00	104.00	134.50	132.50	133.50	79.65	74.00	76.83
22	RDN 98-2-3-5-14	96.50	100.00	98.25	121.00	128.50	124.75	111.65	108.60	110.13
23	RDN 01-2-10-9	106.00	105.50	105.75	136.50	136.00	136.25	88.10	94.80	91.45
24	KJT 2	110.50	112.50	111.50	140.00	143.00	141.50	78.75	84.55	81.65
25	EK 70	85.50	85.00	85.25	108.50	109.00	108.75	102.65	114.75	108.70
26	Patni	99.50	102.00	100.75	124.50	132.00	128.25	140.75	149.70	145.23
27	Diwani	106.00	106.50	106.25	135.50	137.00	136.25	121.35	137.15	129.25
28	Shyamjeer	114.50	112.00	113.25	145.50	142.00	143.75	93.05	112.20	102.63
29	Juhibengal	109.00	107.50	108.25	139.00	137.00	138.00	116.45	130.85	123.65
30	Improved Pusa Basmati 1	105.00	101.50	103.25	135.00	130.50	132.75	85.15	92.35	88.75
31	Kasturi	106.50	111.50	109.00	136.50	142.00	139.25	87.60	92.35	89.98
32	Karnal Local	106.50	116.50	111.50	136.00	147.50	141.75	83.85	93.90	88.88
33	SD 7	103.50	105.50	104.50	133.50	135.50	134.50	114.40	113.75	114.08
34	SD 17	109.00	104.00	106.50	138.50	134.00	136.25	105.60	102.75	104.18
35	Sugandhamati	102.00	102.00	102.00	132.00	131.50	131.75	85.05	97.25	91.15

Table 4.1 cont...

S. No.	Genotype	Days to 50 % flowering (number)			Days to maturity (number)			Plant height (cm)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
36	Basmati 386	105.50	102.00	103.75	136.00	131.50	133.75	117.00	132.50	124.75
37	MC 4	106.00	111.00	108.50	136.50	141.50	139.00	110.80	124.80	117.80
38	Haryana Basmati	106.50	114.50	110.50	135.50	145.00	140.25	101.10	96.20	98.65
39	Mahisugandha	109.00	112.00	110.50	138.00	142.50	140.25	102.65	101.65	102.15
40	Khalibagh	106.50	107.50	107.00	135.00	138.00	136.50	81.10	84.95	83.03
41	Jagatpuri	107.00	108.50	107.75	136.50	138.50	137.50	78.00	87.25	82.63
42	Vikram	104.00	110.50	107.25	132.00	141.00	136.50	95.75	114.50	105.13
43	Velkat	119.00	121.50	120.25	148.50	153.50	151.00	107.30	118.25	112.78
44	Tulshi tall	116.50	120.50	118.50	146.50	152.00	149.25	108.20	114.40	111.30
45	Champakali	91.00	91.00	91.00	116.00	115.00	115.50	90.75	98.50	94.63
46	Siddhagiri	114.50	121.00	117.75	143.00	152.00	147.50	128.15	139.30	133.73
47	RDN 97-2	103.00	106.00	104.50	130.50	136.00	133.25	94.60	97.50	96.05
48	RDN 99-12	102.50	110.50	106.50	131.50	141.50	136.50	80.35	92.30	86.33
49	RDN 99-14	113.00	110.50	111.75	143.00	141.50	142.25	89.10	90.80	89.95
50	RTN purple	107.00	106.00	106.50	136.50	136.00	136.25	76.00	91.35	83.68
51	Taroari Basmati	100.50	104.00	102.25	129.50	134.50	132.00	107.65	130.00	118.83
52	RDN 99-18	114.00	119.50	116.75	133.00	150.50	141.75	88.10	82.95	85.53
53	RDN 02-80	96.50	96.00	96.25	121.00	121.00	121.00	67.75	71.15	69.45
54	Ajara Local 1	106.50	120.00	113.25	135.50	150.50	143.00	122.95	135.80	129.38
55	Ajara Local 3	105.00	115.50	110.25	135.00	146.00	140.50	142.75	162.20	152.48
56	IGT 13857	118.00	117.50	117.75	148.50	148.50	148.50	139.65	131.35	135.50
57	Pavsai	118.50	124.00	121.25	148.00	154.00	151.00	142.55	134.55	138.55
58	Kunchi	115.00	122.50	118.75	144.50	153.50	149.00	156.90	146.80	151.85
59	Ambemohar 102	104.50	102.00	103.25	133.50	131.00	132.25	132.15	144.70	138.43
60	Antersai	120.50	129.00	124.75	150.50	160.00	155.25	130.05	145.15	137.60
61	Nalabhat	109.00	108.50	108.75	139.00	139.00	139.00	152.20	144.05	148.13
62	Sonsali	119.50	127.50	123.50	149.50	159.00	154.25	145.80	132.25	139.03
63	Pinjarwadi Local	97.00	108.00	102.50	121.50	138.50	130.00	106.00	109.30	107.65
64	LK 248	97.00	94.00	95.50	121.00	119.00	120.00	88.45	95.35	91.90
65	Pomendi Local	102.00	97.00	99.50	131.00	121.50	126.25	90.55	95.45	93.00
	Range	61.00 - 120.50	63.50 - 129.00	62.25 - 124.75	86.00 - 150.50	86.00 - 160.00	86.00 - 155.25	67.75 - 156.90	61.95 - 162.20	65.03 - 152.48
	Mean	104.05	106.18	105.12	131.97	135.11	133.54	104.53	109.95	107.24
	S.E. (+/-)	0.83	1.40	2.15	0.98	1.41	2.88	3.67	4.49	4.01
	C.D. at 5%	2.35	3.95	6.08	2.78	3.98	8.15	10.37	12.69	11.34
	C.V. (%)	1.13	1.86	2.90	1.05	1.48	3.05	4.97	5.78	5.29

Loc. I : ARS, Radhanagari; Loc. II : ARS, Lonavala

Table 4.1 cont...

S. No.	Genotype	Panicle length (cm)			Spikelets per panicle (number)			Spikelet fertility (%)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
1	Heera	16.85	17.15	17.00	77.00	76.40	76.70	91.80	92.41	92.10
2	Halvi Sal 17	17.80	17.60	17.70	107.70	115.70	111.70	95.61	95.68	95.65
3	RDN 185-2	17.70	18.35	18.03	114.90	118.70	116.80	95.89	95.49	95.69
4	Phule Radha	21.20	20.45	20.83	182.10	209.80	195.95	92.52	93.85	93.19
5	RTN 1	21.45	20.70	21.08	123.50	133.20	128.35	93.51	93.32	93.42
6	Ratna	22.35	22.10	22.23	126.10	135.40	130.75	95.81	95.11	95.46
7	Phule Samruddhi	21.40	22.15	21.78	152.90	164.10	158.50	92.88	94.63	93.76
8	Jaya	23.10	23.35	23.23	135.20	140.60	137.90	94.73	94.28	94.50
9	Phule Maval	21.10	23.10	22.10	128.70	140.20	134.45	94.55	93.80	94.17
10	Pawana	21.95	22.60	22.28	121.50	140.30	130.90	92.49	90.88	91.68
11	Indrayani	22.30	22.85	22.58	145.00	160.60	152.80	92.67	93.66	93.17
12	Bhogavati	25.15	24.00	24.58	140.40	158.40	149.40	94.02	95.01	94.52
13	Basmati 370	23.45	24.25	23.85	103.10	117.00	110.05	92.70	93.81	93.26
14	Pusa Basmati 1	24.85	25.40	25.13	130.50	132.40	131.45	92.80	94.25	93.52
15	Ambemohar 157	26.50	25.70	26.10	232.10	246.10	239.10	95.68	96.77	96.23
16	Ghansal	26.65	25.85	26.25	216.50	209.80	213.15	95.51	95.32	95.42
17	Kalajirga	23.95	24.85	24.40	200.20	213.20	206.70	96.30	96.36	96.33
18	Vivek Dhan 82	26.90	26.10	26.50	142.10	135.50	138.80	95.06	95.36	95.21
19	Badshabhog	26.35	26.20	26.28	230.70	237.70	234.20	95.92	96.00	95.96
20	Kothimbire	22.30	24.15	23.23	158.40	168.90	163.65	96.59	95.79	96.19
21	BPT 5204	17.90	17.50	17.70	160.80	149.60	155.20	94.15	95.07	94.61
22	RDN 98-2-3-5-14	26.30	24.95	25.63	196.40	203.20	199.80	96.47	95.96	96.22
23	RDN 01-2-10-9	22.35	22.30	22.33	185.60	182.10	183.85	95.36	93.57	94.47
24	KJT 2	22.10	22.50	22.30	152.00	152.70	152.35	94.53	93.49	94.01
25	EK 70	18.15	19.50	18.83	126.10	137.90	132.00	93.99	92.66	93.32
26	Patni	22.15	25.00	23.58	116.00	142.90	129.45	94.50	93.77	94.14
27	Diwani	22.85	27.25	25.05	212.80	219.40	216.10	96.23	95.81	96.02
28	Shyamjeer	21.55	24.00	22.78	200.30	153.60	176.95	94.66	94.96	94.81
29	Juhibengal	22.65	26.25	24.45	156.10	171.70	163.90	95.84	95.98	95.91
30	Improved Pusa Basmati 1	22.10	22.65	22.38	116.40	123.70	120.05	90.80	94.09	92.45
31	Kasturi	24.75	24.85	24.80	130.60	135.20	132.90	94.25	94.07	94.16
32	Karnal Local	25.20	25.45	25.33	134.60	132.80	133.70	94.65	94.05	94.35
33	SD 7	20.85	21.75	21.30	98.90	126.20	112.55	84.40	90.82	87.61
34	SD 17	26.15	25.15	25.65	170.30	163.00	166.65	95.00	93.21	94.10
35	Sugandhamati	19.60	22.55	21.08	139.10	137.90	138.50	94.02	91.54	92.78

Table 4.1 cont...

S. No.	Genotype	Panicle length (cm)			Spikelets per panicle (number)			Spikelet fertility (%)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
36	Basmati 386	23.25	25.50	24.38	128.80	140.30	134.55	92.93	93.08	93.00
37	MC 4	24.65	25.90	25.28	137.00	135.40	136.20	94.24	92.90	93.57
38	Haryana Bas mati	24.20	24.45	24.33	138.60	151.70	145.15	94.09	94.45	94.27
39	Mahisugandha	24.60	25.10	24.85	155.80	159.20	157.50	94.48	94.45	94.46
40	Khalibagh	23.95	25.25	24.60	151.70	144.70	148.20	93.93	93.43	93.68
41	Jagatpuri	17.35	18.70	18.03	151.20	148.40	149.80	93.12	93.27	93.20
42	Vikram	17.85	20.50	19.18	135.80	148.50	142.15	94.24	93.87	94.06
43	Velkat	23.10	23.55	23.33	189.60	179.60	184.60	96.72	94.99	95.86
44	Tulshi tall	20.50	22.60	21.55	170.20	174.70	172.45	94.94	94.61	94.78
45	Champakali	18.65	19.20	18.93	112.70	119.90	116.30	93.00	93.47	93.24
46	Siddhagiri	26.15	26.60	26.38	158.40	167.70	163.05	96.14	96.24	96.19
47	RDN 97-2	20.35	20.55	20.45	203.40	162.20	182.80	96.74	94.33	95.54
48	RDN 99-12	20.30	21.80	21.05	163.60	154.10	158.85	93.86	92.72	93.29
49	RDN 99-14	21.75	22.85	22.30	162.10	167.30	164.70	94.01	94.62	94.31
50	RTN purple	21.35	22.45	21.90	117.70	133.20	125.45	94.48	93.54	94.01
51	Taroari Bas mati	20.30	22.60	21.45	122.10	119.30	120.70	93.61	92.78	93.19
52	RDN 99-18	23.05	21.05	22.05	152.90	136.30	144.60	95.94	95.08	95.51
53	RDN 02-80	18.55	18.95	18.75	110.80	119.70	115.25	93.93	94.81	94.37
54	Ajara Local 1	24.35	27.15	25.75	199.50	212.30	205.90	96.14	95.52	95.83
55	Ajara Local 3	26.10	28.55	27.33	193.40	207.40	200.40	96.23	94.98	95.60
56	IGT 13857	26.25	28.85	27.55	157.00	138.60	147.80	95.39	93.00	94.20
57	Pavsal	23.45	24.35	23.90	147.00	136.00	141.50	94.96	94.63	94.80
58	Kunchi	20.80	26.30	23.55	151.60	146.80	149.20	96.25	95.44	95.84
59	Ambemohar 102	21.05	23.45	22.25	140.90	155.40	148.15	91.27	91.60	91.44
60	Antersal	23.30	27.70	25.50	143.90	172.20	158.05	92.70	92.28	92.49
61	Nalabhat	25.95	26.65	26.30	146.80	143.70	145.25	94.13	91.79	92.96
62	Sonsali	26.30	26.40	26.35	173.50	176.20	174.85	96.48	96.37	96.43
63	Pinjarwadi Local	26.75	26.25	26.50	160.80	160.20	160.50	92.69	92.76	92.72
64	LK 248	21.05	22.15	21.60	124.20	125.30	124.75	94.60	92.71	93.65
65	Pomendi Local	23.50	23.60	23.55	154.80	162.30	158.55	95.41	95.32	95.37
	Range	16.85 - 26.90	17.15 - 28.55	17.00 - 27.55	77.00 - 232.10	76.40 - 246.20	76.70 - 239.10	84.40 - 96.40	90.82 - 96.77	87.61 - 96.43
	Mean	22.56	23.50	23.03	151.08	155.15	153.11	94.35	94.15	94.25
	S.E. (+/-)	0.47	0.43	0.73	5.03	5.74	6.70	0.40	0.40	0.68
	C.D. at 5%	1.33	1.22	2.05	14.22	16.22	18.94	1.13	1.13	1.92
	C.V. (%)	2.95	2.61	4.46	4.71	5.23	6.19	0.60	0.60	1.02

Loc. I : ARS, Radhanagari; Loc. II : ARS, Lonavala

Table 4.1 cont...

S. No.	Genotype	Tillers per plant (number)			Test weight (g)			Grain length (mm)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
1	Heera	7.00	7.00	7.00	26.92	27.03	26.97	6.54	6.45	6.50
2	Halvi Sal 17	5.50	5.40	5.45	27.68	28.11	27.90	5.18	5.16	5.17
3	RDN 185-2	5.40	5.50	5.45	27.73	27.32	27.52	5.18	5.27	5.23
4	Phule Radha	7.00	7.10	7.05	14.13	14.10	14.11	5.27	5.21	5.24
5	RTN 1	7.20	7.00	7.10	28.56	28.70	28.63	6.65	6.47	6.56
6	Ratna	8.70	8.00	8.35	22.93	22.68	22.81	6.23	6.06	6.15
7	Phule Samruddhi	6.80	7.30	7.05	22.38	22.49	22.44	6.36	6.48	6.42
8	Jaya	6.40	7.60	7.00	26.40	26.62	26.51	5.73	5.76	5.75
9	Phule Maval	8.10	6.80	7.45	29.64	29.64	29.64	7.42	7.49	7.46
10	Pawana	7.80	7.80	7.80	24.77	24.51	24.64	6.53	6.62	6.58
11	Indrayani	6.60	6.90	6.75	21.66	21.45	21.56	6.52	6.62	6.57
12	Bhogavati	7.90	8.70	8.30	21.54	21.34	21.44	7.77	7.66	7.72
13	Basmati 370	7.90	6.90	7.40	21.12	20.67	20.90	6.51	6.74	6.63
14	Pusa Basmati 1	7.70	7.00	7.35	21.79	21.70	21.75	7.80	7.69	7.75
15	Ambemohar 157	5.80	5.90	5.85	13.27	13.29	13.28	4.46	4.42	4.44
16	Ghansal	6.50	6.20	6.35	12.69	12.38	12.54	4.36	4.48	4.42
17	Kalajirga	7.10	6.90	7.00	17.49	17.37	17.43	4.57	4.50	4.54
18	Vivek Dhan 82	6.20	6.20	6.20	31.09	31.02	31.05	6.31	6.32	6.32
19	Badshabhog	6.40	6.60	6.50	11.10	11.44	11.27	4.35	4.33	4.34
20	Kothimbire	8.10	7.20	7.65	17.20	17.12	17.16	3.84	3.92	3.88
21	BPT 5204	7.20	6.80	7.00	14.53	14.49	14.51	5.31	5.37	5.34
22	RDN 98-2-3-5-14	9.00	7.50	8.25	19.39	19.41	19.40	5.46	5.43	5.45
23	RDN 01-2-10-9	6.80	7.20	7.00	22.96	22.81	22.89	6.85	6.76	6.81
24	KJT 2	7.60	7.70	7.65	22.22	21.95	22.09	6.56	6.72	6.64
25	EK 70	9.20	8.20	8.70	20.19	20.05	20.12	6.52	6.73	6.63
26	Patni	6.70	7.00	6.85	33.70	33.71	33.70	5.92	5.81	5.87
27	Diwani	7.90	6.50	7.20	9.51	9.41	9.46	4.22	4.31	4.27
28	Shyamjeer	7.00	7.00	7.00	9.93	9.75	9.84	5.04	5.15	5.10
29	Juhibengal	7.40	7.10	7.25	21.60	21.57	21.59	5.60	5.54	5.57
30	Improved Pusa Basmati 1	8.40	7.10	7.75	22.71	22.44	22.58	7.23	7.12	7.18
31	Kasturi	7.10	6.00	6.55	21.78	21.62	21.70	7.06	7.11	7.09
32	Karnal Local	8.40	7.80	8.10	23.34	23.26	23.30	6.87	7.05	6.96
33	SD 7	8.50	8.00	8.25	21.01	20.96	20.99	7.13	7.03	7.08
34	SD 17	7.00	6.80	6.90	25.39	25.16	25.27	7.96	7.94	7.95
35	Sugandhamati	8.40	7.40	7.90	21.17	21.45	21.31	7.54	7.51	7.53

Table 4.1 cont...

S. No.	Name of genotype	Tillers per plant (number)			Test weight (g)			Grain length (mm)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
36	Basmati 386	7.80	6.70	7.25	18.88	19.06	18.97	6.63	6.52	6.58
37	MC 4	7.30	5.40	6.35	26.00	25.81	25.91	7.41	7.27	7.34
38	Haryana Bas mati	8.50	7.60	8.05	22.27	22.18	22.22	6.88	6.95	6.92
39	Mahisugandha	6.40	6.90	6.65	26.61	26.53	26.57	7.24	7.05	7.15
40	Khalibagh	8.80	8.00	8.40	19.90	19.76	19.83	7.96	8.14	8.05
41	Jagatpuri	8.00	8.70	8.35	15.44	15.75	15.60	5.44	5.48	5.46
42	Vikram	7.50	6.90	7.20	19.57	19.59	19.58	5.19	5.06	5.13
43	Velkat	7.30	7.10	7.20	18.33	18.07	18.20	5.49	5.55	5.52
44	Tulshi tall	7.20	6.20	6.70	18.96	18.99	18.98	5.63	5.59	5.61
45	Champakali	9.30	9.60	9.45	15.18	15.34	15.26	6.38	6.48	6.43
46	Siddhagiri	6.10	5.80	5.95	30.13	30.10	30.12	5.91	5.94	5.93
47	RDN 97-2	7.80	7.10	7.45	19.96	19.83	19.90	5.34	5.24	5.29
48	RDN 99-12	7.00	7.30	7.15	22.69	22.56	22.63	6.45	6.45	6.45
49	RDN 99-14	8.90	8.10	8.50	21.95	21.99	21.97	6.64	6.79	6.72
50	RTN purple	8.40	8.00	8.20	21.24	21.10	21.17	6.28	6.32	6.30
51	Taroari Bas mati	8.60	7.80	8.20	22.41	22.44	22.42	7.19	7.30	7.25
52	RDN 99-18	9.00	7.50	8.25	21.96	21.82	21.89	5.85	6.02	5.94
53	RDN 02-80	8.30	9.30	8.80	17.00	17.20	17.10	5.55	5.53	5.54
54	Ajara Local 1	7.00	6.80	6.90	12.72	12.80	12.76	4.44	4.50	4.47
55	Ajara Local 3	9.00	8.50	8.75	13.81	13.62	13.71	4.70	4.52	4.61
56	IGT 13857	10.00	8.80	9.40	25.38	25.43	25.41	6.17	6.22	6.20
57	Pavsal	9.80	8.40	9.10	24.40	24.30	24.35	5.20	5.26	5.23
58	Kunchi	8.10	7.50	7.80	27.98	28.10	28.04	6.39	6.51	6.45
59	Ambemohar 102	7.50	8.80	8.15	18.08	18.26	18.17	5.51	5.41	5.46
60	Antersal	7.70	7.60	7.65	23.57	23.58	23.58	5.90	6.03	5.97
61	Nalabhat	6.30	5.60	5.95	27.59	27.62	27.61	6.58	6.57	6.58
62	Sonsali	6.90	7.10	7.00	23.52	23.44	23.48	5.92	5.70	5.81
63	Pinjarwadi Local	6.10	6.00	6.05	27.36	27.39	27.38	6.48	6.47	6.48
64	LK 248	7.40	7.40	7.40	26.16	26.25	26.20	6.11	6.20	6.16
65	Pomendi Local	6.70	7.10	6.90	28.49	28.08	28.28	5.67	5.75	5.71
	Range	5.40 - 10.00	5.40 - 9.60	5.45 - 9.45	9.51 - 33.70	9.41 - 33.71	9.46 - 33.70	3.84 - 7.96	3.92 - 8.14	3.88 - 8.05
	Mean	7.56	7.23	7.39	21.65	21.60	21.62	6.08	6.09	6.09
	S.E. (+/-)	0.44	0.32	0.35	0.09	0.10	0.09	0.11	0.13	0.05
	C.D. at 5%	1.25	0.91	0.97	0.26	0.27	0.25	0.32	0.36	0.15
	C.V. (%)	8.29	6.29	6.60	0.60	0.64	0.59	2.65	2.93	1.26

Loc. I : ARS, Radhanagari; Loc. II : ARS, Lonavala

Table 4.1 cont...

S. No.	Genotype	Grain breadth (mm)			Length : Breadth ratio			Seed yield per plant (g)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
1	Heera	2.51	2.48	2.50	2.61	2.60	2.60	9.56	5.13	7.35
2	Halvi Sal 17	2.91	2.92	2.92	1.78	1.77	1.77	13.08	10.13	11.61
3	RDN 185-2	2.91	2.79	2.85	1.78	1.89	1.83	17.23	11.07	14.15
4	Phule Radha	1.65	1.55	1.60	3.19	3.38	3.28	18.29	16.60	17.45
5	RTN 1	2.47	2.52	2.50	2.69	2.57	2.63	18.72	12.16	15.44
6	Ratna	2.13	2.06	2.10	2.93	2.94	2.93	17.46	17.49	17.48
7	Phule Samruddhi	1.88	1.91	1.90	3.38	3.39	3.39	20.55	18.69	19.62
8	Jaya	2.72	2.60	2.66	2.11	2.22	2.16	20.79	17.72	19.26
9	Phule Maval	1.92	1.96	1.94	3.87	3.82	3.84	19.00	15.48	17.24
10	Pawana	2.13	2.09	2.11	3.07	3.17	3.12	19.64	20.42	20.03
11	Indrayani	1.96	1.98	1.97	3.33	3.34	3.34	19.06	19.36	19.21
12	Bhogavati	2.10	2.05	2.08	3.70	3.74	3.72	20.86	20.60	20.73
13	Basmati 370	1.96	1.92	1.94	3.32	3.51	3.42	14.48	10.94	12.71
14	Pusa Basmati 1	1.68	1.65	1.67	4.64	4.66	4.65	17.01	13.77	15.39
15	Ambemohar 157	2.36	2.40	2.38	1.89	1.84	1.87	13.79	14.55	14.17
16	Ghansal	2.06	2.12	2.09	2.12	2.11	2.12	12.52	13.30	12.91
17	Kalajirga	2.10	2.15	2.13	2.18	2.09	2.13	13.86	16.24	15.05
18	Vivek Dhan 82	2.66	2.61	2.64	2.37	2.42	2.40	18.26	18.02	18.14
19	Badshabhog	2.08	2.12	2.10	2.09	2.04	2.07	13.70	14.99	14.35
20	Kothimbire	2.69	2.69	2.69	1.43	1.46	1.44	16.47	17.68	17.08
21	BPT 5204	1.91	1.89	1.90	2.78	2.84	2.81	17.96	12.19	15.08
22	RDN 98-2-3-5-14	2.30	2.31	2.31	2.37	2.35	2.36	20.56	17.80	19.18
23	RDN 01-2-10-9	1.89	1.85	1.87	3.63	3.65	3.64	18.85	15.28	17.07
24	KJT 2	1.93	1.94	1.94	3.40	3.46	3.43	16.40	16.58	16.49
25	EK 70	2.07	1.94	2.01	3.15	3.47	3.31	11.25	10.85	11.05
26	Patni	2.71	2.78	2.75	2.18	2.09	2.14	19.32	17.04	18.18
27	Diwani	2.08	2.12	2.10	2.03	2.03	2.03	17.07	14.73	15.90
28	Shyamjeer	2.07	2.06	2.06	2.53	2.50	2.52	16.64	17.54	17.09
29	Juhibengal	2.04	2.04	2.04	2.74	2.72	2.73	13.59	14.20	13.90
30	Improved Pusa Basmati 1	1.79	1.80	1.80	4.04	3.96	4.00	12.48	13.83	13.16
31	Kasturi	1.88	1.86	1.87	3.76	3.82	3.79	15.78	14.23	15.01
32	Karnal Local	1.79	1.77	1.78	3.84	3.98	3.91	16.20	16.56	16.38
33	SD 7	1.84	1.87	1.86	3.88	3.76	3.82	10.22	10.59	10.41
34	SD 17	1.74	1.75	1.75	4.57	4.54	4.56	17.93	15.14	16.54
35	Sugandhamati	1.71	1.68	1.70	4.41	4.47	4.44	11.20	11.70	11.45

Table 4.1 cont...

S. No.	Genotype	Grain breadth (mm)			Length : Breadth ratio			Seed yield per plant (g)		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
36	Basmati 386	1.87	1.90	1.89	3.55	3.43	3.49	12.94	14.23	13.59
37	MC 4	1.84	1.83	1.84	4.03	3.98	4.00	17.46	14.87	16.17
38	Haryana Bas mati	1.88	1.88	1.88	3.66	3.70	3.68	18.52	17.92	18.22
39	Mahisugandha	2.20	2.23	2.22	3.29	3.16	3.23	18.27	17.72	18.00
40	Khalibagh	1.61	1.58	1.60	4.95	5.15	5.05	15.16	15.37	15.27
41	Jagatpuri	1.91	1.89	1.90	2.85	2.90	2.87	20.28	18.74	19.51
42	Vikram	2.01	1.98	2.00	2.58	2.56	2.57	18.31	18.15	18.23
43	Velkat	2.10	2.11	2.11	2.61	2.63	2.62	16.94	16.97	16.96
44	Tulshi tall	2.19	2.19	2.19	2.57	2.55	2.56	17.27	17.46	17.37
45	Champakali	1.89	1.90	1.90	3.38	3.41	3.39	8.00	5.37	6.69
46	Siddhagiri	2.73	2.76	2.75	2.17	2.15	2.16	21.41	21.28	21.35
47	RDN 97-2	2.51	2.50	2.51	2.13	2.10	2.11	23.58	19.18	21.38
48	RDN 99-12	1.93	1.93	1.93	3.34	3.34	3.34	20.71	18.41	19.56
49	RDN 99-14	1.89	1.84	1.87	3.51	3.69	3.60	19.09	19.16	19.13
50	RTN purple	1.83	1.83	1.83	3.43	3.46	3.44	17.90	14.68	16.29
51	Taroari Bas mati	1.95	1.90	1.93	3.69	3.84	3.76	13.66	12.55	13.11
52	RDN 99-18	2.61	2.64	2.63	2.24	2.28	2.26	17.91	17.00	17.46
53	RDN 02-80	1.94	1.91	1.92	2.87	2.90	2.88	12.73	10.02	11.38
54	Ajara Local 1	2.13	2.14	2.14	2.08	2.10	2.09	16.76	17.27	17.02
55	Ajara Local 3	2.12	2.11	2.12	2.22	2.14	2.18	16.59	16.42	16.51
56	IGT 13857	2.41	2.51	2.46	2.56	2.48	2.52	18.23	13.01	15.62
57	Pavsal	2.33	2.41	2.37	2.23	2.18	2.21	16.69	16.38	16.54
58	Kunchi	2.23	2.22	2.23	2.87	2.93	2.90	18.80	18.09	18.45
59	Ambemohar 102	1.81	1.81	1.81	3.04	2.99	3.02	16.09	16.20	16.15
60	Antersal	2.38	2.37	2.38	2.48	2.54	2.51	18.72	17.56	18.14
61	Nalabhat	2.05	2.02	2.04	3.21	3.25	3.23	15.39	15.67	15.53
62	Sonsali	2.07	2.08	2.08	2.86	2.74	2.80	17.28	16.59	16.94
63	Pinjarwadi Local	2.15	2.11	2.13	3.01	3.07	3.04	15.93	15.02	15.48
64	LK 248	1.94	2.00	1.97	3.15	3.10	3.13	16.91	14.97	15.94
65	Pomendi Local	2.41	2.37	2.39	2.36	2.43	2.39	16.53	16.76	16.65
	Range	1.61 - 2.91	1.55 - 2.92	1.60 - 2.92	1.43 - 4.94	1.46 - 5.15	1.44 - 5.05	8.00 - 23.58	5.13 - 21.28	6.69 - 21.38
	Mean	2.14	2.14	2.14	2.95	2.97	2.96	16.67	15.47	16.07
	S.E. (+/-)	0.21	0.22	0.15	0.12	0.19	0.09	0.77	0.86	0.99
	C.D. at 5%	0.61	0.63	0.42	0.35	0.53	0.24	2.19	2.43	2.81
	C.V. (%)	14.14	14.85	9.76	5.96	8.88	4.06	6.57	7.87	8.75

Loc. I : ARS, Radhanagari; Loc. II : ARS, Lonavala

4.1.1.1.5 Spikelets per panicle

The observations presented in Table 4.1 reveal that the mean performance of 65 genotypes showed wide variation for number spikelets per panicle. It ranged from 77.00 to 232.10 at Location I and 76.40 to 246.20 at Location II with 76.70 to 239.10 over two locations. The average number of spikelets per panicle was 151.08, 155.15 and 153.00 at Location I, Location II and pooled, respectively. The genotype Ambemohar 157 recorded highest number of spikelets at both the locations (232.10 and 246.10 at Location I, and Location II, respectively) followed by Badshabhog (230.70 and 237.70 at Location I and Location II, respectively). On the contrary, Heera reported least number of spikelets per panicle (77.00, 76.40 and 76.70 at Location I, Location II and pooled, respectively) followed by SD 7 (98.90) and Basmati 370 (103.10) at Location I and Halvi Sal 17 (115.70) and Basmati 370 (117.00) at Location II. Fifteen genotypes had significantly higher number of spikelets per panicle than the population mean.

4.1.1.1.6 Spikelet fertility

The data on mean performance of spikelet fertility (Table 4.1) indicated 84.40 to 96.74 per cent spikelet fertility at Location I and 90.82 to 96.77 per cent at Location II. At Location I, the genotype RDN 97-2 reported highest (96.74 %) spikelet fertility followed by Velkat (96.72 %) and Kothimbire (96.59 %), whereas the genotype SD 7 reported lowest spikelet fertility (84.40 %) followed by Improved Pusa Basmati 1 (90.80 %) and Ambemohar 102 (91.27 %). However, at Location II, highest spikelet fertility was observed in genotype Ambemohar 157 (96.77 %) followed by Sonsali (96.37 %) and Kalajirga (96.36 %). At this location, the least spikelet fertility of 90.82 per cent was found in SD 17 followed by Pawana and Sugandhamati wherein it was 90.88 and 91.54 per cent respectively. The pooled results showed 87.61 to 96.43 per cent spikelet fertility among the genotypes under study. The genotype Sonsali showed highest fertility (96.43 %) followed by Kalajirga (96.33 %) and Ambemohar 157 (96.23 %) whereas, the genotype SD 7 reported lowest fertility (87.61 %) followed by Ambemohar 102 (91.44 %) and Pawana (91.68 %).

4.1.1.1.7 Tillers per plant

The results depicted in Table 4.1 show that 65 rice genotypes varied in number of tillers per plant from 5.45 to 9.45 with the mean of 7.39. At Location I, the number of tillers per plant ranged between 5.40 and 10.00 with mean performance of 7.50, whereas it was between 5.40 and 9.60 at Location II with the mean of 7.23. The genotype IGT 13857 produced highest number of tillers per plant (10.00) at Location I followed by Pavsai (9.80) and Champakali (9.30), while RDN 185-2 produced least number of tillers per plant (5.40) followed by Halvi Sal 17 (5.50) and Ambemohar 157 (5.80). At Location II, the highest number of tillers per plant were observed in Champakali (9.60) followed by RDN 2-80 (9.30) and Ambemohar 102 (8.80), whereas the genotype Halvi Sal 17 and MC 4 showed least number of tillers per plant (5.40) followed by RDN 185-2 (5.50) and Nalabhat (5.60). The pooled performance indicated that the genotype Champakali had highest number of tillers per plant (9.45) followed by IGT 13857 (9.40) and Pavsai (9.10), whereas the genotype Halvi Sal 17 had minimum number of tillers per plant (5.45) followed by RDN 185-2 (5.45) and Ambemohar 157 (5.85). Eighteen genotypes recorded significantly higher number of tillers per plant than population mean (7.56) at Location I, nine at Location II (7.23) and eight in pooled performance (7.39).

4.1.1.1.8 Test weight

The substantial variation for test weight (Table 4.1) was observed in the material studied. The pooled performance of the genotypes ranged between 9.46 and 33.70 g with the general mean of 21.62 g. The genotype Diwani showed minimum test weight of 9.51, 9.41 and 9.46 g at Location I, Location II and pooled, respectively followed by Shyamjeer (9.93, 9.75 and 9.84 g) and Badshabhog (11.10, 11.44 and 11.27 g at Location I, Location II and pooled, respectively). Highest test weights of 33.70, 33.71 and 33.70 g were observed in Patni at Location I, Location II and pooled, respectively. This was followed by Vivek Dhan 82 and Siddhagiri wherein the test weights were 31.09, 31.02 and 31.05 g and 30.13, 30.10 and 30.12 g at Location I, Location II and pooled mean, respectively. Thirty-two genotypes had significantly lower test weight than the population mean (21.62 g) in the pooled performance.

4.1.1.1.9 Grain length

The data presented in Table 4.1 indicate wide range for grain length in the pooled performance over two locations. It ranged between 3.88 and 8.05 mm with population mean of 6.08 mm. The average grain length, range of grain length and performance of individual genotype over two locations was almost similar. Khalibagh recorded longest grain lengths of 7.96, 8.14 and 8.05 mm at Location I, Location II and pooled, respectively followed by SD 17 (7.96, 7.94 and 7.95 mm at Location I, Location II and pooled, respectively) and Pusa Basmati 1 (7.80, 7.69 and 7.75 mm at Location I, Location II and pooled, respectively). Whereas Kothimbire had shortest grain lengths of 3.84, 3.92 and 3.88 mm at Location I, Location II and pooled, respectively followed by Diwani (4.22, 4.31 and 4.27 mm at Location I, Location II and pooled, respectively) and Badshabhog (4.35, 4.33 and 4.34 mm at Location I, Location II and pooled, respectively). Thirty-four genotypes showed significantly longer grains than population mean (6.09 mm). Among 65 genotypes, 46 showed grain length ≥ 5.5 mm, wherein 35 had grain length ≥ 6.0 mm and 25 had grain length ≥ 6.5 mm.

4.1.1.1.10 Grain breadth

The pooled performance for grain breadth (Table 4.1) of 65 genotypes ranged from 1.60 to 2.92 mm, with the pooled mean of 2.14 mm. The genotypes Phule Radha and Khalibagh reported minimum grain breadth (1.60 mm) followed by Pusa Basmati 1 (1.67 mm) and Sugandhamati (1.70 mm). The genotype Halvi Sal 17 had the thick grain (2.92 mm) followed by RDN 185-2 (2.85 mm) and Siddhagiri (2.75 mm). Twelve genotypes recorded significantly broader and five significantly thinner grain breadth than the population mean (2.11 mm).

4.1.1.1.11 Length : Breadth ratio

The mean performance for Length : Breadth ratio of grain (Table 4.1) indicated considerable variation within the genotypes. The average L:B ratio of 2.96 was observed at Location I with the range varied from 1.43 to 4.94, whereas it was 2.98 with the range from 1.46 to 5.15 at Location II. The pooled L:B ratio ranged between 1.44 and 5.05 with the population mean of 2.97. The genotype Khalibagh showed highest L:B ratios of 4.94 and 5.15 at Location I and Location II followed

by Pusa Basmati 1 (4.64 and 4.66 at Location I and Location II, respectively) and SD 17 (4.57 and 4.54 at Location I and Location II, respectively). Kothimbire recorded lowest L:B ratio of 1.43 and 1.46 at Location I and Location II, respectively) followed by Halvi Sal 17 (1.78 and 1.77 at Location I and Location II, respectively). Among 65 genotypes, 25 recorded significantly higher L:B ratio than population mean (2.97). Thirty-one genotypes had slender grain with L:B ratio $\geq$ 3.00.

4.1.1.1.12 Seed yield per plant

The result presented in Table 4.1 reveal that the mean seed yield per plant for 65 genotypes over two locations ranged between 6.69 and 21.38 g, with the population mean of 16.07 g. The seed yield per plant at Location I ranged between 8.00 and 23.58 g with the average yield of 16.67 g, whereas it ranged between 5.13 and 21.28 g at Location II with the mean of 15.47 g. The genotype RDN 97-2 recorded highest grain yield (23.58 g) at Location I followed by Siddhagiri (21.41 g) and Bhogavati (20.86 g). The genotype Champakali produced lowest grain yield (8.00 g) at this location followed by Heera (9.56 g) and SD 7 (10.22 g). At Location II, Siddhagiri (21.28 g) recorded highest grain yield per plant followed by Bhogavati (20.60 g) and Pawana (20.42 g), whereas grain yield recorded was the lowest in the case of the genotype Heera (5.13 g) followed by Champakali (5.37 g) and RDN 02-80 (10.02 g). The pooled performance of seed yield per plant was highest in RDN 97-2 (21.38 g) followed by Siddhagiri (21.35 g) and Bhogavati (20.73 g), whereas lowest yield was recorded by Champakali (6.69 g) followed by Heera (7.35 g) and SD 7 (10.41 g). Thirteen genotypes recorded significantly higher grain yield per plant than location mean over Location I (16.67 g) as well as Location II (15.47 g). In the pooled performance over two locations 11 genotypes recorded significantly higher grain yield per plant than population mean (16.07 g).

4.1.1.2 Analysis of variance

The mean sum of squares due to replication, treatment (genotype) and error component for 12 agro-morphological characters at two locations and pooled over two locations are given in Table 4.2. The mean sum of squares due to treatments was found highly significant for all the characters under study.

Table 4.2 Pooled analysis of variance of agro-morphological characters in rice

Source of variation	Degrees of freedom	Mean sum of s quare											
		Days to 50 % flowering			Days to maturity			Plant height			Panicle length		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
Replication	1	1.11	0.28	148.62	0.12	1.51	320.12	5.48	34.94	955.00	0.02	0.91	28.482
Treatment	64	205.50**	256.53**	221.74**	287.59**	370.32**	312.33**	1060.86**	1083.40**	1039.92**	14.72**	15.25**	13.93**
Error	64	1.39	3.90	9.27	1.94	3.98	16.63	26.94	40.34	32.21	0.44	0.38	1.057

Source of variation	Degrees of freedom	Mean sum of s quare											
		Spikelets per panicle			Spikelet fertility			Tillers per plant			Test weight		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
Replication	1	0.49	56.10	536.53	0.72	0.07	1.24	0.01	0.19	3.62	0.02	0.01	0.07
Treatment	64	2144.32**	2001.70**	1983.14**	7.06**	3.82**	4.52**	2.10**	1.67**	1.64**	56.70**	56.60**	56.63**
Error	64	50.69	65.91	89.88	0.32	0.32	0.92	0.39	0.21	0.24	0.02	0.02	0.02

Source of variation	Degrees of freedom	Mean sum of s quare											
		Grain length			Grain breadth			L:B ratio			Seed yield per plant		
		Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled	Loc. I	Loc. II	Pooled
Replication	1	0.04	0.03	0.003	0.03	0.15	0.001	0.02	0.15	0.01	1.81	0.19	47.06
Treatment	64	1.94**	1.95**	1.94**	0.27**	0.27**	0.23**	1.22**	1.23**	1.21**	9.12**	5.67**	17.70**
Error	64	0.03	0.03	0.01	0.09	0.10	0.04	0.03	0.07	0.01	0.49	0.43	1.98

Loc. I : ARS, Radhanagari; Loc. II : ARS, Lonavala

** - Significance at 1 per cent.

4.1.1.3 Parameters of genetic variability

Range of variability, estimates of genotypic and phenotypic coefficients of variation, heritability percentage in broad sense, genetic advance and genetic advance expressed as percentage of mean for 12 agro-morphological characters under study pooled over two locations are presented in Table 4.3.

4.1.1.3.1 Coefficient of variation

The estimates for genotypic coefficient of variation (GCV) were lower than the phenotypic coefficient of variation (PCV) for all the characters. Among the traits studied, the characters length : breadth ratio had highest GCV and PCV of 26.12 and 26.44, respectively followed by test weight (21.62 and 24.61), plant height (20.93 and 21.59) and spikelets per panicle (20.09 and 21.03), whereas spikelet fertility exhibited the lowest GCV (1.42) as well as PCV (1.75). The highest difference between GCV and PCV values was observed in the character grain breadth (3.04) followed by seed yield per plant (2.07), while test weight and grain length (0.05) showed lowest difference between the GCV and PCV values.

4.1.1.3.2 Heritability (broad sense) percentage

According to Robinson's (1966) classification, heritability obtained in the present investigation for all the characters could be classified as very high heritability, except for spikelet fertility and grain breadth showing high heritability, indicating the scope for selection of these traits. The highest heritability was reported in test weight (99.90 %) followed by grain length (99.40 %) and length : breadth ratio (97.60 %). The lowest heritability was found in spikelet fertility (66.10 %) followed by grain breadth (67.70 %).

4.1.1.3.3 Genetic advance

The character, spikelets per panicle showed the highest genetic advance (60.57) followed by plant height (44.83) and days to maturity (23.75). The lowest genetic advance was observed for grain breadth (0.51) followed by tillers per plant (1.50) and L:B ratio (1.57). Genetic advance expressed as percentage of mean was highest in L:B ratio (53.18) followed by test weight (50.67).

Table 4.3 Variability parameters of rice genotypes for agro-morphological characters

S. No.	Character	Range	Mean	GCV (%)	PCV (%)	Heritability % (b.s.)	Genetic advance	G.A. as % of mean
1	Days to 50% flowering	62.25 – 124.75	105.12	9.81	10.22	92.00	20.36	19.37
2	Days to maturity	86.00 – 155.25	135.11	9.11	9.60	89.90	23.75	17.78
3	Plant height	65.03 – 152.48	107.24	20.93	21.59	94.00	44.83	41.80
4	Panicle length	17.00 – 27.55	23.03	11.01	11.88	85.90	4.84	21.03
5	Spikelets per panicle	76.70 – 239.10	153.12	20.09	21.03	91.30	60.57	39.56
6	Spikelet fertility	87.61 – 96.43	94.25	1.42	1.75	66.10	2.25	2.38
7	Tillers per plant	5.45 – 9.45	7.39	11.34	13.12	74.70	1.49	20.19
8	Test weight	9.46 – 33.70	21.62	24.61	24.61	99.90	10.96	50.67
9	Grain length	3.88 – 8.05	6.09	16.15	16.20	99.40	2.02	33.16
10	Grain breadth	1.60 – 2.92	2.14	14.13	17.17	67.70	0.51	23.95
11	Length : Breadth ratio	1.44 – 5.05	2.96	26.12	26.44	97.60	1.57	53.18
12	Seed yield per plant	6.69 – 21.38	16.07	17.45	19.52	79.90	5.16	32.13

4.1.1.4 Divergence based on agro-morphological characters

Genetic divergence in 65 genotypes of rice was measured following Mahalanobis (1936) D^2 statistic. The D^2 values ranged between 4.21 and 24015.45 for the pair of genotypes studied (Appendix I). The lowest D^2 value (4.21) was observed between the genotypes Phule Samruddhi and RDN 99-12 followed by Ghansal and Ajara Local 1 (9.82) and Halvi Sal 17 and RDN 185-2 (15.30). The highest D^2 value was found between the genotypes Patni and Diwani (24015.45) followed by Patni and Shyamjeer (22864.02).

4.1.1.4.1. Cluster formation

Cluster formation was done by following Tocher's method as described by Rao (1952). Sixty-five rice genotypes under study were grouped into 10 clusters. The clusters along with genotypes included in them are presented in Table 4.4 and Fig. 4.1. The cluster I was the largest that had 22 genotypes followed by cluster III (14 genotypes), cluster IV (12 genotypes), cluster II (7 genotypes), cluster VIII (4 genotypes) and cluster IX (2 genotypes), whereas the cluster V, VI, VII and X had single genotype.

4.1.1.4.2 Intra and inter-cluster distances

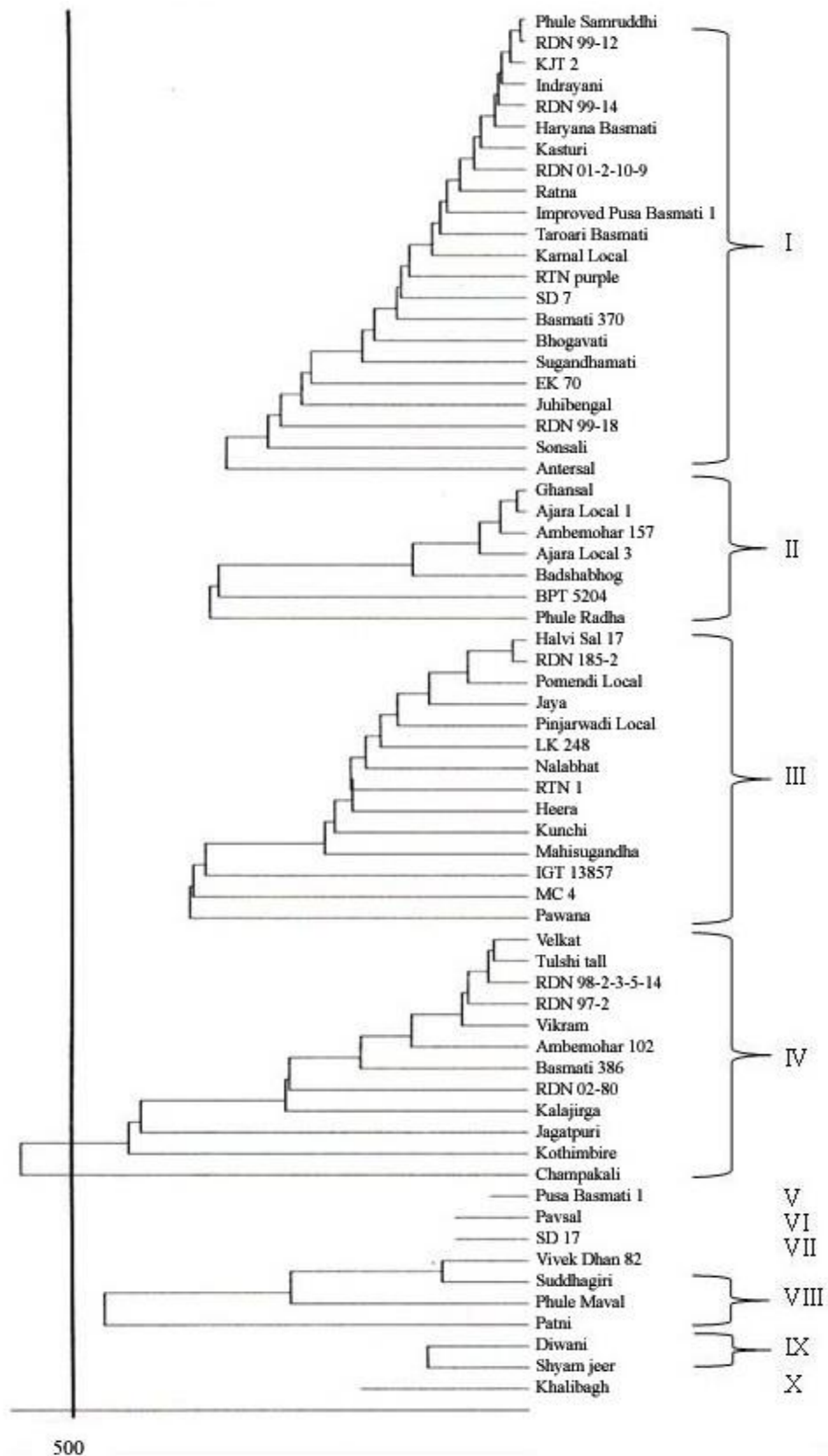
Intra and inter-cluster D^2 and D values were worked out using D^2 values from divergence analysis. These values are presented in Table 4.5.

The minimum intra-cluster distance was found in cluster IX ($D^2 = 111.94$) followed by cluster I ($D^2 = 191.55$) and cluster II ($D^2 = 256.64$). The maximum intra cluster distance was noticed in the cluster VIII ($D^2 = 457.10$) followed by cluster IV (350.06) and cluster III ($D^2 = 282.58$). The cluster V, VI, VII and X had single genotype.

The maximum inter-cluster distance was observed between cluster VIII and IX ($D^2 = 19165.63$) followed by cluster II and VIII ($D^2 = 13656.26$), cluster III and IX ($D^2 = 12624.77$) and cluster VII and IX ($D^2 = 11664.00$). The minimum inter-cluster distance was observed in the cluster V and X ($D^2 = 184.69$) followed by cluster I and V ($D^2 = 377.53$), cluster I and VI ($D^2 = 509.41$) and cluster V and VII ($D^2 = 554.60$).

Table 4.4 Distribution of rice genotypes into different clusters based on agro-morphological characters.

Cluster	No. of genotypes	Genotype	Characters
I	22	Phule Samruddhi, RDN 99-12, KJT 2, Indrayani, RDN 99-14, Haryana Basmati, Kasturi, RDN 01-2-10-9, Ratna, Improved Pusa Basmati 1, Taroari Basmati, Karnal Local, RTN purple, SD 7, Basmati 370, Bhogavati, Sugandhamati, EK 70, RDN 99-18, Sonsali, Antersal, Juhibengal.	Dwarf plant type, long-slender grain, medium test weight.
II	7	Ghansal, Ajara Local 1, Ambemohar 157, Ajara Local 3, Badshabhog, BPT 5204, Phule Radha.	Short grains, low L:B ratio, low test weight, long panicle, more number of spikelets per panicle, low to medium test weight.
III	14	Halvi Sal 17, RDN 185-2, Pomendi Local, Jaya, Pinjarwadi Local, LK 248, Nalabhat, RTN1, Heera, Kunchi, Mahisugandha, IGT 13857, MC 4, Pawana	Early flowering and maturity, coarse grain type, low to medium L:B ratio, high test weight.
IV	12	Velkat, Tulshi tall, RDN 98-2-3-5-14, RDN 97-2, Vikram, Ambemohar 102, Basmati 386, RDN 02-80, Kalajirga, Jagatpuri, Kothimbire, Champakali.	Mixed performing group, intermediate in characters. Contains short- slender and long – slender grain type, early & midlate maturity, dwarf and tall plant type.
V	1	Pusa Basmati 1.	Extra long-slender grain, high L:B ratio.
VI	1	Pavsal.	Very late maturity, tall plant type, high no. of tillers, coarse grain type, high yield.
VII	1	SD 17	Extra long-slender grain, high L:B ratio.
VIII	4	Vivek Dhan 82, Siddhagiri, Phule Maval, Patni.	Long-bold grain, high test weight.
IX	2	Diwani, Shyamjeer.	Short-slender grain, very low test wt. (< 10 gm), tall plant type, late maturity.
X	1	Khalibagh.	Dwarf plant type, long-slender grain, high L:B ratio, low test weight.

Fig. 4.1 Clustering of rice genotypes based on morphological analysis

Cluster I showed maximum inter-cluster distance with cluster IX ($D^2 = 6947.22$) followed by cluster II ($D^2 = 3940.07$) and cluster VIII ($D^2 = 3516.49$), whereas, it recorded minimum inter-cluster distance with cluster V ($D^2 = 377.53$) followed by cluster VI, X, VII, IX and III (Table 4.5). This cluster reported intra-cluster distance of 191.55, indicating moderate genetic variability between the genotypes grouped in this cluster.

Cluster II recorded maximum inter-cluster distance with cluster VIII ($D^2 = 13656.26$) followed by cluster III ($D^2 = 8277.36$), cluster VII ($D^2 = 7638.76$) and cluster VI ($D^2 = 5095.10$), whereas minimum inter-cluster distance was reported with cluster IX ($D^2 = 641.61$) followed by cluster IV ($D^2 = 1296.00$) and cluster X ($D^2 = 3666.30$).

The intra-cluster distance of 282.58 was recorded in cluster III. This cluster recorded maximum inter-cluster distance with cluster IX ($D^2 = 12624.77$) followed by cluster IV ($D^2 = 3642.12$) and cluster X ($D^2 = 2770.97$). The cluster VI had least distance from this cluster with inter-cluster distance of 638.07 followed by cluster VII ($D^2 = 668.74$) and cluster VIII ($D^2 = 998.56$).

The range of minimum and maximum inter-cluster D^2 values for cluster IV was from 1128.96 (cluster I) to 7394.28 (cluster VIII), which was followed by cluster II, X and V and cluster III, IX and VII, respectively (Table 4.5). The intra-cluster distance of 350.06 was recorded in this cluster.

Cluster V had maximum inter-cluster distance with cluster IX ($D^2 = 7572.48$) followed by cluster II ($D^2 = 4563.00$) and cluster VIII ($D^2 = 3976.56$). This cluster showed minimum inter-cluster distance with cluster X ($D^2 = 184.69$) followed by cluster I ($D^2 = 377.53$) and cluster VII ($D^2 = 554.60$). As this cluster had single genotype, its intra-cluster distance was zero.

Cluster VI with single genotype reported inter-cluster D^2 values from 509.41 for cluster I to 8602.56 for cluster IX. Cluster II ($D^2 = 5095.10$), cluster VIII ($D^2 = 2328.06$) and cluster X ($D^2 = 1944.81$) reported higher inter-cluster distances from cluster VI, whereas cluster III ($D^2 = 638.07$), cluster VII ($D^2 = 1087.68$) and cluster V ($D^2 = 1168.96$) showed relatively less distance from this cluster.

Table 4.5 Average intra and inter cluster D^2 and D (in parenthesis) values for agro-morphological characters

[illegible]

Cluster VII showed minimum inter-cluster distance with cluster V ($D^2 = 554.60$) followed by cluster III ($D^2 = 668.74$) and cluster I ($D^2 = 820.25$), whereas the cluster IX reported maximum inter-cluster distance with this cluster ($D^2 = 11664.00$) followed by cluster II ($D^2 = 7638.76$) and cluster IV ($D^2 = 3306.25$).

Cluster VIII with four genotypes recorded maximum intra-cluster distance of 457.10. This cluster was closest to cluster III ($D^2 = 998.56$) followed by cluster VII ($D^2 = 1832.70$) and cluster VI ($D^2 = 2328.06$), whereas it had longest distance from cluster IX ($D^2 = 19165.63$) followed by cluster II ($D^2 = 13656.26$), cluster IV ($D^2 = 7394.28$) and cluster X ($D^2 = 5776.00$).

The intra-cluster distance of 111.94 was recorded between the two genotypes grouped into cluster IX (Diwani and Shyamjeer). This cluster showed maximum inter-cluster distance with cluster VIII ($D^2 = 19165.63$) followed by cluster III, VII, VI, V, I and X. However, it had minimum inter-cluster distance with cluster II ($D^2 = 641.61$) followed by cluster IV ($D^2 = 3090.24$).

Cluster X, with single landrace Khalibagh was found to be closest to cluster V ($D^2 = 184.69$) followed by cluster I ($D^2 = 724.69$), whereas it was at longest distance from cluster IX ($D^2 = 6144.99$) followed by cluster VIII ($D^2 = 5776.00$).

4.1.1.4.3 Cluster means

Cluster means for 12 characters are presented in Table 4.6. It revealed wide range of variability among the clusters for plant height, days to 50 per cent flowering, days to maturity, spikelets per panicle, tillers per plant, test weight, grain length, grain breadth and L:B ratio.

Days to 50 per cent flowering

Cluster V was earliest (98.25 days) in flowering followed by Cluster III (98.82 days), whereas cluster VI (121.25 days) was very late. The variation in the cluster means for the remaining clusters was observed from 101.38 to 109.75 days.

Table 4.6 Mean performance of 10 clusters for agro-morphological characters

S. No.	Character	Cluster number									
		I	II	III	IV	V	VI	VII	VIII	IX	X
1	Days to 50% flowering	107.36	109.00	98.82	105.50	98.25	121.25	106.50	101.38	109.75	107.00
2	Days to maturity	136.55	138.00	125.91	133.90	123.75	151.00	136.25	128.25	140.00	136.50
3	Plant height	99.59	123.43	104.78	108.03	98.28	138.55	104.18	124.06	115.94	83.03
4	Panicle length	22.85	24.32	22.75	21.67	25.13	23.90	25.65	24.64	23.91	24.60
5	Spikelets per panicle	143.80	206.27	134.44	159.68	131.45	141.50	166.65	141.44	196.53	148.20
6	Spikelet fertility	93.76	95.26	93.99	94.52	93.52	94.80	94.10	94.93	95.41	93.68
7	Tillers per plant	7.73	6.91	6.88	7.79	7.35	9.10	6.90	6.61	7.10	8.40
8	Test weight	22.05	13.17	26.97	17.98	21.75	24.35	25.27	31.13	9.65	19.83
9	Grain length	6.65	4.69	6.27	5.41	7.75	5.23	7.95	6.39	4.68	8.05
10	Grain breadth	2.01	2.05	2.34	2.11	1.67	2.37	1.75	2.52	2.49	1.60
11	Length : Breadth ratio	3.40	2.35	2.75	2.62	4.65	2.21	4.56	2.63	2.08	5.05
12	Seed yield per plant	16.07	15.35	15.69	16.04	15.39	16.54	16.54	18.73	16.50	15.27

Days to maturity

Days to maturity showed the range of cluster means from 123.75 to 151.00 days. Cluster V was earliest (123.75 days) among all for maturity followed by cluster III (125.91 days). The cluster VI was very late in maturity (151.00). The cluster means for other clusters ranged from 128.25 to 140.00 days.

Plant height

The mean performance of clusters for plant height ranged between 83.03 and 138.55 cm. Cluster X was found to be dwarfest (83.03 cm) followed by Cluster V (98.28 cm), whereas cluster VI was tallest (138.55 cm) followed by cluster VIII (124.06 cm) and cluster II (123.43 cm).

Panicle length

Mean panicle length was observed to be maximum in cluster VII (25.65 cm) followed by cluster V (25.13 cm) and cluster VIII (24.64 cm), whereas it was minimum in cluster IV (21.67 cm) followed by cluster III (22.75 cm) and cluster I (22.85 cm).

Spikelets per panicle

Highest spikelets per panicle was observed in Cluster II (206.27) followed by cluster IX (196.53) and cluster VII (166.65), whereas it was lowest for the cluster V (131.45) followed by cluster III (134.44) and cluster VI (141.50)

Spikelet fertility

Cluster means for spikelet fertility exhibited less variation among the clusters. The means for this character ranged between 93.68 (cluster X) and 95.41 (cluster IX).

Tillers per plant

Cluster VI showed highest mean performance (9.10) for tillers per plant followed by cluster X (8.40). The lowest mean for this character was reported in the cluster VIII (6.61) followed by cluster III (6.88), cluster VII (6.90) and cluster II (6.91).

Test weight

Mean performance of test weight showed wide range. Cluster VIII (31.13 g) showed highest test weight followed by cluster III (26.97 g) and cluster VII (25.27 g). Cluster IX showed minimum test weight (9.65 g) followed by cluster II (13.17 g) and cluster IV (17.98 g).

Grain length

Cluster X showed maximum grain length (8.05 cm) followed by cluster VII (7.95 cm) and cluster V (7.75 cm), whereas minimum grain length was observed in cluster IX (4.68 cm) followed by cluster II (4.69 cm) and cluster VI (5.23 cm).

Grain breadth

The mean performance of clusters for grain breadth ranged between 1.60 mm and 2.52 mm. The minimum grain breadth was observed in cluster X (1.60 mm) followed by cluster V (1.67 mm) and cluster VII (1.75 mm). The cluster VIII (2.52 mm) had the thick grain followed by cluster VI (2.37 mm) and cluster III (2.34 mm).

Length : Breadth ratio

The length : breadth ratio of grain exhibited wide range of variation for cluster means. It ranged between 2.08 (cluster IX) and 5.05 (cluster X). The cluster V (4.65) and cluster VII (4.56) also reported higher L:B ratio, whereas cluster VI (2.21) and cluster II (2.35) had lower L:B ratio.

Seed yield per plant

The highest seed yield per plant was reported by cluster VIII (18.73 g) followed by cluster VI and cluster VII (16.54 g), whereas lowest yield was recorded by cluster X (15.27 g) followed by cluster II (15.35 g) and cluster V (15.39 g).

4.1.1.4.4 Contribution of various characters for divergence

The per cent contribution of the 12 characters studied for total divergence is presented in Table 4.7. Major contribution to total divergence was observed for test weight (79.04 %) followed by grain length (12.93 %) and length : breadth ratio (5.91 %). The contribution of other characters was less.

Table 4.7 Per cent contribution of 12 agro-morphological characters for divergence in rice.

S. No.	Character	Number of times appearing I in ranking	Per cent contribution
1	Days to 50 % flowering	10	0.48
2	Days to maturity	0	0.00
3	Plant height	24	1.15
4	Panicle length	0	0.00
5	Spikelets per panicle	2	0.10
6	Spikelet fertility	2	0.10
7	Tillers per plant	1	0.05
8	Test weight	1644	79.04
9	Grain length	269	12.93
10	Grain breadth	4	0.19
11	L:B ratio	123	5.91
12	Seed yield per plant	1	0.05
	Total	2080	100.00

4.1.2 Genetic diversity in rice genotypes for pathological traits

The experimental material was evaluated in randomised block design with two replications in Uniform Blast Nursery at Agricultural Research Station, Lonavala, Dist. Pune (MS) for five pathological traits of blast disease. The data obtained was subjected to statistical analysis. The results obtained are presented below.

4.1.2.1 Mean performance of genotypes for pathological traits

The mean performance of the 65 genotypes under study is presented in Table 4.8. The trait wise results of the mean performance are documented below.

4.1.2.1.1 Infection type

The infection type in 65 genotypes was ranged from 1.00 to 9.00 with the mean of 5.44. The genotype RDN 97-2, LK 248 and RDN 02-80 (1.00) showed least infection followed by RDN 98-2-3-5-14 (2.00). The highest infection was observed in EK70 and Ambemohar 102 (9.00) followed by Antersal, Velkat, Sugandhamati and Phule Radha (8.50). None of the genotypes exhibited an immune (0 score) reaction. Three genotypes showed resistant (1 score) reaction, whereas 13 genotypes had the infection score up to 3 indicating moderate resistance to blast.

Table 4.8 : Mean performance of 65 rice genotypes to pathological characters of blast disease

S. No.	Genotype	Infection type *	Diseased leaf area (%)	Lesion density (per cm ²)	Lesion size (mm ²)	Per cent disease intensity
1	Heera	3.00	5.74	0.00	0.20	35.19
2	Halvi Sal 17	6.00	12.59	2.97	3.93	65.87
3	RDN 185-2	7.50	16.85	2.97	4.32	75.40
4	Phule Radha	6.50	11.64	2.40	4.46	71.94
5	RTN 1	8.00	22.84	2.43	9.19	83.87
6	Ratna	5.00	4.02	0.55	5.60	48.15
7	Phule Samruddhi	3.00	10.18	0.00	0.20	33.33
8	Jaya	6.50	19.80	2.97	6.46	74.34
9	Phule Maval	8.50	26.27	3.40	7.03	84.92
10	Pawana	5.50	4.82	0.90	2.82	52.12
11	Indrayani	3.00	6.89	0.00	0.33	36.00
12	Bhogavati	3.00	2.26	0.64	0.45	31.75
13	Basmati 370	4.50	3.82	1.77	5.41	52.12
14	Pusa Basmati 1	4.50	4.49	1.13	3.47	50.53
15	Ambemohar 157	8.00	45.20	3.80	11.68	84.67
16	Ghansal	5.50	5.71	1.37	3.05	59.00
17	Kalajirga	5.00	4.73	0.54	4.42	52.38
18	Vivek Dhan 82	3.00	9.74	0.00	0.20	33.33
19	Badshabhog	6.00	8.94	1.30	4.28	56.35
20	Kothimbire	5.50	10.18	1.53	3.65	59.79
21	BPT 5204	6.50	17.52	2.10	5.99	71.16
22	RDN 98-2-3-5-14	2.00	2.26	0.00	0.20	16.14
23	RDN 01-2-10-9	6.50	15.86	1.70	7.90	64.81
24	KJT 2	3.00	2.52	0.00	0.20	32.54
25	EK 70	9.00	81.71	5.77	14.65	98.42
26	Patni	4.50	5.74	0.74	5.76	46.03
27	Diwani	6.00	12.21	1.27	8.21	63.23
28	Shyamjeer	7.00	12.62	1.80	6.56	70.11
29	Juhibengal	3.50	3.75	0.10	2.43	36.51
30	Improved Pusa Basmati 1	3.50	7.83	0.04	1.60	38.89
31	Kasturi	6.50	12.05	1.07	10.39	67.72
32	Karnal Local	5.00	4.96	0.30	9.10	45.24
33	SD 7	5.50	9.00	0.97	7.48	61.64
34	SD 17	6.50	12.53	1.37	5.58	67.73
35	Sugandhamati	8.50	40.04	3.47	12.58	91.54

Table 4.8 cont...

S. No.	Genotype	Infection type	Diseased leaf area (%)	Lesion density (per cm ²)	Lesion size (mm ²)	Per cent Disease Intensity
36	Basmati 386	2.50	1.88	0.07	1.55	26.46
37	MC 4	5.00	9.81	0.54	7.94	51.59
38	Haryana Bas mati	3.00	5.61	0.00	0.20	28.17
39	Mahisugandha	3.00	9.87	0.00	0.20	30.69
40	Khalibagh	5.50	9.48	0.94	8.72	57.14
41	Jagatpuri	8.50	57.16	4.55	12.33	91.54
42	Vikram	3.00	9.24	0.00	0.20	33.33
43	Velkat	8.50	46.79	3.20	14.21	92.59
44	Tulshi tall	8.00	25.72	1.80	13.88	88.36
45	Champakali	8.00	23.41	2.83	8.10	84.66
46	Siddhagiri	6.00	11.46	0.90	10.60	60.59
47	RDN 97-2	1.00	1.52	0.00	0.20	11.11
48	RDN 99-12	3.00	20.28	0.00	0.20	26.19
49	RDN 99-14	4.00	8.38	0.14	1.22	49.20
50	RTN purple	6.50	16.82	2.94	5.41	72.75
51	Taroari Bas mati	5.00	8.99	0.93	6.83	52.38
52	RDN 99-18	7.00	19.72	2.44	7.57	78.04
53	RDN 02-80	1.00	1.48	0.00	0.20	11.91
54	Ajara Local 1	7.00	16.38	1.60	8.66	62.44
55	Ajara Local 3	8.00	36.17	2.57	9.23	83.73
56	IGT 13857	4.50	5.91	0.50	9.35	44.44
57	Pavsal	7.50	25.62	2.84	9.03	78.57
58	Kunchi	8.00	35.89	3.33	10.07	87.30
59	Ambemohar 102	9.00	39.03	4.54	7.87	94.44
60	Antersal	8.50	56.07	3.34	16.82	92.59
61	Nalabhat	3.00	5.44	0.00	0.20	30.69
62	Sonsali	5.50	8.53	1.30	5.59	62.44
63	Pinjarwadi Local	5.50	7.04	0.87	4.43	55.55
64	LK 248	1.00	0.41	0.00	0.20	11.11
65	Pomendi Local	4.00	3.44	0.30	6.40	42.86
	Range	1.00 – 9.00	0.41 – 81.71	0.00 – 5.77	0.20 – 16.82	11.11 – 98.42
	Mean	5.40	15.46	1.44	5.65	57.01
	S.E. (+/-)	0.45	2.40	0.74	0.15	1.23
	C.D. at 5%	1.28	6.79	2.09	0.43	3.48
	C.V. (%)	11.83	5.97	18.56	15.12	11.26

* Based on 0 – 9 score

4.1.2.1.2 Diseased leaf area

The diseased leaf area (Table 4.8 and 4.10) showed wide range varying between 0.41 and 81.71 per cent with general mean of 15.46 per cent. Minimum diseased leaf area was recorded in the genotype LK 248 (0.41 %) followed by RDN 2-80 (1.48 %) and RDN 97-2 (1.52 %). The genotype EK 70 (81.71 %) had highest diseased leaf area followed by Jagatpuri (57.16 %) and Antersal (56.07 %). Thirty-eight genotypes showed significantly lower diseased leaf area than population mean (15.46 %).

4.1.2.1.3 Lesion density

The observations presented in Table 4.8 and 4.10 indicated 1.41 mean lesion density with the range from 0.00 to 5.77. The genotypes RDN 98-2-3-5-14, KJT 2, RDN 97-2, Heera, Phule Samruddhi, Indrayani, Vivek Dhan 82, Haryana Basmati, Mahisugandha, Vikram, RDN 99-12, RDN 2-80, Nalabhat and LK 248 showed minimum lesion density (0.00 per cm²) followed by Improved Pusa Basmati1 (0.04 per cm²). The lesion density was found highest in EK 70 (5.77 per cm²) followed by Jagatpuri (4.55 per cm²) and Ambemohar 102 (4.54 per cm²). Thirty-one genotypes had significantly lower lesion density than the population mean (1.44) among which 14 genotypes showed only non-sporulating lesions.

4.1.2.1.4 Lesion size

The range for the lesion size in the 65 genotypes under study was observed from 0.20 to 16.82 mm² with the mean lesion size of 5.65 mm². The minimum lesion size (0.20 mm²) was observed in the genotypes RDN 98-2-3-5-14, KJT 2, RDN 97-2, RDN 99-12, RDN 02-80, Heera, Phule Samruddhi, Vivek Dhan 82, Haryana Basmati, Mahisugandha, Vikram, Nalabhat and LK 248 followed by Indrayani (0.33 mm²) and Bhogavati (0.45 mm²). The genotype Antersal (16.82 mm²) showed highest lesion size followed by EK 70 (14.65 mm²) and Velkat (14.21 mm²). Twenty genotypes recorded significantly lower lesion size than population mean (5.65 mm²)

4.1.2.1.5 Per cent disease intensity (PDI)

The variation in the character disease intensity (Table 4.8 and 4.10) was observed from 11.11 to 98.42 per cent with the mean of 57.01 per cent. The minimum PDI was exhibited by RDN 97-2 and LK 248 (11.11 %) followed by RDN 2-80 (11.91 %) and RDN 98-2-3-5-14 (16.14 %). The highest PDI was recorded by EK 70 (98.42 %) followed by Ambemohar 102 (94.44 %) and Antersal and Velkat (92.59 %). Twenty-three genotypes showed significantly lower PDI than the population mean of 57.01 %.

4.1.2.2 Analysis of variance

The mean sum of squares due to replication, treatment (genotype) and error component for five pathological characters are given in Table 4.9. The mean sum of squares due to treatment was found highly significant for all pathological characters.

Table 4.9 Analysis of variance of pathological characters of blast disease of rice

Source of variation	Degrees of freedom	Mean sum of squares				
		Infection type	Diseased leaf area	Lesion density	Lesion size	Per cent disease intensity
Replication	1	7.877	1.452	0.005	0.035	55.616
Treatment	64	9.300**	493.374**	3.977**	37.575**	1065.663**
Error	64	0.408	3.027	0.046	1.098	11.564

** Significance at 1 per cent.

4.1.2.3 Parameters of genetic variability

Range of variability, estimates of genotypic and phenotypic coefficients of variation, heritability percentage in broad sense, genetic advance and genetic advance expressed as percentage of mean for five pathological characters under study are presented in Table 4.10.

Table 4.10 Variability parameters of 65 genotypes for pathological characters

S. No.	Character	Range	Mean	GCV	PCV	Heritability % (b.s.)	Genetic advance	G.A. as % of mean
1	Infection type	1.00 – 9.00	5.40	39.05	40.80	91.59	4.16	76.98
2	Diseased leaf area	0.41 – 81.71	15.46	54.37	54.67	98.78	23.55	110.81
3	Lesion density	0.00 – 5.77	1.44	73.70	74.00	97.73	8.14	147.38
4	Lesion size	0.20 – 16.82	5.65	75.62	77.86	94.32	8.54	151.29
5	Per cent Disease Intensity	11.11 - 98.42	57.01	40.27	40.71	97.85	46.78	82.06

4.1.2.3.1 Coefficient of variation

It was observed that the estimates for genotypic coefficient of variation (GCV) were lower than the phenotypic coefficient of variation (PCV) for all the characters. The characters, infection type had lowest GCV and PCV of 39.05 and 40.80, respectively which was followed by per cent disease intensity (40.27 and 40.71). The character lesion size showed highest GCV (75.62) and PCV (77.86) followed by lesion density (73.70 and 74.00). The highest difference between GCV and PCV values was observed in the case of lesion size (2.24) followed by infection type (1.75). The lowest difference between the GCV and PCV values was observed in per cent disease intensity (0.44) (Table 4.10).

4.1.2.3.2 Heritability (b.s.) percentage

According to Robinson's (1966) classification, heritability obtained in the present investigation for all the characters could be classified under very high heritability. Among the pathological characteristics studied, the character diseased leaf area (98.78 %) reported highest heritability followed by per cent disease intensity (97.85 %), whereas it was lowest for infection type (91.59 %) (Table 4.10).

4.1.2.3.3 Genetic advance

Genetic advance (Table 4.10) was highest in per cent disease intensity (46.78) followed by diseased leaf area (23.55) and was lowest for infection type (4.16). However, when it was expressed as the percentage of mean value it was highest in lesion size (151.29) followed by lesion density (147.38).

4.1.2.4. Divergence based on pathological characteristics

Genetic divergence in 65 genotypes of rice for pathological characters for blast resistance was measured following Mahalanobis (1936) D^2 statistic. The D^2 values between the genotypes studied ranged between 0.03 and 1496.95 (Appendix II). The lowest D^2 value (0.03) was observed between the genotypes RDN 97-2 and RDN 02-80 followed by Phule Samruddhi and Vivek Dhan 82 (0.04), Vivek Dhan 82 and Vikram (0.05) and Phule Samruddhi and Vikram (0.15), whereas highest D^2 value (1496.95) was observed between the genotypes EK 70 and LK 248 followed by EK 70 and RDN 97-2 (1473.96), EK 70 and RDN 02-80 (1471.74) and RDN 98-2-3-5-14 and EK 70 (1432.22).

4.1.2.4.1 Cluster analysis

Clustering was done following Tocher's method as described by Rao (1952). Sixty-five genotypes were grouped into seven clusters. These clusters along with genotypes included in them are presented in Table 4.11 and Fig. 4.2. The cluster I was the largest comprising of 27 genotypes followed by cluster II (26 genotypes) and cluster IV (8 genotypes). The clusters III, V, VI and VII had single genotype.

4.1.2.4.2 Intra and inter-cluster distances

Intra and inter-cluster D^2 and D values were worked out using D^2 values from divergence analysis. These are presented in Table 4.12.

The minimum intra-cluster distance was found in cluster II ($D^2 = 32.38$) followed by cluster I ($D^2 = 33.52$) and cluster IV ($D^2 = 37.95$). The clusters III, V, VI and VII with single genotype, had no intra-cluster distance.

Table 4.11 Distribution of rice genotypes into different clusters based on pathological traits.

Cluster	Number of genotypes	Genotypes	Characteres
I	27	RDN 97-2, RDN 02-80, LK 248, RDN 98-2-3-5-14, Basmati 386, Haryana Basmati, Nalabhat, KJT 2, Bhogavati, Heera, Juhibengal, Indrayani, Vikram, Mahisugandha, Vivek Dhan 82, Improved Pusa Basmati 1, Phule Samruddhi, RDN 99-14, Pomendi Local, Basmati 370, Kalajirga, Patni, Pawana, Ratna, Pusa Basmati 1, Pinjarwadi Local, MC 4.	Resistant, moderately resistant and moderately susceptible to blast (infection type 1 to 5).
II	26	RDN 01-2-10-9, Ajara Local 1, Diwani, Shyamjeer, Kasturi, Siddhagiri, Khalibagh, SD 7, Sonsali, SD 17, Taroari Basmati, Badshabhog, Kothimbire, Ghansal, BPT 5204, Phule Radha, RDN 99-18. Halvi Sal 17, RTN purple, RDN 185-2, Jaya, RTN 1, Champakali, Pavsal, IGT 13857, Karnal Local.	Moderately susceptible to susceptible to blast (infection type 5 to 8).
III	1	Phule Maval	Highly susceptible to blast (infection type 9), low lesion density & PDI.
IV	8	Sugandhamati, Kunchi, Ambemohar 157, Velkat, Ambemohar 102, Ajara Local 3, Antersal, Jagatpuri.	Highly susceptible to blast (infection type 8 to 9).
V	1	Tulsi tall	Susceptible to blast (infection type 8), high lesion density & low PDI
VI	1	RDN 99-12	Moderately resistant (infection type 3), high PDI.
VII	1	EK 70.	Highly susceptible to blast (infection type 9), high lesion size & high PDI.

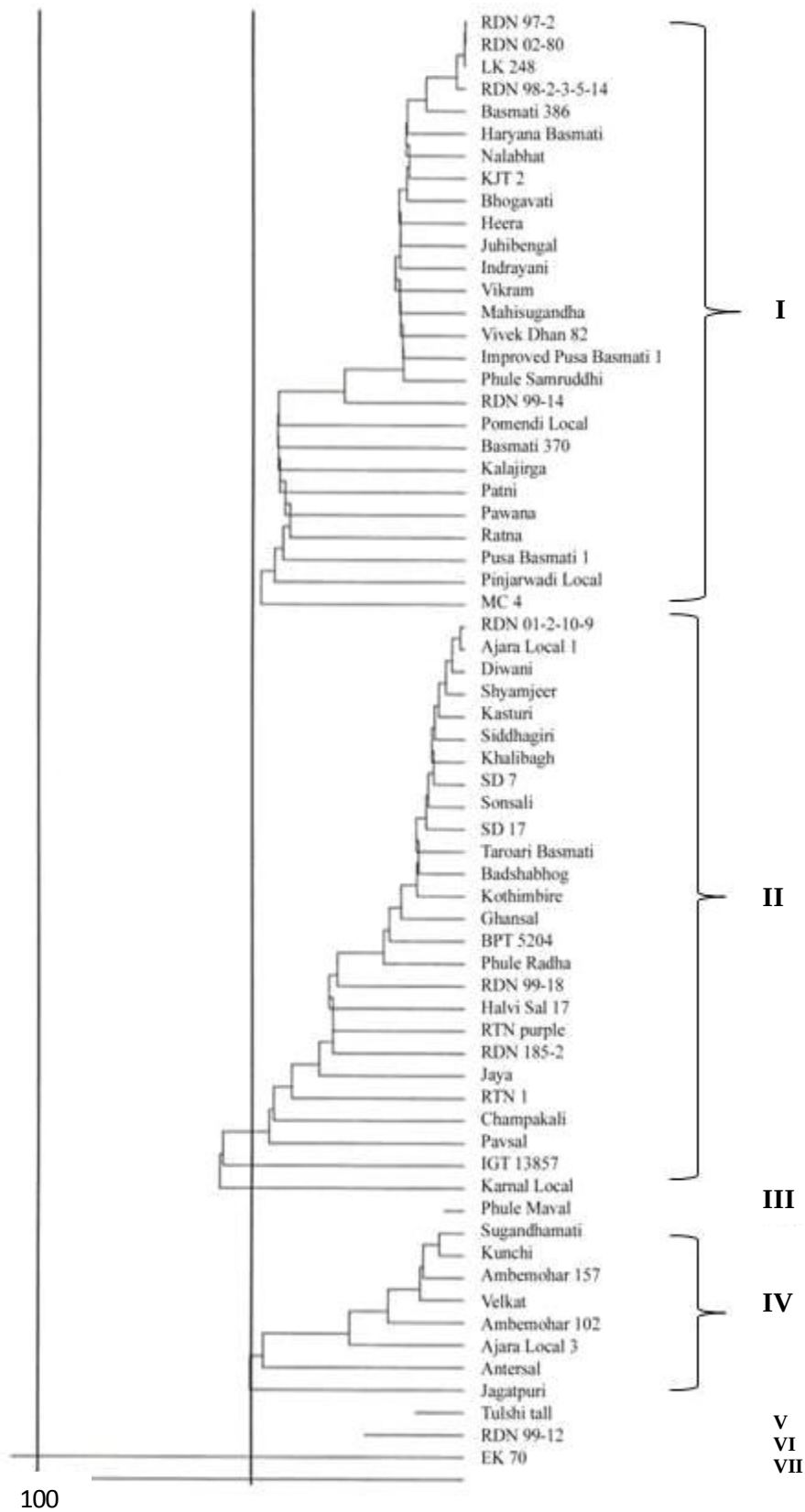
Fig. 4.2 Clustering of rice genotypes based on pathological analysis

Table 4.12 Average intra and inter-cluster D^2 and D (in parentheses) values for pathological traits

Cluster number	I	II	III	IV	V	VI	VII
I	33.52 (5.79)	118.37 (10.88)	277.89 (16.67)	478.30 (21.87)	260.50 (16.14)	82.81 (9.10)	1273.78 (35.69)
II		32.38 (5.69)	73.45 (8.57)	219.93 (14.83)	68.23 (8.26)	216.97 (14.73)	882.09 (29.70)
III			0.00 (0.00)	74.30 (8.62)	35.28 (5.94)	353.06 (18.79)	541.96 (23.28)
IV				37.95 (6.16)	104.04 (10.20)	470.46 (21.69)	275.56 (16.60)
V					0.00 (0.00)	347.82 (18.65)	634.03 (25.18)
VI						0.00 (0.00)	1094.29 (33.08)
VII							0.00 (0.00)

The maximum inter-cluster distance was observed between cluster I and VII ($D^2 = 1273.78$) followed by cluster VI and VII ($D^2 = 1094.29$), cluster II and VII ($D^2 = 882.09$) and cluster V and VII ($D^2 = 634.03$). The minimum inter-cluster distance was observed in the cluster III and V ($D^2 = 35.28$) followed by cluster II and V ($D^2 = 68.23$), cluster II and III ($D^2 = 73.45$) and cluster III and IV ($D^2 = 74.30$).

Cluster I showed maximum inter-cluster distance from cluster VII ($D^2 = 1237.78$) followed by cluster IV ($D^2 = 478.30$). However, cluster I recorded minimum inter-cluster distance from cluster VI ($D^2 = 82.81$) followed by cluster II ($D^2 = 118.37$).

The range of minimum and maximum inter-cluster D^2 values for cluster III were from 35.28 to 541.96. The cluster VII recorded highest inter-cluster distance from cluster III ($D^2 = 541.96$) followed by cluster VI ($D^2 = 353.06$) and cluster I ($D^2 = 277.89$). Cluster V was found closer to cluster III ($D^2 = 35.28$) followed by cluster II ($D^2 = 73.45$) and cluster IV ($D^2 = 74.30$).

Cluster IV showed maximum inter-cluster distance from cluster I ($D^2 = 478.30$) followed by cluster VI ($D^2 = 470.46$). This cluster showed minimum inter-cluster distance from cluster III ($D^2 = 74.30$) followed by cluster V ($D^2 = 104.04$)

Cluster V with single genotype showed inter-cluster D^2 values from 35.28 for cluster III to 634.03 for cluster VII. Cluster VI ($D^2 = 347.82$) and cluster I ($D^2 = 260.50$) showed higher inter-cluster distances from cluster V. Whereas, cluster II ($D^2 = 68.23$) and cluster IV ($D^2 = 104.04$) showed relatively less distance from this cluster.

Cluster VI with single genotype RDN 99-12, was closest to cluster I ($D^2 = 82.81$). It had maximum distance from cluster VII ($D^2 = 1094.29$) followed by cluster IV ($D^2 = 470.46$), cluster III ($D^2 = 353.06$) and cluster V ($D^2 = 347.82$).

Cluster VII, consisting of single genotype EK 70 was found to be most distinct among all the clusters. This cluster showed the D^2 values ranging from 275.56 (cluster IV) to 1273.78 (cluster I). The cluster VI ($D^2 = 1094.29$) and cluster II ($D^2 = 882.09$) were at longer distance from the cluster VII, whereas it was relatively closer to cluster III ($D^2 = 541.96$) and cluster V ($D^2 = 634.03$).

4.1.2.4.3 Cluster means

Cluster means for five pathological characters are presented in Table 4.13. It revealed wide range of variability among the clusters for all the pathological characters viz., infection type, diseased leaf area, lesion density, lesion size and per cent disease intensity.

Table 4.13 Mean performance of clusters for blast resistance traits

S. No.	Character	Cluster number						
		I	II	III	IV	V	VI	VII
1	Infection type	3.41	6.29	8.50	8.38	8.00	3.00	9.00
2	Diseased leaf area	36.21	65.83	84.92	89.90	88.36	26.19	98.42
3	Lesion density	2.08	6.94	7.03	11.85	13.88	0.20	14.65
4	Lesion size	0.24	1.76	3.40	3.60	1.80	0.00	5.77
5	Per cent disease intensity	5.29	13.52	26.27	44.54	25.72	20.28	81.71

Infection type

Cluster means for the character infection type were highest in cluster VII (9.00) followed by cluster III (8.50) and cluster IV (8.38) while, lowest in cluster VI (3.00) followed by cluster I (3.41).

Diseased leaf area

Cluster VI had minimum diseased leaf area (26.19 %) followed by cluster I (36.21 %), whereas it was highest in the cluster VII (98.42 %) followed by cluster IV (89.90 %) and cluster V (88.36 %).

Lesion density

Lowest lesion density was noticed in cluster VI (0.20 per cm²) followed by cluster I (2.08 per cm²). On the contrary, highest mean for lesion density was recorded in cluster VII (14.65 per cm²) followed by cluster V (13.88 per cm²) and cluster IV (11.85 per cm²).

Lesion size

The data presented in Table 4.13 indicate that the mean performance of the clusters for lesion size ranged between 0.00 and 5.77 mm². Cluster VI had minimum lesion size (0.00 mm²) followed by cluster I (0.24 mm²). Cluster VII showed highest lesion size (5.77 mm²) followed by cluster IV (3.60 mm²) and cluster III (3.40 mm²).

Per cent disease intensity (PDI)

The trait, per cent disease intensity exhibited wide range of variation for cluster means. It varied from 5.29 (cluster I) to 81.71 (cluster VII). The cluster IV also showed higher PDI (44.54), whereas cluster II (13.52) and cluster VI (20.28) had lower PDI.

4.1.2.4.4 Contribution of various characters for divergence

The per cent contribution to the total divergence of the five pathological characters studied is presented in Table 4.14. It was observed that per cent disease intensity contributed highest (35.24 %) followed by lesion density (32.88 %) and diseased leaf area (21.15 %). Infection type contributed least (0.39 %) to total divergence followed by lesion size (10.34 %).

Table 4.14 Per cent contribution of pathological observations for divergence

S. No.	Character	No. of times appearing 1 st in ranking	Per cent contribution
1	Infection type	8	0.39
2	Diseased leaf area (%)	440	21.15
3	Lesion density (per cm ²)	684	32.88
4	Lesion size (mm ²)	215	10.34
5	Per cent Disease Intensity	733	35.24
	Total	2080	100.00

4.2 Experiment II : Genetic divergence study through molecular markers

Study of genetic diversity analysis of rice genotypes using molecular markers is one of the objectives of the study. In Experiment I, sixty-five rice genotypes were evaluated on the basis of plant agro-morphological characters. The expression of morphological traits was highly influenced by the environment. Since molecular marker characterization of genotypes is independent of $G \times E$ interaction, it may be an efficient and effective tool to understand and explain the genetic variability and to make use of it in crop improvement programme. In the present investigation, 50 rice genotypes for the molecular evaluation were selected from 65 genotypes of Experiment I by discarding 15 most similar genotypes based on the field diversity analysis. The present study was conducted using 25 ISSR and 17 SSR primers to understand the genetic diversity and inter-relationship among the genotypes. Results obtained are presented in this section.

4.2.1 ISSR Polymorphism

The genomic DNA of 50 rice genotypes was subjected to PCR amplification using 25 ISSR primers. Out of these, 13 primers produced polymorphic amplicons were selected for genetic diversity analysis by taking into consideration the repeatability, sharpness and intensity of alleles.

A total of 103 reproducible amplicons with an average of 7.92 fragments per primer were produced using 13 ISSR primers. All the markers displayed polymorphic amplicons. Of the total amplicons, 100 (97.08 %) were polymorphic for more than one variety and three amplicons were monomorphic (Table 4.15).

Table 4. 15 Details of the ISSR primers used and polymorphism status

S. No.	Details	Number
1	Total number of primers used	25
2	Number of polymorphic primers	13
3	Total number of loci	103
4	Total number of polymorphic loci	100
5	Total number of monomorphic loci	3
6	Total number of unique loci	0
7	Percentage of polymorphic loci	97.08

The observations recorded in Table 4.16 indicate that among 100 polymorphic fragments, 19 were highly polymorphic, 66 moderately polymorphic and 15 fragments showed low polymorphism. The number of amplicons generated by each primer varied from 3 to 12. Maximum number of amplicons were amplified by the primer ISSR815 and ISSR823 (12 amplicons) followed by ISSR827 (11 amplicons) and ISSR810 and ISSR812 (10 amplicons), whereas least number of amplicons were amplified by the primer ISSR808 and ISSR826 (3 amplicons). The amplification pattern of some ISSR primers is shown in Plate V.

The percentage of polymorphism varied with each primer ranging from 66.66 to 100 per cent. The primers ISSR809, ISSR810, ISSR811, ISSR813, ISSR814, ISSR815, ISSR818, ISSR821, ISSR826 and ISSR827 recorded maximum (100 %) polymorphic amplicons, whereas, the primer ISSR808 recorded the lowest (66.66 %) (Table 4.16).

The primer ISSR821, ISSR823 and ISSR827 gave highest number of highly polymorphic fragments (3) followed by ISSR810, ISSR811, ISSR812 and ISSR813

(2), whereas the primers ISSR814 and ISSR815 reported single highly polymorphic fragment. The number of moderately polymorphic fragments were observed between 0 (ISSR826) and 9 (ISSR815). The moderately polymorphic fragments were found highest in ISSR 815 (9) followed by ISSR 809 and ISSR 810 (8). ISSR 826 showed all three fragments with low polymorphism. Single monomorphic fragment was reported by the primers ISSR808, ISSR812 and ISSR823. None of the primers reported unique allele in any genotype under study (Table 4.16).

Table 4.16 Polymorphism status of ISSR primers

S. No.	Name of primer	Total number of loci	Polymorphic loci			Mono-morphic loci	Unique loci	Per cent polymorphism
			High	Moderate	Low			
1	ISSR808	3	0	1	1	1	0	66.66
2	ISSR809	9	0	8	1	0	0	100.00
3	ISSR810	10	2	8	0	0	0	100.00
4	ISSR811	9	2	7	0	0	0	100.00
5	ISSR812	10	2	6	1	1	0	90.00
6	ISSR813	8	2	5	1	0	0	100.00
7	ISSR814	4	1	3	0	0	0	100.00
8	ISSR815	12	1	9	2	0	0	100.00
9	ISSR818	6	0	4	2	0	0	100.00
10	ISSR821	6	3	3	0	0	0	100.00
11	ISSR823	12	3	6	2	1	0	91.67
12	ISSR826	3	0	0	3	0	0	100.00
13	ISSR827	11	3	6	2	0	0	100.00

High : Fragment present in 40 to 60 per cent genotypes

Moderate : Fragment present in 10 to 40 or 60 to 90 per cent genotypes

Low : Fragment present in < 10 and > 90 per cent genotypes (Chimote *et al.*, 2004)

All the 50 genotypes evaluated for the molecular diversity with 13 ISSR primers showed variation in the amplification pattern. The maximum number of amplicons (61 amplicons) were observed in Phule Samruddhi followed by Ratna and RTN-1 (58 amplicons) and Kalajirga (57 amplicons). Whereas Vikram (22 amplicons) reported least number of amplicons followed by Tulshi tall (26 amplicons) and Sonsali and Pomendi Local (27 amplicons) (Appendix III).

4.2.1.1 Divergence analysis in rice genotypes using ISSR markers:

Dendrogram constructed with NTSYSpc 2.02i on the basis of ISSR polymorphism revealed the pattern of relatedness among 50 rice genotypes. Clustering analysis based on Unweighted Pair Group Method using Arithmetic Averages (UPGMA) using Dice similarity coefficient grouped 50 rice genotypes into three major clusters. Cluster I had highest number of genotypes (38) followed by cluster II (11), whereas cluster III had a single genotype. The major clusters I and II were further grouped into sub-clusters. The details of the genotypes included in each sub-cluster are presented in Table 4.17 and depicted in dendrogram (Fig. 4.3) and 2D diagram (Fig. 4.4). The similarity coefficient values between the genotypes were presented in Appendix V.

The results depicted in Table 4.17 show that, cluster I was the largest consisting of 38 genotypes. The similarity coefficient between any two genotypes in this cluster ranged between 44 and 90 per cent (Appendix V). This cluster was further subdivided into eight sub-clusters viz., A – H having varying degrees of similarity. The major sub-cluster F differentiated at similarity coefficient of 0.66 comprised of 13 genotypes followed by sub-cluster B differentiated at similarity coefficient of 0.73 comprised of 11 genotypes. All the genotypes grouped in sub-cluster B and F were improved varieties.

Table 4.17 Distribution of rice genotypes into different clusters based on ISSR analysis

Cluster	Sub-cluster	Number of genotypes	Genotypes	character
I	A	3	Heera, Halvi Sal17, RDN 185-2	Improved varieties, early maturity, coarse grain type.
	B	11	RTN 1, Ratna, Jaya, Pawana, Indrayani, Phule Samruddhi, Phule Maval, Bhogavati, RDN01-2-10-9, BPT 5204, RDN 98-2-3-5-14	Improved varieties with TN 1 or IR 8 in ancestry, dwarf plant type, long-slender or medium-slender grain type,
	C	2	Patni, EK 70	Landrace and local selection, long grain length.
	D	1	Kalajirga	Landrace, tall plant type, short-bold grain .
	E	2	Vivek Dhan 82, KJT 2	Improved varieties, long grain type.
	F	13	Phule Radha, RDN 97-2, Siddhagiri, LK 248, RDN 99-12, RDN 99-14, MC 4, Haryana Basmati, RTN purple, Kasturi, Khalibagh, SD 17, Sugandhamati	Mostly improved with mixed performance.
	G	3	Antersal, RDN 02-80, Sonsali	Landraces except RDN 02-80, medium-slender grain type.
	H	3	Champakali, Taroari Basmati, Nalabhat	Landraces or local selection, long-slender grain type.
II	A	1	Vikram	Landrace, medium-slender grain type.
	B	10	Pusa Basmati 1, Mahisugandha, Diwani, Badshabhog, Kothimbire, Shyamjeer, Basmati 386, Ghansal, Pavsal, Tulshi tall	Mostly scented landraces, slender grain type.
III		1	Pomendi Local	Landrace.

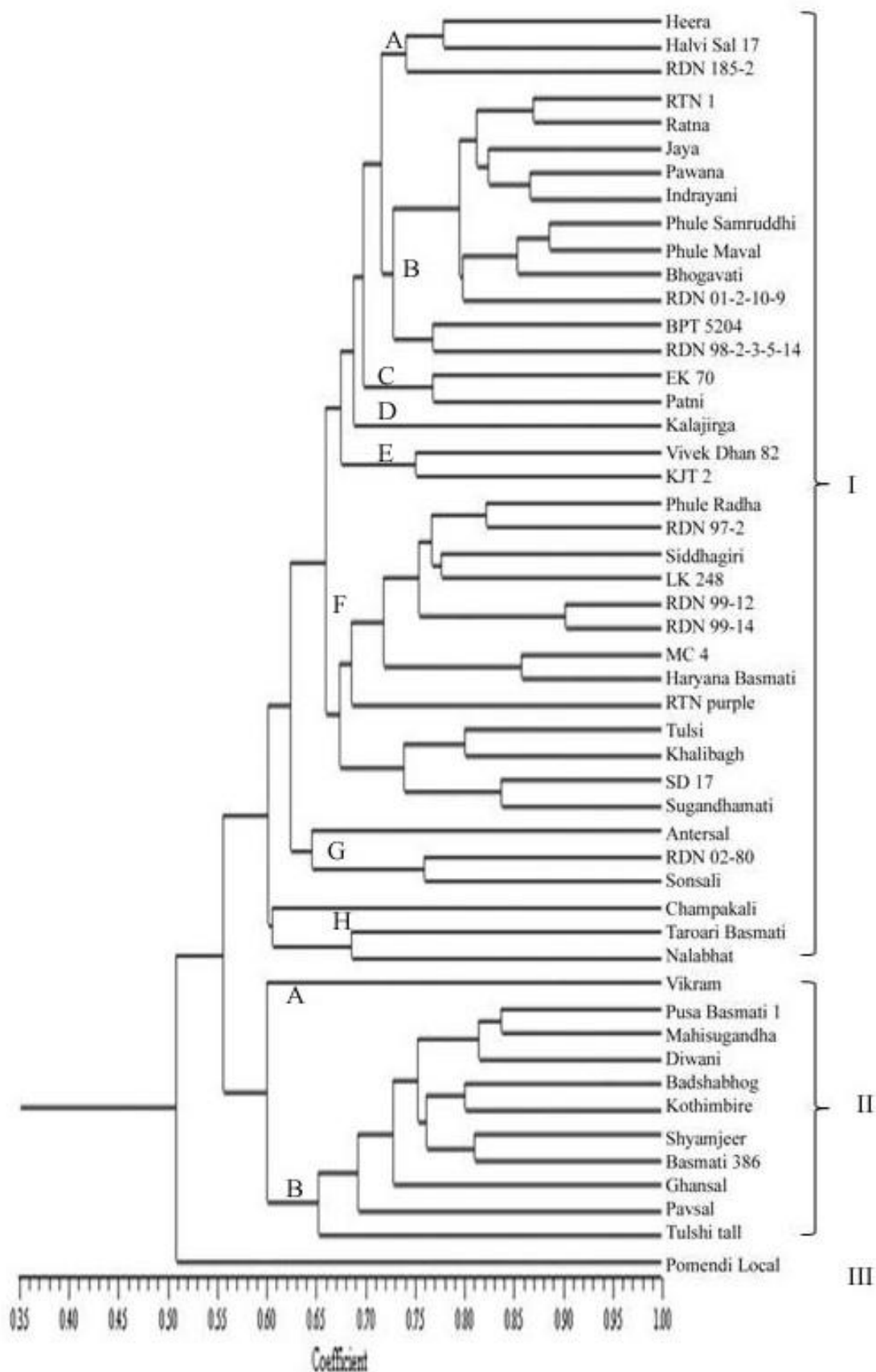
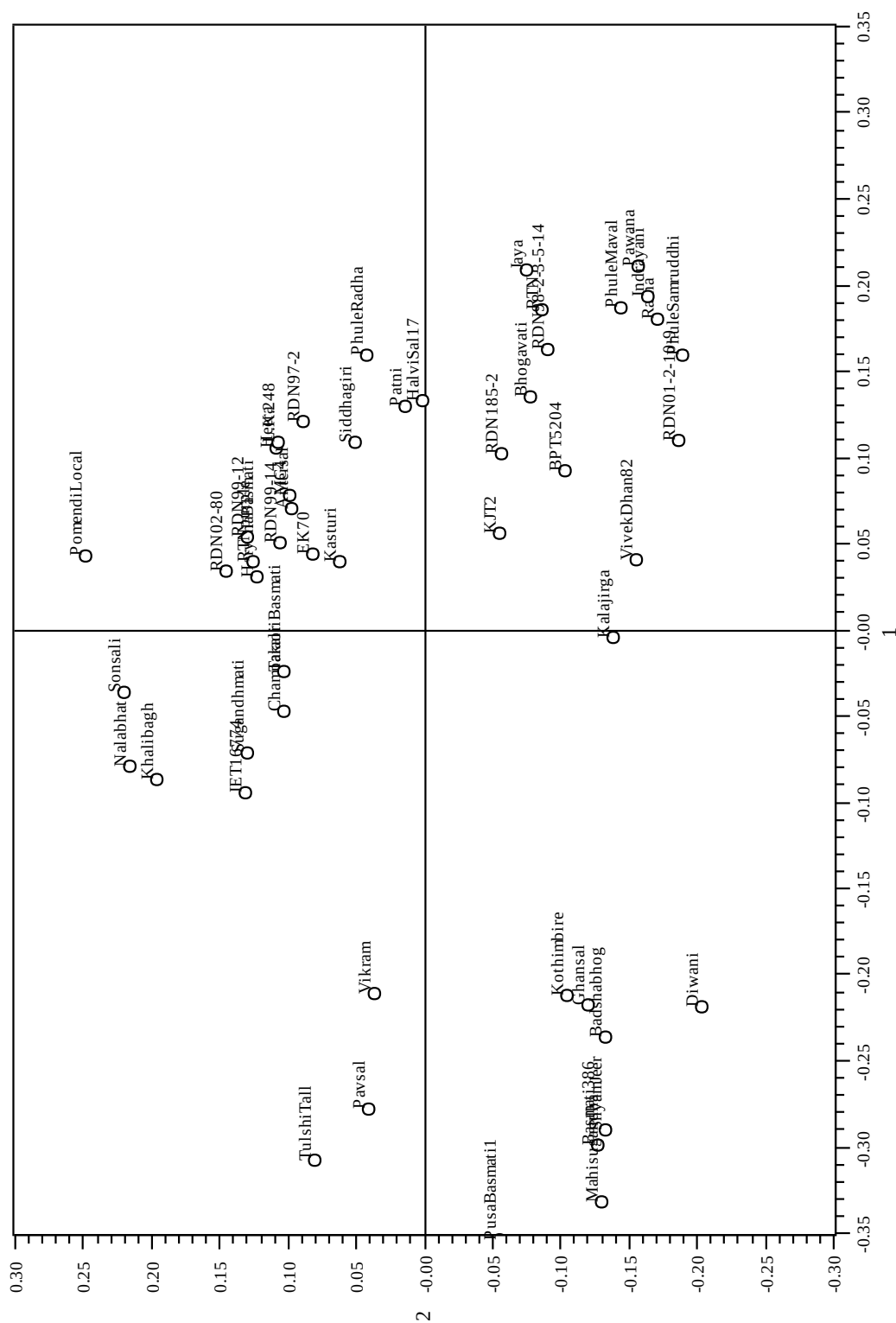
Fig. 4.3 UPGMA based dendrogram of rice genotypes using ISSR markers

Fig. 4.4 2D scatter plot analysis of rice genotypes using ISSR markers



Cluster II consisted of 11 genotypes and was divided into two sub-clusters at the similarity coefficient of 0.60. All the genotypes in this cluster are local selections or landraces, except Pusa Basmati 1 and Mahisugandha. Sub-cluster A of this cluster consist of single landrace Vikram, differentiated from other genotypes at similarity index value 0.60. The similarity coefficient between any two genotypes in the sub-cluster B ranged from 0.45 to 0.84.

Cluster III consist of single landrace, Pomendi Local differentiated from all other genotypes at similarity coefficient of 0.51.

The grouping identified by 2D scatter plot analysis based on principle co-ordinate analysis (PCoA) of 50 genotypes reflected the clustering as obtained in the dendrogram. Cluster I of the dendrogram consisting of 38 genotypes grouped in eight sub-clusters was found to be most diverse in PCoA. Sub-cluster A of this cluster I was differentiated by 1st component of PCoA, grouping the genotypes in I (+/+) and II (+/-) quarter of PCoA diagram. All other sub-clusters of the cluster I of dendrogram were differentiated by both 1st and 2nd component of PCoA grouping them independently in separate quarters. Sub-clusters B and E of the dendrogram were grouped into II (+/-) quarter, whereas the genotypes from sub-cluster C, F and G were grouped into I (+/+) quarter. The single genotype from sub-cluster D was placed in III (-/-) quarter.

Cluster II from the cluster diagram was differentiated by 1st component of PCoA grouping the genotypes in III (-/-) and IV (-/+) quarters, whereas the cluster III with single genotype Pomendi Local was differentiated by 1st and 2nd component, grouping the genotype in I (+/+) quarter.

4.2.2 SSR polymorphism

Fifty rice genotypes were analyzed by 17 SSR markers. Fifteen markers yielded distinct, detectable amplicons of variable intensities. The amplification profiles of 15 SSR primers are summarized in Tables 4.18. A total of 187 scorable amplicons were detected among 50 rice genotypes. The average number of alleles per locus was 12.47 with a range from 2 (RM11) to 23 (RM204).

Table 4.18 Details of the SSR primers used and polymorphism status

S. No.	Details	Number
1	Total number of markers used	17
2	Number of polymorphic markers	15
3	Total number of alleles	187
4	Total number of polymorphic alleles	178
5	Total number of monomorphic alleles	0
6	Total number of unique alleles	9
7	Percentage of polymorphic alleles	95.18

Table 4.19 Polymorphism pattern obtained using SSR primers

Sr. No	Name of primer	Total number of alleles	Polymorphic alleles			Mono-morphic alleles	Unique alleles	Percentage polymorphic alleles (%)
			High	Moderate	Low			
1	RM11	2	0	2	0	0	0	100.00
2	RM21	3	2	1	0	0	0	100.00
3	RM44	20	1	14	5	0	0	100.00
4	RM72	5	0	5	0	0	0	100.00
5	RM162	9	1	3	4	0	1	88.88
6	RM204	23	3	11	7	0	2	91.30
7	RM209	8	0	8	0	0	0	100.00
8	RM215	5	0	3	1	0	1	80.00
9	RM224	15	6	4	4	0	1	93.33
10	RM241	17	5	10	0	0	2	88.24
11	RM246	22	4	16	2	0	0	100.00
12	RM259	20	0	19	1	0	0	100.00
13	RM307	4	0	3	1	0	0	100.00
14	TRS26	15	2	10	3	0	0	100.00
15	TRS33	19	1	12	4	0	2	89.47

High : Band present in 40 to 60 per cent genotypes

Moderate : Band present in 10 to 40 or 60 to 90 per cent genotypes

Low : Band present in < 10 and > 90 per cent genotypes (Chimote *et al.*, 2004)

The data in Table 4.19 reveal that among 187 scorable alleles, 178 were polymorphic (95.18 %) in which 25 alleles were highly polymorphic, 121 alleles moderately polymorphic and 32 alleles with low polymorphism. Nine unique alleles were observed in SSR analysis, which were genotype-specific in the present study. The percentage of polymorphism of SSR markers was observed from 80.00 to 100 per cent. The primers RM11, RM21, RM44, RM72, RM209, RM246, RM259, RM307 and TRS26 reported maximum (100 %) polymorphic alleles, whereas RM215 reported minimum (80.00 %).

Maximum scorable alleles were observed for RM204 (23 alleles) followed by 22 alleles using RM246 and 20 alleles using RM44 and RM259 (Table 4.19). The least number of alleles were observed for RM11 (2 alleles) followed by RM21 (3 alleles) and RM307 (4 alleles). The maximum number of highly polymorphic alleles were recorded by using the primer RM224 (6 alleles) followed by RM241 (5 alleles) and RM246 (4 alleles). The primer RM259 recorded highest number of moderately polymorphic alleles (19 alleles) followed by RM246 (16 alleles) and RM44 (14 alleles). None of SSR marker reproduced monomorphic allele (Table 4.19). The amplification pattern of some SSR primers is shown in Plate VI.

The SSR marker RM162 showed nine alleles with one highly polymorphic, three moderately polymorphic, four low polymorphic and a unique allele of ~500 bp in the variety RDN 02-80 (Plate VI).

The SSR marker RM204 generated two unique, genotype specific alleles. Among these, one allele of ~205 bp was observed in Taroari Basmati, whereas another of ~200 bp was noticed in RDN 97-2 (Plate VI).

The microsatellite marker RM215 generated five loci, of which three were highly polymorphic, one with low polymorphism and one unique locus. The unique locus was observed in the genotype Ratna of ~385 bp.

RM224 generated 15 distinct alleles having six highly polymorphic, four each with moderate and low polymorphism and one unique allele. The unique allele of ~150bp was reported in a landrace Patni (Plate VI).

Two unique alleles of ~95 bp and ~105 bp were generated in RDN 02-80 by the microsatellite marker RM241. This marker also generated five highly polymorphic and 10 moderately polymorphic alleles in 65 rice genotypes (Plate VI).

TRS33 generated 19 alleles, of which 17, were polymorphic and two were unique. The unique alleles were observed in Sonsali (~580 bp) and BPT 5204 (~180 bp) (Plate VI).

4.2.2.1 Divergence analysis in rice genotypes using SSR markers

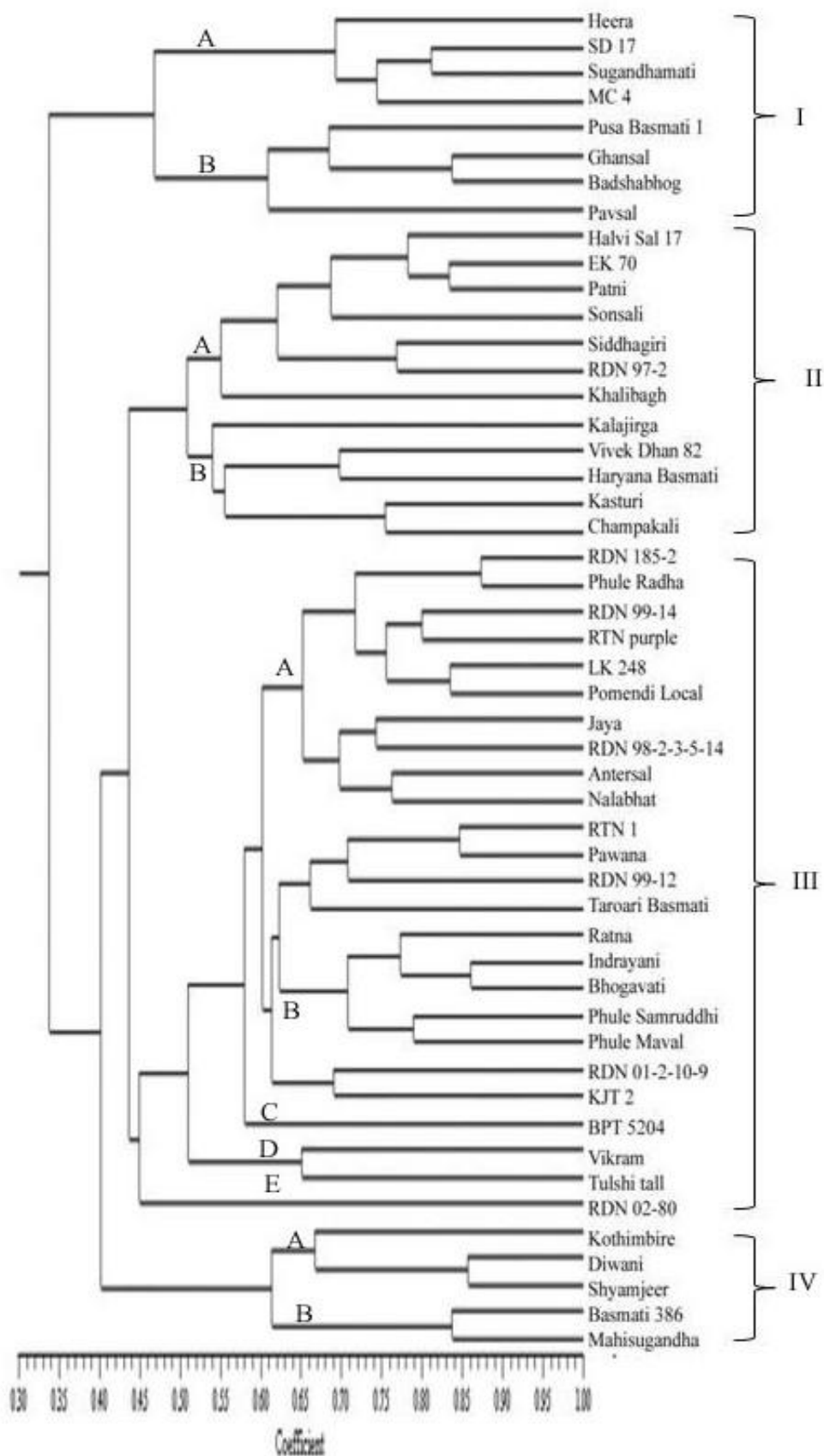
Dendrogram constructed using NTSYSpc 2.02i on the basis of SSR polymorphism indicated the relatedness among 50 rice genotypes. Clustering based on UPGMA grouped 50 rice genotypes into four major clusters. Cluster III had highest number of genotypes (25) followed by cluster II (12). Cluster I had eight genotypes, whereas five genotypes were grouped into cluster IV. These major clusters were further grouped into sub-clusters. The details of the genotypes included in each group are presented in Table 4.20 and depicted in dendrogram (Fig. 4.5) and 2D diagram (Fig. 4.6). The similarity coefficient values between the genotypes are presented in Appendix VI.

Cluster I consisted of eight genotypes and it was grouped into two sub-clusters, A and B with four genotypes each. These two sub-clusters were differentiated at similarity coefficient of 0.47 (Fig. 4.5). The variation in the similarity coefficient between any two genotypes within sub-cluster A was observed between 0.64 and 0.81, whereas the range for similarity coefficient among the genotypes in the sub-cluster B was observed between 0.51 and 0.84 (Appendix VI).

Twelve genotypes were grouped into cluster II, which was further separated into two sub-clusters, A and B, at similarity of 51 per cent. This cluster consisted of most of the local genotypes. The range of similarity coefficient for sub-cluster A was observed between 0.40 and 0.83, whereas it ranged between 0.49 and 0.75 for sub-cluster B (Appendix VI).

Table 4.20 Distribution of rice genotypes into different clusters based on SSR analysis

Cluster	Sub-cluster	Number of genotypes	Genotypes	Characters
I	A	4	Heera, SD 17, Sugandhamati, MC4	Basmati line Karnal Local as parent or in ancestry except Heera.
	B	4	Pusa Basmati 1, Ghansal, Badshabhog, Pavsai	Landraces except Pusa Basmati 1
II	A	7	Halvi Sal 17, EK 70, Patni, Sonsali, Siddhagiri, RDN 97-2, Khalibagh	Landraces or local selection except RDN 97-2 and Vivek Dhan 82.
	B	5	Kalajirga, Vivek Dhan 82, Haryana Basmati, Kasturi, Champakali	
III	A	10	RDN185-2, Phule Radha, RDN99-14, RTN purple, LK 248, Pomendi Local, Jaya, RDN 98-2-3-5-14, Antersal, Nalabhat	Almost all improved varieties with TN 1 in parentage.
	B	11	RTN 1, Pawana, RDN 99-12, Tarori Basmati, Ratna, Indrayani, Bhogavati, Phule Samruddhi, Phule Maval, RDN 01-2-10-9, KJT 2	Almost all improved varieties with IR 8 or IR 28 in parentage.
	C	1	BPT 5204	Improved variety with TN 1 in parentage.
	D	2	Vikram, Tulshi tall	Landraces.
	E	1	RDN 02-80	Improved variety with IR 8 in ancestry.
IV	A	3	Kothimbire, Diwani, Shyamjeer.	Landraces, short grain length, scented.
	B	2	Basmati 386, Mahisugandha.	Scented Basmati, long-slender grain type.

Fig. 4.5 UPGMA based dendrogram of rice genotypes using SSR markers

Cluster III was the largest and most diverse. This cluster consisted of 25 genotypes including most of the improved varieties under study. All the genotypes in this cluster were initially grouped at 45 per cent similarity differentiating the variety RDN 02-80 (sub-cluster E). Similarly, another pair of genotypes, Vikram and Tulshi tall (sub-cluster D) differentiated from remaining genotypes at 51 per cent similarity. Thereafter, a unique genotype, BPT 5204, grouped separately at 58 per cent similarity forming sub-cluster C. Two major sub-clusters of cluster III viz., A and B, with 10 and 11 genotypes, respectively were differentiated at 60 per cent similarity. The range of similarity index between any two genotypes grouped in sub-cluster A was observed between 0.54 and 0.87, whereas it was between 0.48 and 0.86 for sub-cluster B.

Cluster IV consisted of five genotypes grouped into two sub-clusters A and B differentiated at 61.50 per cent similarity. Sub-cluster A consisted of three local types with similarity between them ranging between 62 and 86 per cent, whereas sub-cluster B had two Basmati type genotypes (Basmati 386 and Sugandhamati) with 84 per cent similarity between them.

The grouping of 50 rice genotypes by 2D scatter plot analysis based on principle co-ordinate analysis (PCoA) were very similar to those identified by UPGMA tree cluster analysis. Among four major clusters formed by tree clustering analysis, three clusters viz., I, II and IV were differentiated by both the components of PCoA (Fig.4.6).

Cluster I and cluster IV of the dendrogram with 8 and 5 genotypes, respectively were differentiated by 1st and 2nd component of PCoA grouping the genotypes in I (+/+) quarter. Cluster III of the dendrogram consisting 25 genotypes was differentiated by 1st component of PCoA differentiating the genotypes in III (-/-) and IV (-/+) quarter, whereas Cluster II consisting of most of the landraces and local genotypes was differentiated by both the axis grouping the genotypes in II (+/-) quarter of PCoA diagram.

4.2.3 Molecular diversity studies using pooled data of ISSR and SSR markers

The binary data from the polymorphic ISSR and SSR primers were used for computing Jaccard's similarity indices. The similarity coefficient based on 13 ISSR and 15 SSR primers ranged between 0.25 and 0.85 (Appendix VII). Thirteen genotypic combinations recorded similarity value ≤ 0.30 and 277 genotypic combinations recorded it ≤ 0.40 showing high diversity among these pairs. Maximum diversity was observed between the landrace Pomendi Local and improved scented (Basmati) variety Pusa Basmati 1 (0.25) followed by an improved breeding line RDN 97-2 and a local land race Tulshi tall (0.26). The genotype Badshabhog recorded highest number of genotypic combinations (26) with similarity index value ≤ 0.40 followed by Pavsai (25), Pusa Basmati 1 (20), Khalibagh (15), Ghansai (15), Tulshi tall (14), Vikram (10), Shyamjeer (9) and RDN 97-2 (9) (Appendix VII).

Maximum similarity was observed between the improved varieties. The highest similarity was observed between the improved varieties Pawana and RTN 1 (0.85) followed by Phule Maval and Phule Samruddhi (0.84), Bhogavati and Indrayani (0.84) and Indrayani and Phule Maval (0.83). In all, 12 genotypic combinations showed the similarity index > 0.80 , whereas 70 genotypic combinations recorded similarity index > 0.75 showing high similarity. The improved variety Pawana recorded highest number of genotypic combinations (6) with the similarity index value ≥ 0.75 followed by Indrayani (5), Phule Maval (5), Bhogavati (4) and RDN 99-14 (4) indicating the similarity in their genetic constitution.

4.2.3.1 Divergence analysis in rice genotypes using pooled data of ISSR and SSR markers

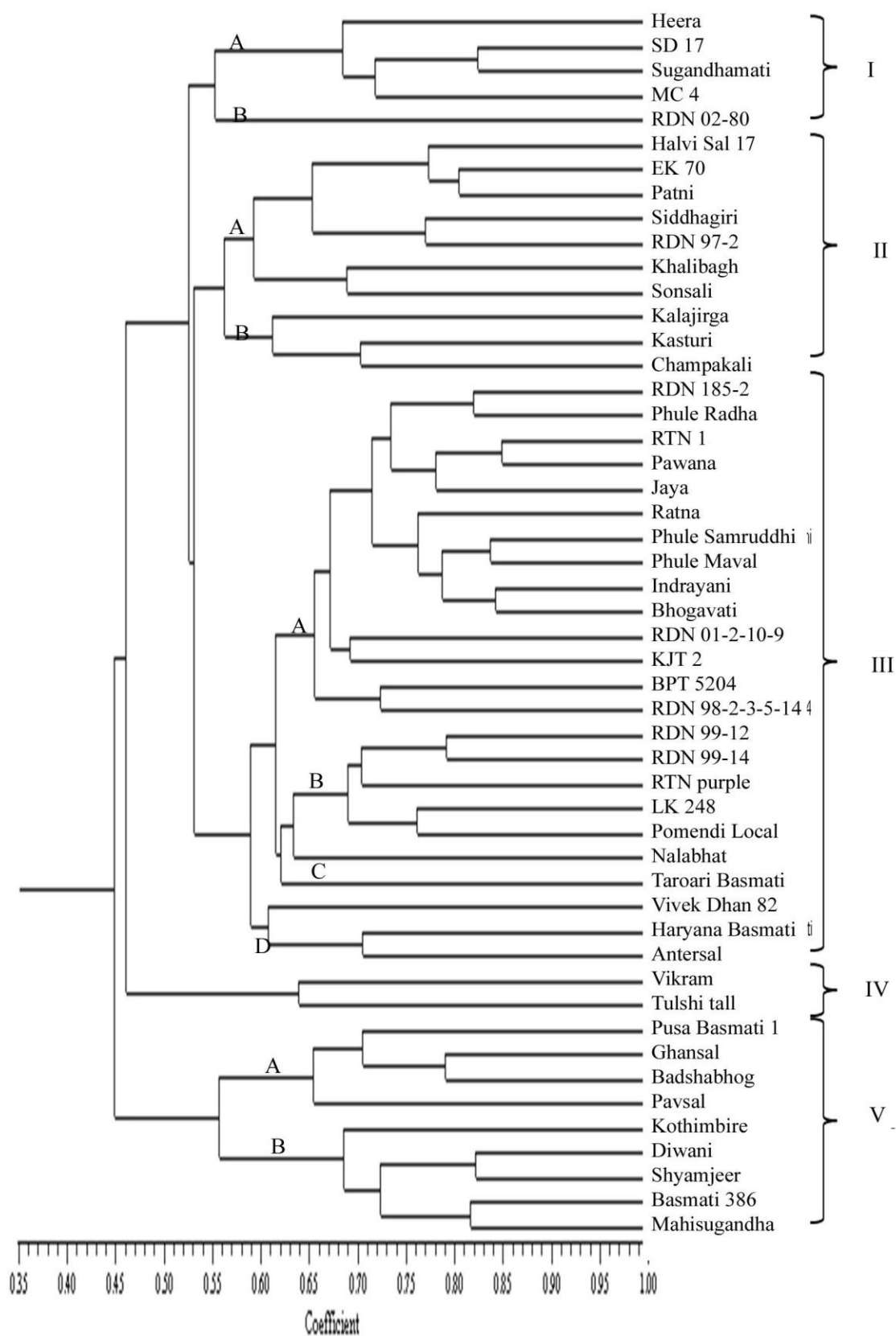
Genetic similarity values between the various rice genotypes obtained from ISSR and SSR data were used to produce a cluster diagram (dendrogram, Fig. 4.7) and two dimensional scale diagram (PCoA analysis, Fig. 4.8), which clearly explained the relationship between 50 genotypes. The Unweighted Pair Group method using Arithmetic Averages (UPGMA) based clustering analysis using Dice similarity coefficient grouped the rice genotypes into five clusters. Cluster III had the highest number of genotypes (24) followed by cluster II (10) cluster V (9), cluster I (5) and

cluster IV (2) The details of the genotypes included in each group are presented in Table 4.21. The similarity coefficient values between the genotypes were presented in Annexure V.

Table 4.21 Distribution of 50 rice genotypes into different clusters based on pooled analysis

Cluster	Sub-cluster	Number of genotypes	Genotypes	Characters
I	A	4	Heera, S.D.17, Sugandhamati, MC-4	Basmati line Karnal Local as parent or in ancestry, except Heera.
	B	1	RDN 02-80	
II	A	7	Halvi Sal 17, EK 70, Patni, Siddhagiri, RDN 97-2, Khalibagh, Sonsali.	Non-scented, landraces or local selections, except RDN 99-2.
	B	3	Kalajirga, Kasturi, Champakali.	Scented landraces except Kasturi
III	A	14	RDN 185-2, Phule Radha, RTN-1, Pawana, Jaya, Ratna, Phule Samruddhi, Phule Maval, Indrayani, Bhogavati, RDN 01-2-10-9, KJT-2, BPT5204, RDN 98-2-3-5-14.	All improved varieties with dwarf plant type, IR-8 or TN1 as parent or in ancestry.
	B	5	RDN 99-12, RDN 99-14, RTN purple, LK 248, Pomendi Local.	Dwarf plant type, midlate to late maturity, medium slender grain type.
	C	2	Nalabhat, Taroari Basmati	Landraces, long-slender grain type, late maturity.
	D	3	Vivek Dhan 82, Haryana Basmati, Antersal.	Tall plant type.
IV		2	Vikram, Tulshi tall	Landraces.
V	A	4	Pusa Basmati-1, Ghansal, Badshabhog, Pavsai	Scented Basmati or non-Basmati genotypes. Mostly landraces.
	B	5	Kothimbire, Diwani, Shyamjeer, Basmati 386, Mahisugandha.	

Fig. 4.7 UPGMA based dendrogram of rice genotypes using pooled data of ISSR and SSR markers



Cluster I consisted of five genotypes with similarity between any two genotypes in the cluster ranging between 41 and 82 per cent (Appendix VII). The cluster was further subdivided into two sub-clusters with similarity coefficient of 0.56. The genotypes in this cluster, except Heera had scented Basmati line Karnal Local in their ancestry, resulting in the similarity in their genotypic constitution.

Cluster II consist of 10 genotypes was divided into two sub-clusters at the similarity coefficient of 0.56. All the genotypes in this cluster were local selections or landraces. The similarity between the genotypes in this cluster ranged between 43 and 80 per cent (Appendix VII). The sub-cluster A comprised of all non-scented local genotypes except RDN 97-2, whereas sub-cluster B comprised of scented local types except Kasturi. Separate grouping of local selections and landraces indicated distinct diversity in their genotypic content than improved varieties.

Cluster III had 24 genotypes that included most of the improved cultivated varieties. The similarity coefficient between any two rice varieties in this cluster ranged from 44 to 85 per cent (Appendix VII). The cluster was further subdivided into several subgroups having varying degrees of similarity. The major sub-group differentiated at similarity coefficient 0.66, comprised of 14 improved varieties, which are of interest because most of them are under cultivation in India (Ratna, Jaya, Indrayani, BPT 5204, RTN 1 etc.). Varieties in this group are dwarf, high yielding and have TN 1 or IR 8 in their ancestry as a source of dwarfing gene. Separate clustering of these genotypes indicated the parental relationship between these genotypes. The other 10 genotypes in the sub-clusters B, C and D were more diverse.

Cluster IV consisted of only two local landraces, which proved to be most unusual genotypes in this collection. These landraces may contain highly unusual combination of alleles and hence did not cluster with genotypes in any of other group in this study.

Cluster V, consisted of nine genotypes with similarity coefficient between any two genotypes in this cluster ranging from 47 to 82 per cent. Further the cluster was subdivided into two sub-clusters, A and B, with 65 and 68.5 per cent similarity,

respectively. The cluster consists of all scented (Basmati and non-Basmati) genotypes.

The grouping identified by 2D scatter plot analysis based on principle co-ordinate analysis (PCoA) of 50 genotypes, reflected the clustering obtained in the dendrogram (Fig 4.7). Cluster I of the dendrogram was found in I quarter (+/+) and located at centre of the 2D scatter plot. Cluster II from the cluster diagram was differentiated by 2nd component of PCoA(Y axis), whereas, cluster III was differentiated by 1st component (X axis). In cluster III, sub-cluster A was densely placed at the centre of III (-/-) quarter indicating narrow diversity among the genotypes, whereas, sub-clusters B, C and D were placed in quarter III (-/-) and IV (-/+). The culture Tulshi tall and Vikram forming cluster IV in cluster diagram were differentiated by 2nd component of PCoA. These two cultures were placed in quarter II (+/-) and III (-/-), respectively. Cluster V from the cluster diagram was differentiated by both 1st and 2nd component of PCoA in 2D scatter diagram and found in quarter II (+/-).

4.2.4 Comparative performance of different methods of diversity studies in rice

In the present investigation 65 rice genotypes were assessed for 12 agro-morphological characteristics to study genetic diversity, whereas, 50 rice genotypes were assessed with ISSR and SSR primers to study molecular diversity. The binary data from polymorphic ISSR and SSR primers was analysed independently as well as pooled to study the genetic diversity among the rice genotypes. The comparative performance of these methods is presented in Table 4.22.

The outcome of three molecular analysis methods showed comparable performance. Wide range of similarity coefficient was observed in all three methods indicating the presence of wide genetic differences among the genotypes under study. The similarity coefficient was highest in ISSR analysis (0.31 to 0.90), whereas the difference between the minimum and maximum similarity index was highest in SSR analysis (0.76). The pooled analysis showed intermediate similarity values as well as difference in minimum and maximum similarity index. Fifty rice genotypes were grouped into three, four and five clusters through ISSR, SSR and pooled analysis, respectively. The independent and separate grouping of improved

varieties was observed in SSR and pooled analysis, whereas they were grouped partly in ISSR analysis.

Table 4.22 Comparison between different methods of diversity studied in rice

S. No.	Particulars	ISSR analysis	SSR analysis	Pooled analysis	Phenotypic analysis
1	Range of similarity coefficient / D^2 value	0.31 - 0.90	0.11 - 0.87	0.25 - 0.85	4.21 – 24015.45
2	Average similarity coefficient/ D^2 value	0.62	0.46	0.53	
3	Number of clusters	3	4	5	10
4	Number of sub-clusters	11	11	11	
5	Independent clustering of improved varieties	Yes (partly)	Yes (clearly)	Yes (mostly)	Yes (partly)
6	Independent clustering of landraces and local selection	Yes (partly)	Yes (fairly)	Yes (mostly)	No
7	Independent clustering of Basmati types	Imperfect	Yes (mostly)	Yes (mostly)	No

The wide range of genetic diversity was also recorded in phenotypic analysis of agro-morphological traits. Sixty-five rice genotypes were grouped into 10 clusters by this method. The grouping of genotypes partly separated improved varieties from other genotypes, however, the independent grouping of landraces and Basmati lines was not observed in this method.

4.3 Experiment III : Genetics of blast resistance in rice

The present investigation was undertaken to identify the gene/QTL and tags using SSR markers for blast resistance in rice. The present study was conducted with the F_2 population developed from the cross between EK 70, a highly susceptible cultivar to blast and RDN 98-2-3-5-14, a blast resistant variety. The results obtained from phenotypic and molecular evaluation of segregating population are discussed below.

4.3.1 Disease reaction in the blast nursery

The F₂ population derived from the cross between EK 70 (susceptible) and RDN 98-2-3-5-14 (resistant) was screened for blast resistance under hot spot condition using standard screening procedures. A total of 150 F₂ plants were randomly selected during early growth stage. The selected plants and parents were examined and scored for infection type as per SES (Anonymous, 2002) for rice blast. Plants with a reaction type score up to 3 were rated as resistant whereas, the plants with a reaction score greater than 3 were rated as susceptible.

The parent RDN 98-2-3-5-14 showed resistance reaction, whereas the parent EK 70 was highly susceptible with typical blast lesions having more than 75 per cent intensity. Among the selected 150 plants, 111 were found to be resistant and 39 susceptible to the pathogen. Genetic analysis was conducted by measuring the goodness of fit to the expected ratio for a single gene model using a Chi-square test. The expected and observed segregation ratios for the F₂ population are shown in Table 4.23. The F₂ population analysis showed a goodness of fit to the expected segregation ratio (3:1) for a single gene model with Chi-square value 0.08 ($P = 0.78$). The result confirmed the hypothesis that resistance reaction in RDN 98-2-3-5-14 to *Magnaporthe grisea* is controlled by a single dominant gene (Table 4.23).

Table 4.23 Phenotypic evaluation of F₂ progenies

S. No.	Category	Observed phenotype	Expected phenotypes (3:1)	χ^2	P
1	Resistant	111	112.5	0.08	0.78
2	Susceptible	39	37.5		
3	Total	150	150		

4.3.2 Molecular marker analysis

4.3.2.1 Parental polymorphism

The resistant (RDN 98-2-3-5-14) and susceptible parent (EK 70) used to develop F₂ mapping population were assessed for polymorphism by SSR primers. Twenty-five SSR markers were tested for amplification quality and polymorphism

among the parents. The sequence information of the primer pairs used for present investigation is presented in Table 3.11. Among these, 14 primers (RM259, RM213, RM11, RM215, RM44, RM25, RM210, RM224, RM4, RM209, RM21, RM204, RM241 and RM250) were found to be polymorphic between two parents.

4.3.2.2 Bulk segregant analysis

Bulk segregant analysis was carried out for rapid identification of markers associated with blast resistance. Based on morphological observations, two bulks; susceptible (B1) and resistant (B2) were generated from F_2 population. Susceptible bulk (B1) comprised of five most susceptible F_2 plants (53, 73, 81, 108 and 139) and resistant bulk (B2) comprised of five most resistant F_2 plants (22, 76, 103, 130 and 147). A pooled DNA sample was prepared for each bulk by mixing of an equal quantity DNA of five respective component F_2 s. The parents and the bulks were screened with 14 SSR markers to identify the polymorphic markers associated with resistance to blast. Among these, two markers viz., RM204 and RM215 were polymorphic between susceptible and resistant parents and their respective bulks (Plate VII). These two primers were used for further analysis of individual F_2 plants.

4.3.2.3 Co-segregation of SSR marker with blast resistance

The two SSR markers, RM204 and RM215 showing polymorphism in parents and bulks were analysed for their co-segregation in F_2 mapping population consisting of 150 F_2 plants.

4.2.3.3.1 Co-segregation of SSR marker RM204

The molecular analysis of susceptible and resistant parents and 150 F_2 plants with SSR marker RM204 showed a fragment of ~174 bp amplified from the resistant plants that was absent in susceptible plants, whereas a fragment of ~110 bp was present in susceptible plants and absent in resistant plants. Both these fragments were present in heterozygous resistant plants. The data for the polymorphism was recorded as '0' for susceptible, '1' for resistant and '2' for heterozygote. About 33 F_2 plants had resistant fragment, 41 had susceptible fragment, whereas 73 F_2 plants showed both the fragments (Table 4.24, Plate VIII).

Genetic analysis was conducted by measuring the goodness of fit to the expected ratio for a single model using a Chi-square test. The marker analysis showed a goodness of fit to the expected segregation ratio (1:2:1) for a single gene model with Chi-square value 0.88 ($P = 0.65$).

Table 4.24 Molecular evaluation of F_2 progenies with SSR marker RM204

S. No.	Category	Observed genotype	Expected genotypes (1:2:1)	χ^2	P
1	Resistant	33	36.75	0.88	0.65
2	Heterozygote	73	73.5		
3	Susceptible	41	36.75		
4	Total	147			

Simple regression analysis was conducted to study the association between the molecular marker RM204 with phenotypic data. The marker RM204 was found to be significantly linked with the phenotypic data for disease resistance ($P < 0.010$, Table 4.25).

Table 4.25 ANOVA for regression analysis for SSR marker RM204 with phenotypic data

S. No.	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	P
1	Regression	1	6.50	6.50	8.61**	0.0038
2	Residual	148	111.79	0.76		
3	Total	149	118.29			

4.2.3.3.2 Segregation of SSR marker RM215

The molecular analysis of susceptible and resistant parents and 150 F_2 plants with SSR marker RM215 showed a fragment of ~150 bp in the resistant parent, whereas a fragment of ~310 bp was present in susceptible plants in addition to ~150 bp fragment. The data for the polymorphism was recorded as '0' for susceptible and '1' for resistant. The molecular expression of heterozygous resistant

plants was similar to that of susceptible plants. Forty-one F_2 plants reported resistant fragment, whereas 107 having susceptible fragment. Genetic analysis was conducted by measuring the goodness of fit to the expected ratio for a single model using a Chi-square test. The marker analysis did not show a good fit to the expected segregation ratio (1:2:1) for a single gene model with Chi-square test. Also, on regression analysis this marker was not found to be associated with blast resistance. Hence, this marker, although was polymorphic in bulk segregants analysis, was not associated with blast resistance in present investigation.

CHAPTER V

DISCUSSION

Rice (*Oryza sativa* L.) is one of the most important cereal crop of the world after wheat. It is the staple food of East, South-East and South Asia, where 90 per cent of the world's rice crop is produced and consumed. Rice is the most important crop of India ranking first area wise in the world, while second in production after China. Although several high yielding varieties and hybrids have been evolved in India during the past few decades the desired increase in production could not be achieved due to various biotic and abiotic factors. Rice production has multifarious problems and the production potential is often limited due to its susceptibility to pests and diseases. Among the important diseases of rice, blast caused by *Magnaporthe grisea* is one of the most devastating and destructive diseases in India causing substantial losses in grain yield. Susceptibility to blast is widespread among commercial cultivars of rice, underscoring the need for improving resistance. Development of resistant genotypes is the long-term strategy to combat this disease. However, to do so more efficiently durable sources of resistance and their inheritance mechanism needs to be elucidated.

Improvement in grain yield per unit area coupled with tolerance to biotic and abiotic stresses are the prime objectives in any breeding programme. It depends on the presence of genetic variability in the available material. The utility of such material in the germplasm could be judged on the basis of the extent of variability available in the material. Majority of economically important characters including grain yield, grain quality traits and disease and pest resistance are amenable for genetic improvement through *inter se* breeding among genetically diverse parents. A systematic breeding programme involves the steps like creating genetic variability, practicing selection and utilization of selected genotypes to evolve promising varieties. The large spectrum of genetic variability and genetic advance among combining genotypes offers a better scope for selection. Estimates of heritability and genetic advance will play an important role in exploiting future research programme of rice improvement. Murthy and Arunachalam (1966) stated that multivariate analysis with "Mahalanobis D^2 statistic" is a powerful tool to know

the clustering pattern to establish the relationship between genetic and geographical divergence and to determine the role of different quantitative characters towards the maximum divergence.

Assessment of genetic diversity in plant species with field evaluation has influence of environmental factors and developmental stage of the crop. Hence the results elucidated based on such studies have limitations of the accuracy of genetic diversity. Molecular approach overcomes these limitations and gives accurate and true measure of the diversity in the material under study.

The molecular markers provide a new alternative selection approaches to enhance genetic improvement in yield and other important traits in rice. Identification of molecular markers linked to genomic regions or QTLs and their proper utilization might enable the breeders to establish marker-assisted selection programme. DNA markers have proved their importance for gene tagging and are very useful in locating and manipulating QTL in a number of crops.

In view of this the present investigation entitled “Genetic and molecular analysis of blast resistance in rice (*Oryza sativa* L.)” was conducted to assess genetic diversity in 65 rice genotypes for agro-morphological and pathological traits, assessing molecular diversity in selected genotypes and to identify QTLs and molecular markers linked to blast resistance in rice. The information obtained on field and molecular variability parameters, divergence studies and genetics of blast resistance are discussed below.

5.1 Genetic variability for agro-morphological and pathological characters

5.1.1 Genetic variability for agro-morphological characters

The extent of genetic variability available in a crop is prerequisite for its improvement. In the present investigation, 65 rice genotypes were evaluated at two locations (ARS, Radhanagari and ARS, Lonavala) to assess the genetic variability for 12 agro-morphological traits during *kharif* 2010.

The analysis of variance revealed highly significant differences for all the 12 characters studied indicating significant variability among the genotypes under study for phenotypic expression. Significant differences among genotypes for

different traits were also reported by Amirthadevarathinam (1990), Reddy *et al.* (1995), Reddy and De (1996), Borbora and Hazarika (1998), Vange *et al.* (1999), Naik *et al.* (2002), Pandey and Awasthi (2002), Vivek *et al.* (2004), Singh *et al.* (2005) and Raut *et al.* (2009b).

Considerable amount of variation was observed for all the characters in the present study at both the locations as well as in pooled performance. In plant breeding programmes, wide range of variation among the genotypes paves the way to bring a desirable improvement in the crops. The present material, therefore, could serve a pool for selection of suitable material in breeding programmes. The significant variation for morphological characters was also reported by Vange *et al.* (1999) and Raut *et al.* (2009b).

Days to 50 per cent flowering showed wide range of variation at both the locations showing the range from 61.00 to 120.50 days at location I and 63.50 to 129.00 days at location II. The results are in conformity with the reports of Naik *et al.* (2002), Pandey and Awasthi (2002), Kumari *et al.* (2003), Mall *et al.* (2005) and Vaithiyalingan and Nadarajan (2006) who also noticed considerable variation for 50 per cent flowering. The genotype Heera was found earliest at both the locations followed by Halvi Sal 17 and EK 70. The genotype Antersal was very late in flowering at both locations followed by Sonsali.

The maturity of the genotypes showed similar trend to that of 50 per cent flowering. The duration of maturity in the genotypes varied from 86.00 to 150.50 days at location I and 86.00 to 160.00 days at location II. The genotype Heera was earliest in maturity followed by Halvi Sal 17 and EK 70, whereas the local type Antersal was very late in maturity followed by Sonsali.

Based on flowering and maturity duration, the genotypes were grouped into three categories as early, midlate and late with 50 per cent flowering up to 95 days, between 95 to 105 days and above 105 days, respectively. At location I, eight genotypes were early, 21 were midlate and 36 were late, whereas, at location II 10, 17 and 38 genotypes were early, midlate and late, respectively. The pooled performance indicated eight genotypes from early group, 22 from midlate group and 35 genotypes from late group. The genotypes had similar performance at both the locations indicating very little influence of locations.

The considerable amount of variation was observed for plant height, which ranged between 67.75 and 156.90 cm at location I and 61.95 and 162.60 cm at location II. Significant variation for plant height was also reported by Chauhan and Tandon (1984), Naik *et al.* (2002), Pandey and Awasthi (2002), Kumari *et al.* (2003), and Mall *et al.* (2005). The genotype RDN 02-80 was found to be dwarfest at location I followed by Heera and RTN purple. While Heera was found to be dwarfest at the location II followed by RDN 02-80 and BPT 5204. The landrace Kunchi was tallest at location I followed by the landrace Nalabhat and Sonsali, whereas at location II another landrace Ajara Local 3 was found tallest followed by Patni and Kunchi. Most of the landraces and local selections in present investigation exhibited taller plant type, whereas the improved varieties had dwarf plant structure. This may be due to the presence of dwarfing gene in improved varieties developed after green revolution. Two local landraces viz., Khalibagh and Jagatpuri showed extreme dwarfness having height of 83.03 and 82.63 cm, respectively. These genotypes may have different genetic combinations and can be used as dwarfing source for further breeding programme.

The performance of the genotypes for panicle length showed little variation over two locations. The pooled performance of the genotypes over two locations reported the panicle length from 17.00 to 27.55 cm. These results are in agreement with the work of Amirthadevarathinam (1990), Mall *et al.* (2005) and Vaithiyalingan and Nadarajan (2006) who also reported high genotypic variation for panicle length. The local types IGT 13857, Ajara Local 3, Pinjarwadi Local, Sonsali, Nalabhat, Ghansal, Ambemohar 157 and Antersal had long panicles measuring 27.55, 27.33, 26.50, 26.35, 26.30, 26.25, 26.10 and 25.50 cm, respectively that are useful for the improvement of panicle length.

The number of grains per panicle is one of the most important traits related to grain yield in rice. Wide variation for this trait was observed in the present study. The number of spikelets per panicle ranged from 77.00 to 232.10 at location I, 76.40 to 246.20 at location II with 76.70 to 239.10 pooled mean over locations, indicating the little influence of location. The genotype Ambemohar recorded highest number of spikelets per panicle (239.10) followed by Badshabhog (234.20),

whereas Heera recorded least number of spikelets (76.70) followed by Basmati 370 (110.05) and Halvi Sal 17 (111.70). The genotypes with long panicle with short grain size had higher number of spikelets per panicle. High variability for this trait was also reported by Amirthadevarathinam (1990), Reddy *et al.* (1995), Reddy and De (1996), Yadav *et al.* (2000), Bidhan *et al.* (2001), Nayak *et al.* (2002), Pandey and Awasthi (2002), Singh *et al.* (2002), Kumari *et al.* (2003), Mall *et al.* (2005), Patil and Sarawgi (2005), Vaithiyalingan and Nadarajan (2006) and Raut *et al.* (2009b).

The pooled range of spikelet fertility (87.66 to 96.77 %) showed narrow variation over two locations. All the genotypes recorded high fertility percentage at both the locations, except Basmati line SD 7 at location I (84.40 %). The genotypes Sonsali (96.43 %), and Kalajirga (96.33 %) reported highest fertility percentage indicating their usefulness in further breeding programme.

Significant differences were observed for number of tillers per plant at both the locations and thereby in pooled performance over two locations. It ranged from 5.40 (RDN 185-2) to 10.00 (IGT 13857) for location I and 5.40 (Halvi Sal 17 and MC 4) to 9.60 (Champakali) at location II. These findings are in consonance with the reports of Reddy *et al.* (1995), Panday and Awasthi (2002), Kumari *et al.* (2003), Vivek *et al.* (2004), Mall *et al.* (2005), Vaithiyalingan and Nadarajan (2006) and Raut *et al.* (2009b) who also noticed significant differences for tillers per plant. The pooled performance over two locations indicated that the genotypes Champakali, IGT 13857 and Pavsai produced more than nine tillers per plant. Further, RDN 2-80, Ajara Local 3, EK70 and RTN purple reported more than 8.50 tillers per plant. These genotypes can be used in future breeding programme for improvement in tiller number per plant.

Different genotypes showed substantial variation in test weight. It ranged from 9.51 to 33.70 g at location I and 9.41 to 33.71 g at location II. The genotype Diwani reported lowest test weight (9.51 and 9.41 g) at both the locations followed by Shyamjeer (9.93 and 9.75 g) and Badshabhog (11.10 and 11.14 g), whereas the genotype Patni had highest test weight (33.30 and 33.71 g) at both the locations followed by Vivek Dhan 82 (31.09 and 31.02 g) and Siddhagiri (30.13 and 30.10 g).

This trait showed very little variation among the genotypes over different locations. Among the genotypes studied, two genotypes (*viz.*, Diwani and Shyamjeer) had test weight below 10 g and three genotypes (Patni, Vivek Dhan 82 and RDN 97-2) recorded test weight more than 30 g showing their usefulness for improvement in the trait in respective directions. Reddy *et al.* (1995), Borbora and Hazarika (1998), Vange *et al.* (1999), Naik *et al.* (2002), Singh *et al.* (2002), Kumari *et al.* (2003), Patil and Sarawgi (2005) and Kumar *et al.* (2007) also reported wide variation for test weight.

Significant differences were observed for grain quality parameters *viz.*, grain length, grain breadth and Length : Breadth ratio in the genotypes studied. The expression of the trait among the genotypes over different locations was almost similar indicating very little influence of the location. The pooled performance over two locations exhibited wide range of grain length. The genotype Khalibagh had longest grains (8.05 mm) followed by SD 17 (7.94 mm) and Pusa Basmati 1 (7.75 mm), on the other hands Kothimbire recorded shortest grain (3.88 mm) followed by Diwani (4.34 mm) and Badshabhog (4.34 mm). As regards to grain breadth, Phule Radha and Khalibagh exhibited finest grains (1.60 mm) followed by Pusa Basmati 1 and Sugandhamati (1.70 mm), whereas Halvi Sal 17 (2.92 mm) had thick grain followed by RDN 185-2 (2.85 mm) and Siddhagiri (2.75 mm). Length : breadth ratio of the grain varied from 1.44 to 5.05. The genotype Khalibagh showed highest L:B ratio (5.05) followed by Pusa Basmati 1 (4.65) and SD 17 (4.56). The foregoing results tally with the work of Marekar and Siddique (1997), Pandey and Awasthi (2002), Vanaja and Babu (2006) and Raut *et al.* (2009b) who also observed significant variability in grain quality parameters.

The differences within genotypes due to seed yield per plant were highly significant. It ranged between 8.00 to 23.58 g with the average of 16.67 g per plant at location I, and between 5.13 and 21.26 g with the average of 15.47 g at location II. The pooled results revealed that 11 genotypes had significantly higher grain yield than the population mean (16.07 g). The genotypes RDN 97-2, Siddhagiri, Bhogavati, Pawana, Phule Samruddhi, RDN 99-12, Jaya and RDN 99-14 produced more than 19.00 g seed yield per plant. Chauhan and Tandon (1984), Reddy *et al.*

(1995), Reddy and De (1996), Borbora and Hazarika (1998), Vange *et al.* (1999), Yadav *et al.* (2000), Bidhan *et al.* (2001), Roy *et al.* (2001), Nayak *et al.* (2002), Pandey and Awasthi (2002), Singh *et al.* (2002), Kumari *et al.* (2003), Vivek *et al.* (2004), Patil and Sarawgi (2005), Vaithiyalingan and Nadarajan (2006), Vanaja and Babu (2006), Kumar *et al.* (2007) and Raut *et al.* (2009b) observed wide variation for seed yield per plant, which is in conformity with the present findings.

5.1.1.1 Genotypic and phenotypic coefficient of variation

The estimation of genetic (heritable) and environmental (non-heritable) components of the total variability facilitate the breeder to choose the desired breeding technique. Johanssen (1909), Nilson Ehle (1909) and East (1916) separated genetic variance from total variance by using environmental variance. Now, it is an accepted fact in all the biometrical studies as it helps in arriving at precise conclusions about the true breeding value of the genotype. Thus, in the present study the components of variation such as genotypic and phenotypic coefficient of variation were calculated with heritability, predicted genetic advance and genetic advance as percentage of mean in respect of 12 agro-morphological characters.

The magnitude of phenotypic coefficient of variation was high as compared to the genotypic coefficient of variation for all the characters studied indicating the important role of environmental variance in expression of different traits in rice. The results obtained by Kumar *et al.* (2001) and Raut *et al.* (2009b) were similar to present findings.

Genotypic and phenotypic coefficients of variation were high for grain length : breadth ratio, test weight, plant height, spikelets per panicle, grain length, grain breadth and seed yield per panicle. The genetic variability in different quantitative characters is measured from the genotypic coefficient of variation (Fisher, 1930). In the present investigation the characters L:B ratio, test weight, plant height, spikelets per panicle, grain length and seed yield per panicle showed high genotypic coefficient of variation indicating good scope for their improvement by selection. These results are in agreement with the reports of Reddy *et al.* (1995), Marekar and Siddique (1997), Singh *et al.* (2002) and Raut *et al.* (2009b) where they

reported similar findings. The magnitude of GCV and PCV were low for days to 50 per cent flowering, days to maturity, panicle length, spikelet fertility and number of tillers per plant. The results were in conformity with those observed by Chauhan and Tandon (1984), Naik *et al.* (2002) and Raut *et al.* (2009b).

The difference between magnitude of genotypic and phenotypic coefficient of variation showed very low difference for all the characters studied, indicating good correspondence between genotypic and phenotypic expression of traits with low level of environmental factors operating on the traits. It was maximum for grain breadth (3.04) followed by seed yield per plant (2.07), tillers per plant (1.78) and spikelets per panicle (1.56), whereas least for test weight (0.007). Verma (2000) also observed relatively low magnitude of difference between GCV and PCV in rice.

5.1.1.2 Heritability (b.s.) and genetic advance

The coefficient of variation reveals the extent of variability present for different characters and it does not indicate the heritable portion. To obtain the knowledge of heritable portion of the variability, it is essential to know the heritability estimates of different attributes.

In the present investigation, all the characters showed high broad sense heritability ranging from 66 per cent for spikelet fertility to 99 per cent for test weight and grain length. High heritability for all the characters studied were also reported by Reddy and De (1996), Bidan *et al.* (2001), Roy *et al.* (2001), Naik *et al.* (2002) and Raut *et al.* (2009b).

High heritability estimates indicates the effectiveness of selection based on phenotypic performance, but does not necessarily mean a high genetic gain for that particular trait. The character with high genetic gain may be attributed to the additive gene effect, which can easily be improved by simple selection (Panse, 1957). On the other hand, high heritability and low genetic advance may be attributed to non-additive gene action and such character may be improved by hybridization.

Heritability estimates in broad sense do not serve as true indicator of the genetic potential of the genotype, since their scope is restricted by their interaction with the environment. As such, the heritability in broad sense includes both additive and non additive gene effect (Hanson *et al.*, 1956). It is advisable to consider the predicted genetic advance along with heritability estimates as a tool in the selection programme for better efficiency in selection. Johnson *et al.* (1955) suggested that the genetic gain will be low when there is non-additive gene action, whereas the genetic advance would be high when there is additive gene action.

In the present investigation high heritability coupled with high genetic advance were observed for spikelets per panicle, plant height, days to maturity and days to 50 per cent flowering. Direct selection for these traits would be effective as heritability and genetic advance might be due to additive gene interaction. Genetic advance for grain yield per plant was low indicating the likelihood of predominance of non additive gene interaction. However, when expressed as percentage of mean, it was fairly high. Verma *et al.* (2000), Kumari *et al.* (2003), Mall *et al.* (2005), Singh *et al.* (2006) and Raut *et al.* (2009b) also reported similar results.

The studies on the genetic parameters for agro-morphological traits indicate that the character grains per panicle needs due attention in the selection programme because it had higher PCV and GCV with less difference between them, very high heritability coupled with high genetic advance and high range of variability to be exploited in the rice improvement programme.

5.1.1.3 Genetic divergence for agro-morphological traits

The important objective in obtaining biometrical measurements is to understand the possibilities of classifying individual genotypes into different groups. The most important and difficult task in initiation of hybridization programme is the selection of genotypes with high *per se* performance among them. Genetic variability estimates offers the possibility of identifying desirable genotypes but unless we have better knowledge about divergence between them it is difficult to expect any extraordinary results from their progenies.

D^2 statistics, a concept developed by Mahalanobis (1936) is important tool to plant breeder to classify the genotypes into different groups based on genetic

divergence between them. It is useful in quantifying degree of divergence between biological populations at genotypic level and assessing the relative contribution of different component traits to the total divergence at both intra and inter-cluster level. Rao (1952) suggested the application of this technique for the assessment of genetic diversity in plant breeding.

5.1.1.3.1 Cluster formation, intra and inter-cluster distances and mean performance

The cluster formation and estimation of intra and inter-cluster divergence provides a basis for selecting genetically diverse parents belonging to different clusters. It is assumed that the statistical distance (D) is the index of genetic diversity. The crossing between strains of widely separated clusters with high inter-cluster diversity appears to be very effective.

In the present study, D^2 values between all possible pairs (2080) of 65 genotypes ranged between 4.21 (Phule Samruddhi and RDN 99-12) and 24015.45 (Patni and Diwani). The high range for D^2 values indicated the presence of great amount of diversity in rice genotypes under study.

Clustering of genotypes following the Tocher's method as described by Rao (1952) led to formation of 10 clusters in the present study. The cluster I included 22 genotypes and was the largest followed by cluster III (14 genotypes), cluster IV (12 genotypes), cluster II (7 genotypes), cluster VIII (4 genotypes) and cluster IX (2 genotypes). The clusters V, VI, VII and X had single genotype.

The intra and inter-cluster D^2 and D values worked out using D^2 values from divergence analysis showed maximum intra cluster distance in the cluster VIII ($D^2 = 457.10$) followed by cluster IV ($D^2 = 350.06$) and cluster III ($D^2 = 282.58$). This indicated that the genotypes in these clusters might have different genetic architecture. The minimum intra-cluster distance was found in cluster IX ($D^2 = 111.94$) followed by cluster I ($D^2 = 191.55$) and cluster II ($D^2 = 256.64$) indicating that the strains of these clusters genetically resemble one another and might have evolved from common gene pool. The clusters V, VI, VII and X with single genotype had no intra-cluster distance.

The maximum inter-cluster distance was observed between cluster VIII and IX ($D^2 = 19165.63$) followed by cluster II and VIII ($D^2 = 13656.26$), cluster III and IX ($D^2 = 12624.77$) and cluster VII and IX ($D^2 = 11664.00$) revealing a wide range of divergence among these clusters. This also suggested that the genetic architecture of the genotypes of one cluster differed significantly from those included in other clusters. Genotypes of distinct clusters separated by high genetic distances could be utilized in breeding programme for obtaining a wide range of variability in the segregating generation. Results of present investigation suggested a possibility for obtaining greater variation in the segregating generations derived from hybridization between genotypes of cluster II, VIII, IX and X. The inter-cluster distances between the cluster V and X ($D^2 = 184.69$), cluster I and V ($D^2 = 377.53$), cluster I and VI ($D^2 = 509.41$) and cluster V and VII ($D^2 = 554.60$) were minimum suggesting genetic relatedness among the genotypes of the concerned clusters.

Cluster means for 12 characters revealed wide range of variability among the clusters for plant height, days to 50 per cent flowering, days to maturity, spikelets per panicle, tillers per plant, test weight, grain length, grain breadth and L:B ratio. These findings are in agreement of the work did by Gahalain (2006), Raut *et al.* (2009a) and Rajesh *et al.* (2010) who also reported wide variability among clusters for yield and most of the yield contributing characters.

The mean performance of the clusters indicated that cluster I with largest numbers of genotypes, mainly comprised of improved varieties having dwarf plant type with long-slender grain type and medium test weight. This group is of interest because of prevailing cultivated scented Basmati (Basmati 370, Taroari Basmati) and non-Basmati (Indrayani, Bhogavati, Phule Samruddhi) varieties.

Cluster II consisted of seven genotypes was characterized by short-slender or short-bold grained genotypes with long panicle length and higher number of spikelets. The widely cultivated fine grain variety BPT 5204 and Phule Radha were grouped in this cluster. In contrast, cluster III consisted of medium-slender or long-bold genotypes with medium grain length, broader grain width and high test weight, whereas the four genotypes with very bold grains having low length : breadth ratio and high grain yield per plant were grouped in the cluster VIII.

The cluster IX comprised of two landraces, one each from Uttar Pradesh (Diwani) and Bihar (Shyamjeer), which had short-slender grains with very low test weight (9.65 gm) grouping them separately from other genotypes. These landraces can be used for breeding very fine grain varieties.

The clusters V, VI, VII and X with a single genotype each represented unique genotypic combinations. The extra long, fine grain genotypes Pusa Basmati 1, SD 17 and Khalibagh grouped separately in cluster V, VII and X, respectively and showed a distinct expression for plant type, duration and test weight. The genotype Pavsai grouped in cluster VI represented a tall plant type with very late maturity and short bold grains. These clusters with single genotypes represented unique allelic combinations with independent evolutionary history that distinguished them from most other varieties and can be used as diverse sources in future breeding programme.

5.1.1.3.2 Relative contribution of characters towards total divergence

The variance of cluster means provides the information on relative importance of characters towards the divergence. The per cent contribution of the twelve characters studied towards total divergence indicated that the test weight contributed to a major part of divergence (79.04 %) followed by grain length (12.93 %) and length : breadth ratio (5.91 %). Higher contribution of test weight and length : breadth ratio was also reported by Bharadwaj *et al.* (2001) and Rather *et al.* (2001). Similarly, Senapati and Sarkar (2005), Reddy *et al.* (2006), Chandra *et al.* (2007) and Raut *et al.* (2009a) also reported test weight as major contributor in total divergence of rice genotypes. In addition, Kole (2000), Roy *et al.* (2002) and Nayak *et al.* (2004) reported grain length contributing major part in total divergence. The major contribution of test weight, grain length and length : breadth ratio to the total divergence suggested that these traits deserve due consideration while choosing parents for breeding programme. The contribution of days to 50 per cent flowering, days to maturity, plant height, panicle length, spikelets per panicle, spikelet fertility, tillers per plant, grain breadth and seed yield per plant to total divergence was much less in present investigation. The fore cited results are in consonance with the reports of Nayak *et al.* (2004) and Raut *et al.* (2009a) wherein they also reported

lower contribution of one or more of these traits to total divergence. On the contrary, Awasthi *et al.* (2005), Devi *et al.* (2006), Gahalain (2006) and Rajesh *et al.* (2010) reported major contribution of grain yield, days to flowering, days to maturity, number of spikelets per panicle towards total divergence. Thus, some discrepancies in the characters contributing to divergence were observed in the present investigation and results of the previous workers. Such discrepancies in the results might be due to the different sets of plant material and also due to environmental variability.

5.1.2 Genetic diversity for pathological characters

Rice blast caused by *Magnaporthe grisea* (Hebert) is one of the most devastating and destructive diseases of rice in India. It is a well known rice disease occurring in every region of the world where the crop is grown. Since last several years, plant breeding has achieved significant progress towards the enhancement of host resistance to blast but the development of durable resistant cultivar is yet to be realized. The use of resistant cultivars is a powerful tool to reduce the use of environmentally destructive pesticides. Using classical plant breeding techniques, plant breeders have developed a number of blast resistant cultivars adapted to different rice growing regions worldwide. However, the rice industry remains threatened by blast disease because of the variability of the rice blast fungus.

Good amount of variability has been reported in rice for various yield and blast resistance traits. Indian rice varieties are evolved through traditional selection, hybridization and back crossing with locally adapted high yielding lines. With the advent of modern cultivars, farmers are replacing the traditional landrace cultivars. Such landraces could be potential genetic resources, with respect to resistance to blast disease causing significant losses in rice growing countries (Yang *et al.*, 2009).

Genetic improvement in any crop relies on the amount of genetic diversity present in different genotypes. The estimation of genetic diversity in different genotypes is therefore first and foremost process in plant breeding (Rajesh *et al.*, 2010). In view of this fact, basic work on breeding resistance and screening of

different genotypes at hot spot is the long term solution to avoid the outbreak and endemic spread of blast disease. Identification of genetically diverse accessions with desired genes is essential for better utilization in crop breeding programme. Hence the present study was undertaken to evaluate 65 rice genotypes for genetic divergence for blast disease. These selected genotypes were evaluated in randomised block design with two replications in Uniform Blast Nursery at Agricultural Research Station, Lonavala for five pathological traits related to blast disease. The results obtained are discussed below.

5.1.2.1 Variability, heritability and genetic advance

The mean performance of rice genotypes for five pathological characters of blast resistance indicated wide variation for all the pathological traits. The variation in the infection type (score) among the genotypes was recorded from 1.0 to 9.0 with the mean of 5.4. None of the genotypes reported immune reaction (0 score). Three genotypes viz., RDN 97-2, LK 248 and RDN 02-80 reported resistant reaction with infection type of 1. Further, the genotype RDN 98-2-3-5-14 showed infection score of 2 as per SES. Thirteen genotypes recorded the infection type up to 3 indicating that these genotypes have allelic combinations showing resistant or moderately resistant reaction to blast.

The character diseased leaf area had wide range from 0.41 to 81.71 per cent. Three genotypes (LK 248, RDN 02-80 and RDN 97-2) showed diseased leaf area below 2 per cent and 14 genotypes recorded less than 5 per cent leaf area affected by the disease indicating the ability of these genotypes to resist the development of the blast.

Density of the sporulating blast lesions on the genotypes varied from 0.00 to 5.77 per cm². Fourteen genotypes showed lesion density of zero, indicating that these genotypes had only non-sporulating lesions thus having genetic potential for blast resistance.

The size of blast lesions varied from 0.20 to 16.83 mm² in the 65 rice genotypes under study. Twenty-two genotypes showed significantly lower lesion size than population mean and among them 13 genotypes had the least (0.20 mm²) lesion size.

Sixty-five genotypes showed wide variation for disease intensity that ranged between 11.11 and 98.42 per cent. Twenty-three genotypes recorded significantly lower PDI than the population mean of 57.01 per cent. The genotypes RDN 97-2 and LK 248 (11.11 %) showed least PDI followed by RDN 2-80 (11.91 %) and RDN 98-2 (16.14 %).

The foregoing results are in conformity with the work did by Moumeni and Leaung (2003), Wu *et al.* (2004), Telebanco-Yanoria *et al.* (2008) and Ghaley *et al.* (2012) who also reported significant variation in different rice genotypes for various blast resistance characters.

The mean sum of squares in the genotypes was found highly significant for all pathological characters. This indicated the presence of significant differences in the genotypes for all the pathological traits. The present material therefore, could serve as a pool for selection of suitable parents for use in breeding programme.

In the present study, the magnitude of phenotypic coefficient of variation was high as compared to the genotypic coefficient of variation for all the characters. This revealed the importance of environmental variance in expression of different traits of blast resistance in rice.

Genotypic and phenotypic coefficients of variation were high for all the pathological characters. High genotypic coefficient of variation indicated good scope for the improvement of these pathological traits by selection.

The difference between magnitude of genotypic and phenotypic coefficient of variation was very low for all the characters under study, indicating a low level of environmental factors operating on the traits. It was maximum for lesion size (2.25) followed by infection type (1.76) while it was least for per cent disease intensity (0.44).

The proportion of genetic variability that is transmitted from parent to progeny is reflected in heritability (Lush, 1949). The highest heritability was observed for the character diseased leaf area (98.78 %) followed by per cent disease intensity (97.85 %) while it was lowest for infection type (91.59 %).

As heritability in broad sense includes both additive and non-additive gene effects, heritability estimates should be considered in conjunction with genetic advance (Johnson *et al.*, 1955). In the present investigation, per cent disease intensity (46.78) reported highest genetic advance. Direct selection based on this trait would be effective as heritability and genetic advance might be due to additive gene interaction.

5.1.2.2 Cluster formation, intra and inter-cluster distances and mean performance

In the present investigation, D^2 values corresponding to the pair-wise comparison between the genotypes ranged from 0.03 to 1496.95. The lowest D^2 value (0.03) was observed between the genotypes RDN 97-2 and RDN 02-80, whereas highest D^2 value (1496.95) was observed between the genotypes EK 70 and LK 248. The high range for D^2 values indicated the presence of great amount of diversity in rice genotypes under study.

Genetic divergence measured as per Mahalanobis D^2 statistic, grouped 65 genotypes of rice into seven clusters following Tocher's method as described by Rao (1952). Cluster I had highest 27 genotypes followed by cluster II (26 genotypes) and cluster IV (8 genotypes). The clusters III, V, VI and VII had single genotype each. Grouping of genotypes into seven clusters suggested the presence of moderate to high genetic diversity for pathological traits in the material under investigation.

Intra and inter-cluster D^2 and D values worked out using D^2 values from divergence analysis showed maximum inter-cluster distance between the cluster I and VII ($D^2 = 1273.78$) followed by cluster VI and VII ($D^2 = 1094.29$), cluster II and VII ($D^2 = 882.09$) and cluster V and VII ($D^2 = 634.03$) suggesting that the genotypes included in these clusters might have different genetic architecture. The minimum inter-cluster distance was observed between cluster III and V ($D^2 = 35.28$) followed by cluster II and V ($D^2 = 68.23$), cluster II and III ($D^2 = 73.45$) and cluster III and IV ($D^2 = 74.30$). The lower inter-cluster distances between these clusters suggest the close genetic proximity of between the genotypes of these clusters.

The intra-cluster distance in the clusters ranged from 32.38 (cluster II) to 37.95 (cluster IV). The clusters III, V, VI and VII with single genotype had intra-cluster distance of zero. Lower intra-cluster distances in present investigation reflect the genotypic relatedness between the genotypes within the cluster.

The cluster means for five characters showed wide range of variability among the clusters for all the pathological characters *viz.*, infection type (score), diseased leaf area, lesion density, lesion size and per cent disease intensity (PDI).

Cluster I with 27 genotypes reported moderately resistant mean performance for infection type (3.41), diseased leaf area (36.21 %), lesion density (2.08 per cm²), lesion size (0.24 mm²) and PDI (5.29 %). The genotypes from this cluster were mostly resistant or moderately resistant to blast. These genotypes may contain one or more genes for blast resistance and can be used as sources of resistance in future breeding programme.

Cluster II, containing 26 genotypes exhibited moderately susceptible to susceptible reaction to blast with moderate to high mean performance for infection type (6.29), diseased leaf area (65.23 %), lesion density (6.94 per cm²), lesion size (1.76 mm²) and per cent disease intensity (13.52 %). The genotypes from this cluster were moderately susceptible to susceptible for blast. The widely adapted popular genotypes with quality attributes from this cluster (Phule Radha, Taroari Basmati, BPT 5204, Jaya etc.) can be used in breeding programme for further improvement.

Cluster III, IV, V and VII recorded higher mean performance for all the observations for blast indicating the genotypes from these clusters were susceptible to highly susceptible for blast. Cluster VI, having single genotype RDN 99-12 recorded moderately resistant reaction of infection type (3.00), very low lesion density (0.20 per cm²) and non-sporulating lesions. However, this genotype had higher lesion density (26.19 %) and PDI (20.28 %). This genotype may contain one or few resistant genes and can be used as diverse source in breeding programme for blast resistance.

In the present investigation, the resistant and moderately resistant cultivars were grouped in cluster I and VI, moderately susceptible and susceptible cultivars

into cluster II and highly susceptible cultivars into cluster III, IV, V and VII. These findings are in agreement with the reports of Telebanco-Yanoria *et al.* (2008) wherein they did separate grouping of resistant and susceptible genotypes into different clusters. The inter-cluster distances revealed the variation in expression of blast resistant characteristics between the genotypes from different clusters. The inter-cluster distances were maximum between the resistant and susceptible clusters like cluster I and VII ($D^2 = 1273.78$) and VI and VII ($D^2 = 1094.29$), whereas it was least between the two susceptible clusters *i.e.* cluster III and V, II and III and III and IV ($D^2 = 35.28, 73.45$ and 74.30 , respectively) and two resistant clusters (clusters I and VI; $D^2 = 82.81$).

Genotypes of distinct clusters separated by high genetic distances could be utilized in breeding programme for obtaining a wide range of variability in the segregating generation. The breeding lines from cluster I (RDN 97-2, RDN 02-80) exhibiting resistance to blast may serve as good donors for blast resistance. The improved varieties from Cluster I (KJT 2, Improved Pusa Basmati 1, Bhogavati and Indrayani) showing moderately resistant reaction to blast and having better agronomic features can be further utilized in breeding programme. The improved line RDN 99-12 reported moderately resistant reaction and clustered separately indicates the unique combinations of alleles in it and can be used for further breeding programme.

The per cent contribution of the five characters studied towards total divergence indicated that the PDI had contributed maximum (35.24 %) towards total divergence followed by lesion density (32.88 %) and diseased leaf area (21.15 %). Infection type contributed least (0.39 %) to the divergence followed by lesion size (10.34 %). This suggested that PDI, lesion density and diseased leaf area should be given due consideration while choosing parents for breeding programme for blast resistance in rice.

The present investigation has enabled the identification of six landraces (Nalabhat, Juhibengal, Vikram, Patni, Pomendi Local and Pinjarwadi Local) and three local selections (LK 248, Kalajirga and Haryana Basmati) as resistant or

moderately resistant to the blast pathogen. Since these cultivars originate from different geographical and cultural regions in the country, they may have different genetic background for resistance. These lines can be used as donor for breeding resistance into the widely cultivated popular rice varieties as well as the genotypes can be intercropped with other susceptible cultivars to maintain the diversity of blast resistant gene in the rice field and reduce the blast infection. The resistant landraces can also be used for future studies on gene mapping and mining of new resistant genes for subsequent introgression into other rice materials.

5.2 Genetic divergence study through molecular markers

Diversity analysis based grouping of genotypes using molecular markers is the prime objective of the study. Sixty-five rice genotypes were grouped into ten and seven clusters on the basis of 12 agro-morphological and five pathological characters, respectively in experiment I using Mahalanobis D^2 statistic. The major limitation in field evaluation is the effect of environment and growth stage on the expression of the character.

Among numerous techniques available for assessing the genetic diversity and relatedness in crop germplasm, molecular markers, which portray genome sequence composition and enabling detection of differences in the genetic constitution among different individuals are highly useful to assess the genetic variability for breeding programme. Unlike morphological markers, they are not influenced by environmental factors and growth stages. Thus, these techniques have been increasingly used to characterize and identify a novel germplasm for use in the crop breeding process (O'Neill *et al.*, 2003). Molecular markers serve as a valuable tool to assess the genetic variation, varietal classification and germplasm identification of rice (Singh *et al.*, 2006).

Fifty rice genotypes selected from 65 genotypes studied in experiment I, by discarding 15 most similar genotypes based on the field diversity analysis were assessed for molecular diversity using ISSR and SSR markers. The results obtained are discussed below.

5.2.1 ISSR polymorphism

The genomic DNA of 50 rice genotypes was subjected to PCR amplification using 25 ISSR primers. Thirteen primers among these produce polymorphic amplicons and were selected for genetic diversity analysis by taking into consideration the repeatability, sharpness and intensity of amplicons. These 13 ISSR primers produced 103 reproducible amplicons with average of 7.92 loci per primer. The number of loci generated by each primer varied from 3 (ISSR808 and ISSR 826) to 12 (ISSR 815 and ISSR 823). The range of loci and average number of loci reproduced in present investigation were in comparison with the earlier studies with random primers. Saini *et al.* (2004) reported 240 loci with an average of 9.88 loci per primer in studies of 18 rice genotypes with 25 ISSR primers, whereas Rabbani *et al.* (2008) reported the range of loci from 4 to 16 by 25 decamer primers in 30 traditional and improved Basmati lines with an average of 8.3 loci per primer. Similarly, Kiani *et al.* (2011) reported the range of loci varying from 4 to 11 among 15 polymorphic primers with an average of 8.2 loci per primers. The details of polymorphism pattern of ISSR primers indicated that a total of 100 (97.08 %), out of 103 ISSR loci were observed to be polymorphic in the present investigation. The extent of polymorphism in the present study was higher as compared to the earlier reports of Saini *et al.* (2004), Shivapriya and Hittalmani (2006), Rabbani *et al.* (2008), Rekha *et al.* (2011) and Skaria *et al.* (2011) who reported 78.33, 74.00, 89.40, 72.90, 72.27 per cent polymorphic loci, respectively with arbitrary primers in rice. The polymorphic loci were classified as high, moderately and low polymorphic (Chimote *et al.*, 2004). Among 100 polymorphic loci 19 loci were highly polymorphic, 66 moderately polymorphic and 15 fragments showed low polymorphism. The percentage of polymorphism varied with each primer varied from 66.66 to 100 per cent. Nine primers revealed 100 per cent polymorphism showing their ability to discriminate the variety.

Of the different varieties, considering all the primers, Phule Samruddhi yielded the highest number of loci (61) followed by Ratna and RTN-1 (58 each) and Kalajirga (57), whereas Vikram (22) showed the least number of loci followed by Tulshi tall (26) and Sonsali and Pomendi Local (27 each). The improved varieties

showed higher number of loci than that of landraces and local selections. This may be due to the more number of alleles accumulated in them from diverse parents. On the contrary landraces showed less number of loci amplified, indicating their distinctive and diverse genetic makeup.

The cluster analysis based on unweighted paired group method of arithmetic means (UPGMA) with 13 ISSR primers allowed the discrimination of cultivars. The dendrogram based on Dice similarity coefficient values calculated using ISSR marker data of 50 rice genotypes clearly indicated low to moderate genetic diversity among the rice genotypes under study. The similarity coefficient for the genotypes varied from 0.31 to 0.90. The range of similarity coefficient was wider than the findings of Rabbani *et al.* (2008) who reported similarity coefficient from 0.62 to 0.96 in 30 Pakistani rice varieties, Kiani *et al.* (2011) who noticed the range of 0.59 to 0.98 in Iranian rice varieties and Rekha *et al.* (2011) who observed it in the range of 0.68 to 0.90 in Kerala rice varieties. The wider range in similarity coefficient indicated large genetic diversity in material studied which may be due to use of more number of genotypes from diverse sources than that of previous reports.

The UPGMA based clustering grouped 50 rice genotypes into three major clusters. Cluster I had maximum number of genotypes (38) followed by cluster II (11), whereas cluster III had a single genotype. Joshi *et al.* (2000) grouped 42 rice genotypes into six clusters based on amplification pattern generated by 11 ISSR primers, whereas Nagaraju *et al.* (2002) grouped 24 Basmati rice genotypes into five clusters based on 12 ISSR primers.

Cluster I was the largest and most diverse cluster consisting of 38 genotypes. This cluster was sub-grouped into eight sub-clusters at varying degree of similarity. The cluster comprised of landraces as well as improved varieties. However, the landraces and improved varieties were grouped into separate sub-clusters. The sub-cluster A, B, E and F had almost all improved varieties, whereas sub-cluster C, D, G and H had most of the landraces or local selections. The genotypes grouped in various sub-clusters showed phenotypic and/or genotypic similarities. The three genotypes from sub-cluster A (Heera, Halvi Sal 17 and RDN 185-2) were early in maturity with coarse grain type, whereas sub-cluster B comprised of 11 improved

varieties with dwarf stature, long-slender or medium-slender grain type with IR 8 or TN 1 (containing dwarfing gene *dee-geo-woo-gen*) in their ancestry. The sub-cluster F with 13 genotypes showed mixed expression for various traits. This subgroup consisted of extra-long slender, scented Basmati type genotypes like Taroari Basmati, Sugandhamati, SD 17, Kasturi, Haryana Basmati and MC 4 as well as non-scented fine grain genotypes like RTN purple, RDN 99-14, RDN 99-12, LK 248 and Phule Radha, medium-slender grained Sonsali, RDN 02-80 and Antersal and coarse grained Siddhagiri and RDN 97-2. In this cluster landraces exhibited more variability with lower similarity coefficient than the improved varieties. The diversity in the landraces was more distinct in 2D scatter plot diagram, in which the landraces were differentiated by both components. The local types Champakali, Nalabhat, Sonsali and Taroari Basmati were grouped in IV quarter (-/+) of 2D diagram, whereas Patni, EK 70 and Antersal were grouped in the I quarter and Kalajirga grouped into III quarter (-/-). The improved varieties were clustered at the centre of I (+/+) and II (+/-) quarter showing relatedness among the genotypes of respective sub-cluster. The independent grouping of landraces and improved varieties indicated the differences in the genetic architecture of these genotypes.

Eleven genotypes grouped in cluster II comprised of all landraces and local selections except Mahisugandha and Pusa Basmati 1. The similarity coefficient between any two genotypes in this cluster varied from 0.45 to 0.84 indicating moderate similarity among the genotypes. This cluster was grouped into two sub-clusters at 60 per cent similarity differentiating the landrace Vikram from other genotypes in the cluster. The landraces grouped in the sub-cluster B of this cluster showed varying degrees of similarity separating the genotype Tulshi tall at 65 per cent similarity and Pavsai at 69.5 per cent similarity. Three landraces viz., Vikram, Pavsai and Tulshi tall were differentiated by both the components of 2D scatter plot analysis placing these genotypes in IV quarter (-/+) of the diagram. These three landraces were tall in stature, very late in maturity with short and bold grains and low grain yield per plant. The distinctness of these landraces from other landraces in the cluster indicated the differences in genetic constitution of these genotypes. All other genotypes in the sub-cluster B reported 64 to 84 per cent similarity between them indicating moderate genetic relationship among the genotypes in the cluster.

The single genotype, Pomendi Local, grouped in cluster III was distinct from other genotypes. This local type from Ratnagiri district of Maharashtra reported least similarity (51 %) with other genotypes under study. The 2D scatter plot analysis showed differentiation of this genotypes in I quarter with both viz., 1st and 2nd component values positive (0.4/0.24). The higher values for 2nd component clearly differentiated this genotype from other genotypes placing it at extreme top in the I quarter of the diagram, which clearly indicated the differences in the genetic constitution of the genotype.

In a nutshell, ISSR primers were found to be useful for differentiating rice genotypes according to their respective genotypic constitution indicating the usefulness of these primers in the fingerprinting of rice genotypes and in understanding the evolutionary relationships of this species. The present investigation was in confirmation of the earlier studies conducted by Joshi *et al.* (2000) who reported the usefulness of ISSR markers for fingerprinting and to study phylogenetic analysis of rice genotypes and Nagaraju *et al.* (2002) on the usefulness of these markers for differentiating traditional and improved lines of rice.

5.2.2 SSR polymorphism

The genomic DNA of 50 rice genotypes was subjected to PCR amplification using 17 SSR markers. Fifteen markers among these were polymorphic producing 187 alleles. The average number of alleles per marker in this study was 12.47, with a range of 2 to 23. This range of alleles per marker was higher than the range 1 to 4 as reported by Singh *et al.* (2011), while it is similar to the range of 3 to 22 as reported by Jain *et al.* (2004) and 2 to 21 by Ghaley *et al.* (2012). The average number of alleles was higher than the earlier findings of Jain *et al.* (2004), Saini *et al.* (2004), Lapitan *et al.* (2007) and Singh *et al.* (2011) who reported 7.8, 5.33, 5.89 and 1.9 alleles per marker, respectively. This may be due to higher genetic differences in the material used in present investigation and/or due to use of PAGE for achieving better resolution of alleles. The details of polymorphism pattern of SSR primers revealed that among 187 scorable alleles, 178 were polymorphic (95.18 %). The extent of polymorphism reported in present study was higher than

56.20 per cent as reported by Singh *et al.* (2011) but was lower than 99.38 per cent reported by Saini *et al.* (2001). The present study revealed a high degree of genetic diversity in rice genotypes which may be due to the multiple agro-ecological and cultural conditions in the growing area and geographical location to domestication.

The present study reported nine unique alleles which were genotype-specific in the investigation. These unique alleles are very useful for cultivar identification and can be used for distinguishing the genotypes from other. Two unique alleles were reported in the genotype RDN 02-80 with the marker RM162 and one with the marker RM241, whereas the traditional Basmati landrace Taroari Basmati and improved variety RDN 97-2 showed unique alleles with the marker RM204. The popular and widely cultivated variety Ratna indicated unique allele with the marker RM215, whereas a landrace Patni recorded it with RM224. While, the landrace Sonsali and most popular South Indian variety, BPT 5204 showed unique allele with the marker TRS33. These alleles identified as unique to a particular accession made that genotype distinct from other genotypes and can be useful for varietal identification in future. The importance of unique and rare alleles for DNA fingerprinting was also highlighted by Nagaraju *et al.* (2002), Jain *et al.* (2004) and Shivapriya and Hittalmani (2006). The number of unique alleles obtained in the present study signifies the power of SSR markers in fingerprinting and diversity analysis, especially between closely related species. This information if used in combination with a database containing SSR-based fingerprints of registered varieties would provide standard method for determining the authenticity a variety. However, owing to the large number of accessions available for each of the species in the genus *Oryza*, it is important to screen the specificity of these alleles over a large sample size before affirming their diagnostic capacity. On the contrary, instead of using single marker for discrimination, multiplexing 2 or 3 markers to get a unique allelic pattern for each variety should be explored.

DNA-based markers serve as powerful tools for discriminating variation within crop germplasm and for studying evolutionary relationships (Gepts, 1993). The multivariate analysis of SSR data obtained from 15 markers in the present study indicated inter-relationship among the rice varieties. The cluster analysis

grouped 50 rice genotypes into four clusters at 42.5 per cent level of similarity. This is in accordance with the work of Lapitan *et al.* (2007) who grouped 24 rice cultivars into three clusters at 40 per cent similarity, whereas Ghaley *et al.* (2012) grouped 352 Bhutan landraces into two clusters at dis-similarity of 0.84 and Singh *et al.* (2011) grouped 50 aromatic rice accessions into eight clusters at similarity index of 0.76, similarly, Jain *et al.* (2004) grouped 69 rice genotypes into four major groups using 80 per cent similarity as the threshold. Cluster III has maximum 25 genotypes followed by cluster II (12), Cluster I (8) and cluster IV (5).

Cluster I comprising of eight genotypes was differentiated from other clusters at 34 per cent similarity, indicating the presence of wide genotypic differences from other clusters. This cluster was sub-grouped at similarity index of 0.46 into two sub-clusters viz., sub-cluster A and B. The sub-cluster A comprising of four genotypes (Heera, SD 17, Sugandhamati and MC 4) and the similarity between any two individuals varied from 64 to 81 per cent. Among these, the genotype MC 4 was selected from a Basmati landrace Karnal Local, whereas two genotypes SD 17 and Sugandhamati have Pusa Basmati 1 in their parentage, which has Karnal local as one of its parent. The similarity between these three genotypes was more than 70 per cent indicating a close relationship in their genetic constitution. The genotype Pusa Basmati 1 was grouped in the sub-cluster B of cluster I. The separate grouping of Pusa Basmati 1 indicated the difference in its genetic architecture. Jain *et al.* (2004) and Singh *et al.* (2011) also reported separate grouping of Pusa Basmati 1 from traditional Basmati lines and indica lines, which is tallying with present investigation. Interestingly, Pusa Basmati 1 was grouped in the sub-cluster B with three popular landraces from three agro-climatic locations viz., Ghansal, a popular landrace from Western Ghat zone of Maharashtra, Badshabhog, a very popular landrace from Orissa and Pavsai, a landrace from Sub-mountain zone of Maharashtra. The similarity coefficient between these genotypes varied from 0.65 to 0.84 indicating moderate variation in their genetic structure. Two dimensional scatter plot analysis indicated the differentiation of cluster I by both the axis, grouping the genotypes in quarter I of the diagram with positive values for both the components. The variation in the 2nd component values clearly indicated the wide variation in the sub-cluster B than that of sub-cluster A.

The Cluster II consist mostly of landraces or local selections and the grouping was at 50 per cent similarity into two sub-clusters, A and B with seven and five genotypes, respectively. The genotypes in both the sub-clusters showed wide range of similarity index from 0.40 to 0.83 for sub-cluster A and 0.49 to 0.75 for sub-cluster B indicating differences in the genetic composition of the genotypes in these sub-clusters. Both these sub-clusters were grouped into II quarter (+/-) of 2D scatter plot diagram. The genotypes from sub-cluster B had narrow range for the value of 1st and 2nd component of the diagram and were clustered at the centre of the diagram, whereas the genotypes from sub-cluster B reported relatively higher values with wide range for both the components and were distributed deep in the II quarter. The grouping of landraces or local selections independent from improved varieties indicates the independent evolutionary mechanisms in the development of these landraces. In addition, these genotypes showed distinct identity with wide range for phenotypic expression from one another. Present study also revealed that there were considerable differences in their genotypic composition too.

In the present investigation, Cluster III was the largest with 25 genotypes that consisted of almost all the improved varieties. Wide range of variation was observed among the genotypes from this cluster with similarity index varying from 0.21 to 0.87. This cluster was grouped into five sub-clusters at varying degrees of similarity. The single genotype RDN 02-80 was differentiated at similarity of 45 per cent to form sub-cluster E. This genotype was developed from the cross between a popular scented variety Indrayani and Pusa Basmati 1. It exhibited dwarf plant type, midlate maturity, higher number of tillers per plant with medium-slender grain type. This genotype was distinctly separated in 2D scatter plot diagram too, differentiated by both the axis and placed in II quarter (+/-) of the diagram. This clearly showed the differences in the genotypic structure of this genotype from other genotypes in the cluster. Two landraces, Vikram and Tulshi tall were differentiated from other genotypes of the cluster at the similarity coefficient value of 0.51 forming sub-cluster D. These two landraces had only 65 per cent similarity between them and were grouped in two different quarters of 2D diagram (Tulshi tall in quarter I with 0.025/ 0.10 value for 1st and 2nd component, whereas Vikram in quarter III of the diagram with -0.09/-0.04 values for 1st and 2nd component) indicating the

differences in their genetic makeup. These two landraces represents two agro-climatic zones (Western Ghat and Konkan) of Maharashtra state and may have independent evolutionary history resulting into differences in their genetic structure.

BPT 5204, the most popular genotype of South India differentiated at 57 per cent similarity from other genotypes in the cluster III forming sub-cluster C. This genotype has unique characteristics, which makes it most popular in South India. This improved variety was developed from triple cross between GEB 24, TN 1 and Mahsuri. The variety had dwarf plant type, medium-slender grain with low test weight and high grain yield. Grouping of this genotype into separate sub-cluster indicated the differences in genetic composition of this variety from other improved varieties.

Sub-cluster A and B consisting of 10 and 11 genotypes, respectively were the most important groups of this cluster accommodating most of the improved varieties. All the improved varieties grouped in sub-cluster A have TN 1 in their parentage, whereas most of the varieties in sub-cluster B have IR 8 or IR 28 in their parentage or ancestry as dwarfing source. The independent grouping of improved varieties according to their parentage indicated the efficiency of SSR markers to distinguish the genotypes based on their parental relationship. The range of the similarity coefficient between any two genotypes in these sub-clusters varied from 0.54 to 0.87 for sub-cluster A and 0.48 to 0.86 for sub-cluster B, indicating moderate to high similarity among the genotypes. The 2D scatter plot analysis reflected the relatedness among the genotypes of this cluster by accumulating them along the X axis in IV quarter. This revealed that the improved varieties have less genetic diversity.

Five genotypes grouped in cluster IV included three landraces viz., Kothimbire, Diwani and Shyamjeer forming sub-cluster A and two Basmati lines viz., Basmati 386 and Mahisugandha forming sub-cluster B. Kothimbire, a landrace from Maharashtra showing 66.5 per cent similarity with other two landraces from sub-cluster A, indicated independent evolutionary history than other two. This genotype had a distant short-bold grain type as against very fine, short slender grain type of Diwani and Shyamjeer. Though, these landraces have phenotypic as well as

genotypic differences they were distinctly different from Basmati landrace Basmati 386 and evolved Basmati Mahisugandha. This indicated the independent and distinct evolutionary mechanism of Basmati lines in India. The independent and distinct nature of Indian Basmati lines was also reported by Jain *et al.* (2004), Saini *et al.* (2004) and Singh *et al.* (2011) which is in consonance with the present findings.

In summary, the present investigation of rice genotypes with SSR markers revealed genetic differences between improved varieties and landraces. The improved varieties showed a limited genetic variability due to related genotypes in their parentage and/or ancestry, on the other hand the landraces had wider genetic differences. The study also indicates independent and long evolutionary history of Indian landraces and Basmati lines. Marker-based differentiation and identification of these genotypes may help for better utilization in further breeding programme.

5.2.3 Molecular diversity studies using pooled data of ISSR and SSR markers

The Jaccard's similarity index computed from binary data from the polymorphic ISSR and SSR primers indicated moderate to high diversity among the rice genotypes under study with similarity coefficient ranging from 0.25 to 0.85. In the present investigation 277 genotypic combinations had similarity value ≤ 0.40 among which 13 had ≤ 0.30 showing high diversity among these genotypic combinations. Maximum diversity was observed between Pomendi Local, a landrace from Maharashtra and improved Basmati variety Pusa Basmati 1 (0.25) followed by an improved line RDN 97-2 and a local landrace Tulshi tall (0.26), Pomendi Local and Ghansal (0.27), KJT 2 and Badshabhog (0.27) and Pavsai and RDN 97-2 (0.27). Most of the landraces reported more number of combinations with similarity index value ≤ 0.40 which included Badshabhog (26 combinations), Pavsai (25 combinations), Khalibagh (15 combinations), Ghansal (15 combinations), Tulshi tall (14 combinations), Vikram (10 combinations) and Shyamjeer (9 combinations). The maximum diversity among the landraces indicated the distinct differences in their genetic architecture. These landraces were from various agro-climatic regions of the country and evolved through long evolutionary processes under different environmental conditions of respective

regions. Interestingly, the improved Basmati variety Pusa Basmati 1 and improved line RDN 97-2 also recorded higher number of combinations (20 and 9, respectively) with similarity value ≤ 0.40 indicating genetic dis-similarity of these varieties with other genotypes in the study. These two genotypes may have different genetic constitution than other genotypes. The distinctness in genetic makeup of Pusa Basmati 1 was also reported by Jain *et al.* (2004) and Singh *et al.* (2011) that tallying with the above mentioned results.

In the present investigation, improved varieties exhibited maximum similarity. The highest similarity was reported between the improved varieties Pawana and RTN-1 (0.85) followed by Phule Maval and Phule Samruddhi (0.84), Bhogavati and Indrayani (0.84) and Indrayani and Phule Maval (0.83). Seventy genotypic combinations recorded similarity index ≥ 0.75 , in which 12 showed the similarity index ≥ 0.80 indicating higher similarity in these genotypic combinations. The improved varieties Pawana (6 combinations), Indrayani (5 combinations), Phule Maval (5 combinations), Bhogavati (4 combinations) and RDN 99-14 (4 combinations) showed higher number of combinations with similarity value ≥ 0.75 indicating the similarity in genetic constitution of these genotypes. All these genotypes had dwarf plant type, long-slender grains and high grain yield. These genotypes had common parents and/or related by ancestry, which reflected in more genotypic similarity between them.

The UPGMA based clustering using Dice similarity coefficient prepared from the pooled data of 13 ISSR and 15 SSR polymorphic markers grouped 50 rice genotypes into five clusters. The clustering pattern of the genotypes using pooled data was comparable to a certain extent to that of SSR data. Cluster III had maximum number of genotypes (24) followed by cluster II (10), cluster V (9), cluster I (5) and cluster IV (2).

Cluster I consist of five genotypes differentiated from other clusters at 52 per cent similarity. All the genotypes in this cluster, except Heera, had Basmati landrace Karnal Local in their ancestry. This cluster was alienated into two sub-clusters at 55 per cent similarity differentiating RDN 02-80 from other genotypes in the cluster. RDN 02-80 has a widely cultivated scented rice variety Indrayani in its parentage

with Pusa Basmati-1. The parental relationship of RDN 02-80 with that of other genotypes in sub-cluster I grouped them together in cluster I. However, the presence of non-Basmati parent in RDN 02-80 might have reflected in its genetic differences with genotypes in sub-cluster A. Common parentage between SD 17 and Sugandhamati resulted in maximum genetic similarity between these two genotypes (84 per cent similarity) and were grouped together in sub-cluster A with MC 4 and Heera. In 2D scatter plot analysis, this cluster was differentiated by both the components of the diagram grouping the genotypes from sub-cluster A in I quarter (+/+) and RDN 02-80 (sub-cluster B) in IV quarter (+/-) of the diagram. In general the grouping in cluster A reflected the parental relationship among the genotypes.

Most of the landraces in present investigation were grouped independently in clusters II, IV and V. Cluster II consisted of 10 genotypes accommodating nine local types from Maharashtra and one improved line RDN 97-2. These genotypes reported wide variation with the similarity coefficient between any two genotypes varying from 0.43 to 0.80. Sub-cluster A, comprised of all non-scented local genotypes, except RDN97-2, whereas sub-cluster B comprised of scented local types except Kasturi, which is improved scented variety. The genetic differences in the landraces were distinct in 2D diagram, showing the genotypes widely distributed by 2nd component with value ranging from -0.04 for Kalajirga to 0.25 for Siddhagiri. The genotypes were grouped in I, II and IV quarter of 2D diagram indicating wide genetic variability among them. Higher genetic differences in these landraces indicated their distinct and independent evolutionary mechanisms.

The improved varieties in present investigation were mostly grouped in the Cluster III, which was the largest cluster in this study. This cluster was differentiated into four sub-clusters at different levels of similarity. Sub-cluster A was the largest with 14 genotypes and all of them were improved varieties with most of them having TN 1 or IR 8 in their parentage. The similarity coefficient between any two rice varieties in this sub-cluster ranged from 56 to 85 per cent. In 2D scatter plot analysis, the genotypes from this sub-cluster were differentiated by both 1st and 2nd component and were densely accumulated in the III quarter (-/-). The higher similarity in genetic architecture of these genotypes indicated the

parental relationship between them. Improved lines from sub-cluster B (RDN 99-12, RDN 99-14) were also grouped in same quarter indicating genetic relatedness with sub-cluster A, whereas two local selections (RTN purple and LK 248) and landrace Pomendi Local from this sub-cluster were distributed in IV quarter (-/+) of the diagram. This indicated the differences in the genetic makeup of these local types from improved varieties.

Two landraces Vikram and Tulshi tall were grouped in Cluster IV indicating distinct differences in their allelic combinations. These two genotypes showed moderate similarity (64 per cent) and were widely distributed in 2D diagram. Tulshi tall a landrace from Kolhapur district of Maharashtra was placed in II quarter of 2D diagram with the values of 0.15 for 1st component and -0.08 for 2nd component, whereas Vikram, a landrace from Sindhudurg district of Maharashtra, was placed in III quarter of diagram with the values of - 0.02 for 1st component and - 0.02 for 2nd component. This revealed wide variation in their genetic constitution which may be due to differences in evolutionary mechanisms under different agro-climates.

Nine genotypes grouped in Cluster V represented scented Basmati and non-Basmati local types from India. The range of similarity coefficient in this cluster varied from 47 to 82 per cent indicating moderate to high variation between the genotypes. These genotypes were scattered in II quarter of 2D diagram with the range for 1st component varying from 0.17 to 0.32 and for 2nd component from - 0.015 to - 0.26 indicating ample genetic differences among the genotypes. These local types belonged to different agro-climatic zones of India and were evolved through different selection indices with shifting effect of biotic and abiotic factors resulting into differences in genetic composition among them.

When taken as a whole, the 50 rice varieties were grouped into five clusters based on the similarity coefficient. Most of the local genotypes and Basmati lines were grouped independently from improved varieties. Close association among scented Basmati germplasm was also reported by Davierwala *et al.* (2000) and Rabbani *et al.* (2008). This analysis suggested that the landraces, local genotypes and Basmati rice have a long and independent history of evolution that distinguishes them from most of the improved varieties.

In summary, SSR and ISSR-based diversity analysis of rice in the present study indicated independent clustering of landraces, scented Basmati and non-Basmati genotypes and improved varieties suggests long, independent and complex pattern of evolution of these germplasm groups. Greater appreciation of genetic diversity in rice gene pool will facilitate the classification, proper maintenance, conservation and utilization of these valuable resources. In addition, marker-based identification and differentiation of rice genotypes may help to maintain the integrity of the lines to benefit farmers and research workers.

5.2.4 Comparative performance of different diversity studies in rice

In the present investigation rice genotypes were assessed for diversity using phenotypic characteristics and molecular markers. The comparative performance of ISSR, SSR and pooled analysis with the phenotypic analysis indicated distinct performance.

The study of molecular diversity between rice genotypes was undertaken with ISSR and SSR primers. The binary data from polymorphic ISSR and SSR primers was analysed independently in ISSR and SSR analysis as well as pooled to calculate Jaccard's similarity indices. These three molecular analysis methods reported comparable results. Wide range of similarity coefficient was observed in all three methods indicating the presence of wide genetic differences among the genotypes under study.

All three methods grouped improved varieties separately from local and Basmati lines. However, the clustering of improved varieties was more precise in SSR analysis, which grouped the improved varieties with the parent TN 1 independently in cluster IIIA to that of IR 8 and IR 28 in cluster IIIB. In ISSR analysis, improved varieties were intermingled with Basmati types and landraces in cluster III, whereas in pooled analysis they were mostly independent and placed in cluster IIIA and IIIB.

The landraces and local selections were grouped fairly independently in all three methods. However, the grouping was more precise in pooled analysis with grouping non-scented landraces in cluster IIA, IIIC, IIID and cluster IV, while scented landraces in cluster IIB and cluster V. Though the landraces were grouped

independent of improved varieties in ISSR and SSR analysis, many of them were grouped intermediary to improved varieties.

In case of Basmati lines, SSR analysis grouped the lines independently in cluster IA, IIB, IVB with exception of Taroari Basmati in cluster IIIB. On the other hand pooled analysis grouped these lines independently in cluster IA, IIIC, IIID and VB. In ISSR analysis, Basmati lines were intermingled with other genotypes in cluster IIIC.

In the nutshell, all three methods *viz.*, ISSR, SSR and pooled analysis distinguished rice genotypes based on their pedigree. All the systems indicated wide range of genotypic differences in landraces than improved varieties and indicated the independent evolutionary mechanisms of Indian landraces and Basmati genotypes. In the study, ISSR markers recorded higher similarity coefficients, whereas SSR markers reported maximum range of diversity with more precise grouping of improved varieties. However, with intermediate range of similarity, more number of independent clusters and proper categorization of different types of genotypes pooled analysis using both ISSR and SSR markers was found more effective in grouping of rice genotypes based on their genotypic constitution, parental relationship and evolutionary mechanisms. The foregoing results tally with the work of Nagaraju *et al.* (2002) who suggested combination of SSR and ISSR markers for rice diversity study.

The diversity analysis based on agro-morphological characters also recorded wide range of D^2 values (4.21 to 24015.45) indicating huge diversity among the genotypes studied. The genotypes were grouped in to 10 clusters. The grouping of genotypes largely reflects the phenotypic similarities between them. The grouping was more or less character specific (*i.e.* plant stature, maturity, grain type *etc.*) than that to ancestry, geographical or evolutionary terms. The improved varieties were grouped independently to some extent, however the landraces, local selections and Basmati lines were intermingled in many clusters.

None of the molecular marker analysis method showed correspondence with phenotypic analysis. The deviation between phenotypic analysis and molecular marker analysis could partially be explained by the fact that different coefficients *i.e.* Mahalanobis D^2 statistic for phenotypic analysis and Jaccard's similarity

coefficient for molecular markers were used in the study. Secondly, the molecular markers measures the genetic variation in DNA sequence which are independent of environmental effect, on the other hand, phenotypic analysis are affected by environmental conditions and show considerable variation. Molecular marker analysis show better resemblance with the pedigree as compared to phenotypic evaluation indicating superiority of molecular analysis over phenotypic analysis in diversity study on rice. These findings are in agreement of the work done by Singh *et al.* (2011) who also reported superiority of molecular markers to that of morphological diversity.

5.3 Genetics of blast resistance in rice

Rice plants are attacked by several diseases caused by various micro-organisms including bacteria, fungi, viruses and nematodes. Among these, blast caused by fungus *Magnaporthe grisea* is one of the major disease causing huge crop losses. The susceptibility of rice to blast is widespread among commercial cultivars, indicating the need for improving resistance. Genetic resistance is the most economically viable and environmentally safe option for control of this disease. Such resistance is conferred by either major resistance genes that offer complete protection against certain races of pathogen or minor genes, which act together to provide quantitative, partial resistance (Wang *et al.*, 1994). Untill now, at least 73 major resistance genes for blast resistance have been reported (Ballini *et al.*, 2008). However, such vertical blast resistance is frequently overcome within few years of intensive cultivation due to selection of new virulent race of the fungus (Ghaley *et al.*, 2012). Strategies for blast control aim at durable resistance by accumulating several resistance genes, partial resistance genes or combination of resistance and partial resistance genes in varieties. However, to do it more efficiently, new sources of durable blast resistance and their inheritance mechanisms should be elucidated. There is need for identification of new resistance and partial resistance genes in genetically diversified rice material, which has developed resistance over thousands of years of co-evolution with the

fungus. Therefore, identification of genes / QTLs and the linked molecular marker(s) for blast resistance in rice is very much essential.

In the present investigation, a cross was made between a blast susceptible genotype EK 70 and a resistant variety RDN 98-2-3-5-14 to produce the mapping population. EK 70, the local selection from Nasik district of Maharashtra having early maturity, tall plant stature and long-slender grains is popular among farmers. However, it is highly susceptible to blast suffering from heavy losses. RDN 98-2-3-5-14, a variety developed from the cross between a local selection Halvi Sal 17 and a improved variety TN 1 has midlate maturity, tall plant stature with medium-slender grain type and is resistant to blast. The genotype was tested at ARS, Lonavala that is a 'hotspot' location during the years 2007 and 2008 where it was found resistant to blast (score < 2) during both the years (Anonymous, 2008, 2009).

The F₂ population of a cross and the parents were evaluated for reaction to blast at ARS, Lonavala during *kharif* 2011. The observations were recorded for infection type as per SES score. The susceptible parent EK 70 showed highly susceptible reaction with lesion type of ≥ 8 , whereas the parent RDN 98-2-3-5-14 was found to be resistant producing lesion type of 1 in the field. This indicated that natural blast inoculum pressure was high enough to allow evaluation of resistance and susceptibility. Among the randomly selected 150 F₂ plants, 111 plants showed resistant reaction (blast score ≤ 3) and 39 plants showed susceptible reaction (blast score > 3). The observed frequencies were tested for goodness of fit with chi-square (χ^2) test to the expected segregation ratio of 3:1 for single gene model, which confirmed that the resistance in RDN 98-2-3-5-14 is governed by single dominant gene. The results obtained in this study are in accordance with the reports of Filippi and Prabhu (1996), Chen *et al.* (1999), Lang *et al.* (2001), Barman *et al.* (2004), Zhou *et al.* (2004), Padmavati *et al.* (2005), Sharma *et al.* (2007), Persaud *et al.* (2007), Lee *et al.* (2009) and Ashkani *et al.* (2011) who also reported that the rice blast is governed by single dominant gene.

During the last few years, the genetics of blast resistance in rice have been extensively studied and many dominant R genes conferring complete resistance to *Magnaporthe grisea* have been identified. The first *Pi* gene in rice was named by

Kiyosawa (1966). To date, approximately 73 major resistance genes have been named and most of them were mapped on rice chromosomes 2, 5, 6, 9, 11 and 12 (Joshi *et al.* 2009). Molecular markers are useful for mapping genes of interest and for marker-assisted breeding against rice blast. Among various types of molecular markers PCR-based SSR markers are more preferable for this purpose (Swarup *et al.* 2006). Therefore, to fulfil the objective of identification of QTLs and the linked molecular marker(s) for blast resistance in rice, the mapping population was evaluated with SSR markers by following bulk segregant analysis method as suggested by Michelmore (1991).

Among 25 SSR markers used for present investigation, 14 markers reported polymorphism between two parents EK 70 and RDN 98-2-3-5-14 and two SSR markers RM204 and RM215 reported polymorphism between susceptible and resistant parents and bulks indicating their possible association with blast resistance in present mapping population. F₂ mapping population was genotyped with these two primers to study their linkage with blast resistance.

Segregation study with the marker RM204 recorded a resistant allele of ~174 bp amplified in 33 plants, whereas a susceptible allele of ~110 bp was amplified in 41 plants. Seventy-three F₂ plants exhibited both the alleles. Genetic analysis with chi-square test indicated goodness of fit to the expected ratio of 1:2:1 for single gene model indicating the association of RM204 with blast resistant gene in the present population. This confirmed that the resistance for blast in present investigation was governed by single dominant gene. The simple regression analysis conducted to study association between phenotypic data of blast and molecular marker data of RM204 indicated that the marker was significantly linked with blast resistance.

The marker RM204 was mapped on short arm of chromosome 6 at 17.2 cM by Chen *et al.* (1997), 31.1 cM by Temnykh *et al.* (2000) and 25.1 cM by Temnykh *et al.* (2001). The blast resistance genes *Pi*-2 (Goto *et al.*, 1981), *Pi* 3(t) (Mackill and Bonman, 1992), *Pi*-8, *Pi* 9(t), *Pi* 13 (t), *Pi*22(t) (Pan *et al.*, 1996), *Pi*27 (Sallaud *et al.*, 2003) and *Pi*-tq1 (Tabian *et al.* 2000) were reported on chromosome 6.

As per Temnykh *et al.* (2001) the marker RM225 mapped on short arm of chromosome 6 at 26.2 cM. The blast resistant genes *Pi22* (Pan *et al.*, 1996) and *Pi27* (Sallaud *et al.*, 2003) were mapped on short arm of chromosome 6. As per Eizenga *et al.* (2006) the marker RM225 was found close to the blast resistant genes *Pi22* and *Pi27*. The marker RM204, which has been identified to be associated with blast resistance in present study was co-segregating with RM225 as per Chen *et al.* (1997) and mapped 1.1 cM away from the marker RM225 by Temnykh *et al.* (2001). It suggests that the marker RM204, which has been identified in present study is linked with the same QTL which has been identified by Temnykh *et al.* (2001).

Wang *et al.* (1994) reported QTLs for blast lesion number and blast diseased leaf area on the short arm of chromosome 6 and the marker RZ398 co-segregated with these QTLs. Chen *et al.* (1997) mapped the markers RZ398, RM204 and RM225 at same position on chromosome 6, whereas Temnykh *et al.* (2001) mapped the markers RZ398 and RM225 at 26.2 cM on chromosome 6 and the marker RM204 at 1.1 cM distance from these two markers. The marker RM204 was found associated with blast resistance in present study. It seems that the QTLs reported by Wang *et al.* (1994) for blast lesion number and blast diseased leaf area and the one identified for blast resistance in present investigation might be the same, as blast lesion number and blast diseased leaf area are highly correlated to blast resistance in field condition. Similarly, Biradar *et al.* (2007) identified a QTL for silicon (Si) content on chromosome 6. The accumulation of silicon in plants helps in disease resistance (Marscher, 1995). The relationship between Si content and blast resistance was first reported by Onodera in 1917. Later on it was demonstrated that Si content in rice straw and husk were inversely proportional to blast disease severity and number of blast lesions. Two mechanisms *viz.*, forming physical barrier in leaf epidermis and producing fungal toxins (Yoshida *et al.*, 1962; Fawe *et al.*, 1998 and Datnof and Rodrigues., 2005) were found associated with Si mediated resistance for blast in rice. The QTL for silicon content identified by Biradar *et al.* (2007) was at similar location to the QTLs reported by Wang *et al.* (1994) chromosome 6. The comparative analysis of the map of chromosome 6 reported by Wang *et al.* (1994), Chen *et al.* (1997), Temnykh *et al.* (2001) and Biradar *et al.*

(2007) suggests that the QTLs reported by Biradar *et al.* (2007) for silicon content and the one identified for blast resistance in present investigation might be the same.

The marker RM215, although was polymorphic in bulk segregants analysis, was found not associated with blast resistance in present investigation.

The finding of this study indicated that the SSR marker RM204 is associated with blast resistance locus in RDN 98-2-3-5-14. In future, markers specific to chromosome 6 will be used for screening the parental lines and polymorphic markers will be used to prepare framework map of the chromosome 6. This map will be used for QTL mapping using phenotypic data of F_2 derived F_3 families. The findings of this study could be directly useful in molecular analysis of segregating generations, breeding lines and varieties having RDN 98-2-3-5-14 as a parent. This approach will take less time and resources than field evaluation. It could prove useful in marker assisted selection in the absence of appropriate testing conditions.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present investigation entitled “Genetic and molecular analysis of blast resistance in rice (*Oryza sativa* L.)” was designed and executed to study the genetic and molecular basis of blast resistance in rice. The experimental material was evaluated under field condition at Agricultural Research Station, Radhanagari, Dist. Kolhapur (MS) and Agricultural Research Station, Lonavala, Dist. Pune (MS), whereas the molecular studies were conducted at State Level Biotechnology Centre, MPKV, Rahuri during the period 2010 to 2012 with the objectives to study genetic variability and divergence in rice genotype for different agro-morphological and pathological traits, to study molecular diversity in rice genotypes using molecular markers and to identify the QTLs and the linked molecular marker(s) for blast resistance in rice

In order to the study genetic variability and divergence, 65 rice genotypes from different eco-geographical origin were evaluated for agro-morphological and pathological traits. The agro-morphological evaluation of the genotypes was carried out at two locations viz., ARS, Radhanagari (MS) and ARS, Lonavala (MS) during *kharif* 2010. Genotypes were examined for 12 agro-morphological traits viz., days to 50 per cent flowering, days to maturity, plant height, panicle length, spikelets per panicle, spikelet fertility, tillers per plant, test weight, grain length, grain breadth, Length: breadth ratio and seed yield per plant. The diversity for pathological traits (blast disease) in rice genotypes was evaluated at blast “hot spot” location, ARS, Lonavala adopting uniform blast nursery (UBN) during *kharif* 2010. Five pathological traits viz., infection type, diseased leaf area, lesion density, lesion size and per cent disease intensity (PDI) were studied.

Wide range of variation was observed for all the 12 agro-morphological and five pathological characters, except fertility percentage. The analysis of variance exhibited statistically significant differences among genotypes for all the characters indicating the presence of substantial amount of variability. Among the agro-morphological characters, length : breadth ratio reported highest GCV and PCV followed by test weight, whereas the character spikelet fertility exhibited the lowest

GCV and PCV. In pathological observations, lesion size showed highest GCV and PCV followed by lesion density, whereas infection type reported lowest GCV and PCV followed by percent disease intensity.

Heritability (broad sense) of all the characters under study was categorized to very high ranging from 66.10 per cent for spikelet fertility to 99.90 per cent for test weight. The genetic advance for morphological characters was found highest for spikelet per panicle (60.57) followed by plant height and days to maturity. However, when expressed in percentage of mean, it was highest for length : breadth ratio followed by test weight. High heritability coupled with high genetic advance observed for spikelets per panicle, plant height, days to maturity and days to 50 per cent flowering advocates high genetic progress in them on direct selection.

Among pathological observations the heritability varied in the range of 91.59 per cent for infection type to 98.78 per cent for diseased leaf area, while genetic advance was ranging from 4.16 for infection type to 46.78 for per cent disease intensity. Further, high heritability coupled with genetic advance was observed for per cent disease intensity and diseased leaf area indicating effectiveness of direct selection based on these traits.

In the evaluation for morphological traits, D^2 values between all possible pairs of 65 genotypes ranged from 4.21 to 24015.45. The genotypes were grouped into ten clusters following Tocher's method. Cluster I had maximum number of genotypes (22) followed by cluster III (14) and cluster IV (12). Among other clusters, the cluster II, IV and IX had 7, 4 and 2 genotypes, respectively, whereas the cluster V, VI, VII and X had single genotype each. The maximum inter-cluster distance was observed between the clusters VIII and IX followed by clusters II and VIII, whereas it was minimum between the cluster V and X followed by that between clusters I and V. Among 12 characters studied, test weight has contributed major part of divergence followed by grain length and length : breadth ratio. The contribution of other characters was least.

For pathological traits, D^2 values were observed from 0.03 to 1496.95. Cluster formation by Tocher's method grouped 65 genotypes into seven clusters. Cluster I was largest with 27 genotypes followed by cluster II (26) and cluster IV

(8). The clusters III, V, VI and VII had single genotype each. The resistant, moderately resistant, susceptible and highly susceptible genotypes were grouped into different clusters. The maximum inter-cluster distance was observed between clusters I and VII, followed by clusters VI and VII, whereas the minimum inter-cluster distance was noticed between the clusters III and V followed by cluster II and V. Among the five pathological traits studied, PDI has contributed highest for total divergence followed by lesion density and diseased leaf area.

A study on molecular diversity in the rice genotypes was one of the objectives of present study. Fifty rice genotypes were assessed with ISSR and SSR markers at State Level Biotechnology Centre, MPKV, Rahuri during 2010 and 2011. Fifty rice genotypes for the molecular evaluation were selected from 65 genotypes studied for field evaluation by discarding 15 most similar genotypes based on the field diversity analysis.

Among the 25 ISSR primers used 13 were polymorphic which produced a total of 103 reproducible amplicons with an average of 7.92 amplicons per primer, in which 100 amplicons (97.08 %) were polymorphic. The number of amplicons generated by each primer varied from three (ISSR808 and ISSR 826) to 12 (ISSR 815 and ISSR 823). The similarity coefficient between the genotypes varied from 0.31 to 0.90 indicating moderate to high diversity among the genotypes. The UPGMA based clustering analysis using Dice similarity coefficient grouped 50 rice genotypes into three major clusters. ISSR analysis grouped landraces, Basmati lines and improved varieties in different sub-clusters. The grouping of the genotypes represents parental relationship.

In case of SSR analysis, from the total of 17 markers, 15 were polymorphic. These markers produced 187 scorable alleles with the average number of 12.47 alleles per locus, among which 178 alleles were polymorphic (95.18 %). The marker RM204 produced highest number of alleles (23) followed by RM246, whereas RM11 produced least number of alleles (2) followed by RM 21. In the present study nine unique alleles were observed in seven genotypes with six SSR markers. These unique alleles are useful for molecular fingerprinting and can be used for distinguishing the genotypes from other. The range of similarity coefficient

varied from 0.11 to 0.87 indicating medium to large genetic diversity among the genotypes. Landraces reported high genetic diversity, whereas improved varieties reported more similarity. The cluster analysis grouped 50 rice genotypes into four clusters at 42.5 per cent similarity. This analysis reflected genetic differences between improved varieties and landraces.

The pooled data of ISSR and SSR was used to compute Jaccard's similarity index indicated moderate to high diversity among the rice genotypes with similarity coefficient ranged from 0.25 to 0.85. Most of the landraces showed more number of combinations with similarity index value ≤ 0.40 indicating distinct differences in their genetic architecture. Further, the improved varieties reported maximum similarity. The highest similarity was noticed between the improved varieties Pawana and RTN-1, followed by Phule Maval and Phule Samruddhi. Parental relatedness among improved varieties may have been reflected in more genotypic similarity between them.

The UPGMA based clustering analysis using Dice similarity coefficient prepared from the pooled data of 13 ISSR and 15 SSR polymorphic markers grouped 50 rice genotypes into five clusters.

Among the three methods used for molecular diversity studies, pooled analysis was more effective in distinguishing rice genotypes on their genotypic constitution, parental relationship and evolutionary mechanisms.

Identification of QTLs and the linked molecular marker(s) for blast resistance in rice was the major objective of present study. For this, the F_2 population of a cross between a blast susceptible genotype EK 70 and a resistant variety RDN 98-2-3-5-14 was evaluated for blast at ARS, Lonavala during *kharif* 2011. Randomly selected 150 F_2 plants were scored for lesion type as per SES scale. Among 150 F_2 plants, 111 plants showed resistant reaction while 39 plants showed susceptible reaction. The observed frequencies were tested for goodness of fit with chi-square (χ^2) test to the expected segregation ratio of 3:1 for single gene model. The results indicated the resistance in RDN 98-2-3-5-14 is governed by single dominant gene.

For molecular marker studies, genomic DNA was isolated from each of the parent and randomly selected 150 F₂ plants using a modified CTAB method. Bulk segregant analysis was used for rapid identification of markers linked to blast resistance. Among 25 SSR markers used for present investigation, 14 markers reported polymorphic reaction between two parents EK 70 and RDN 98-2-3-5-14 and two SSR markers RM204 and RM215 reported polymorphism between susceptible and resistant parent and bulks indicating their possible association with blast resistance in present mapping population.

Segregation study with the marker RM204 showed a fragment of ~174 bp amplified from 33 plants and a fragment of ~110 bp in 41 plants, whereas 73 F₂ plants reported both the fragments. Genetic analysis with chi-square test indicated goodness of fit to the expected ratio of 1:2:1 for single gene model. The simple regression analysis indicated that the marker data with RM204 was significantly linked with phenotypic evaluation. This shows that the marker RM204 was significantly associated with blast resistance in present mapping population. Segregation study with the marker RM215 indicated that, although this marker was polymorphic in bulk segregants analysis, was found not associated with blast resistance.

Conclusions

Based on present study, following conclusions have been drawn

1. Wide range of variation was observed for all agro-morphological and pathological characters.
2. The genotypes showed substantial degree of variability for all agro-morphological and pathological characters.
3. Heritability (broad sense) was high for all agro-morphological and pathological characters. Very high heritability coupled with high genetic advance observed for the spikelets per panicle and plant height advocated high genetic progress for them.

4. D^2 statistic indicated that the genotypes studied were genetically diverse. The 65 genotypes were grouped in 10 clusters based on agro-morphological characters and in 7 clusters based on pathological characters.
5. The molecular diversity study with ISSR, SSR and pooled analysis reported wide variability among the genotypes.
6. Maximum diversity was observed between the landraces, whereas the improved varieties reported more similarity.
7. The SSR analysis identified nine unique alleles in seven genotypes with six SSR markers. These unique alleles are useful for molecular fingerprinting and can be used for distinguishing the genotypes.
8. The ISSR, SSR and pooled analysis grouped rice genotypes in three, four and five major clusters, respectively.
9. The molecular diversity analysis was better than phenotypic diversity for independent clustering of landraces, scented Basmati and non-Basmati genotypes and improved varieties. Molecular marker analysis showed better resemblance with the pedigree as compared to phenotypic evaluation indicating superiority of molecular analysis over phenotypic analysis in diversity study on rice.
10. Among the three methods used for molecular diversity studies, pooled analysis was more effective in distinguishing rice genotypes on their genotypic constitution, parental relationship and evolutionary mechanisms.
11. The F_2 population derived from the cross between a blast susceptible genotype EK 70 and a resistant variety RDN 98-2-3-5-14 segregated in 3:1 ratio indicating the resistance in RDN 98-2-3-5-14 is governed by single dominant gene.
12. Among the 25 SSR markers studied, two markers viz., RM204 and RM215 reported polymorphism between susceptible and resistant parents and bulks indicating their possible association with blast resistance in present mapping population. However, only RM204 was found significantly associated with the blast resistance.

Future line of work:

In the present investigation based on the mean performance and estimates of morphological, pathological and molecular diversity studies of rice genotypes the genetically diverse genotypes be selected for further breeding programme. These genotypes should be from different clusters identified through morphological studies with higher mean performance for grain yield and/or having special quality features. While selecting the genotypes from pathological diversity analysis preference should be given to the clusters with resistant characteristics. The genotypes should be selected from molecular analysis based on diverse genetic constitution and high genetic diversity. Taking into consideration all these guiding principle following genotypes from different clusters have been selected.

S. No	Genotype	Characters
1	Phule Samruddhi	Midlate maturity, dwarf plant stature, long-slender grain type, moderately resistant to blast and high yield.
2	Bhogavati	Midlate maturity, dwarf plant stature, long-slender grain type, moderately resistant to blast and high yield.
3	RDN 99-12	Midlate maturity, dwarf plant stature, long-bold grain type and high yield.
4	RDN 98-2	Midlate maturity, medium- slender grain, resistant to blast, long panicle and high yield.
5	LK 248	Midlate maturity, dwarf plant stature, long- slender grain type and resistant to blast.
6	Vivek Dhan 82	Early maturity, tall plant stature, long- bold grain type and moderately resistant to blast.
7	Siddhagiri	Late maturity, tall plant stature, Bold grain type and high yield.
8	Diwani/ Shyamjeer	Late maturity, tall plant stature, short-slender grain type and very low test weight.
9	Pusa Bas. 1	Early maturity, dwarf plant stature, long panicle, and extra-Long slender scented grain.
10	Pavsal	Late maturity, tall plant stature, more number of tillers per plant, bold grain type and high yield.
11	S.D. 17	Extra-long slender scented grain and high L:B ratio.

12	Khalibagh	Late maturity, dwarf plant stature, extra-long slender grain type and high L:B ratio.
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The further breeding programme may be designed using these parents as per the specific requirements.

The present study reported nine unique SSR alleles which were genotype-specific in the investigation. The diagnostic capacity of these SSR alleles be confirmed over a large sample size. On confirmation, these unique alleles are very useful for cultivar identification and can be used for distinguishing the genotypes from other.

In the present investigation SSR marker RM204 was found associated with blast resistance in rice. In future, markers specific to chromosome 6 will be used for screening the parental lines and polymorphic markers will be used to prepare framework map of the chromosome 6. This map will be used for QTL mapping using phenotypic data of F_2 derived F_3 families.

This marker can be used for further research programme for confirming blast resistance in genotypes, segregating generations and improved lines. On confirmation these markers could be directly useful in analysis of segregating generations, breeding lines and varieties for blast resistance. This approach will take less time and resources than field evaluation. It could prove useful in marker assisted selection in the absence of appropriate testing conditions.

CHAPTER VII

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CHAPTER VIII

VITA

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of

DOCTOR OF PHILOSOPHY

in

AGRICULTURAL BOTANY

(GENETICS AND PLANT BREEDING)

2013

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- Professional** :
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