

**GENETIC DIVERSITY FOR COLD
TOLERANCE IN RICE (*Oryza sativa* L.)**

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B.Sc. (Ag.)

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GENETIC DIVERSITY FOR COLD TOLERANCE IN RICE (*Oryza sativa* L.)

By

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B.Sc. (Ag.)

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DECLARATION

I, **Mr. D. SRINIVAS** , hereby declare that the thesis entitled “**GENETIC DIVERSITY FOR COLD TOLERANCE IN RICE (*Oryza sativa* L.)**” submitted to Acharya N. G. Ranga Agricultural University for the Degree of **MASTER OF SCIENCE IN AGRICUTLURE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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Mr. D. SRINIVAS has satisfactorily prosecuted the course of research and that the thesis entitled “**GENETIC DIVERSITY FOR COLD TOLERANCE IN RICE (*Oryza sativa* L.)**” submitted, is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by his for a degree of any University.

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This is to certify that the thesis entitled **“GENETIC DIVERSITY FOR COLD TOLERANCE IN RICE”** submitted in partial fulfillment of the requirements for the degree of **“MASTER OF SCIENCE IN AGRICUTLURE”** of the **Acharya N. G. Ranga Agricultural University, Hyderabad**, is a record of the bonafide research work carried out by **Mr. D.SRINIVAS**, under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

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

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percent
°C	:	Degree Celsius
μ	:	micro
μg	:	micro gram
μl	:	micro liter
AFLP	:	Amplified Fragment Length Polymorphism
ASG	:	Aromatic Short Grain
bp	:	base pair
BPH	:	Brown Plant Hopper
cM	:	centi Morgan
cm	:	centimeter
CTAB	:	Cetyl Trimethyl Ammonium Bromide
ddW	:	double distilled water
dGTP	:	2'-deoxyguanosine 5'-triphosphate
DNA	:	Deoxyribo Nucleic Acid
dNTPs	:	2'-deoxy Nucleotide 5'- Tri-Phosphates
DRR	:	Directorate of Rice Research
EDTA	:	Ethylene Diamine Tetra Acetic Acid
FAO	:	Food and Agricultural Organization
Fig	:	Figure
g	:	gram
HCl	:	Hydrogen Chloride

HYV	:	High Yielding Varieties
ICAR	:	Indian Council of Agricultural Research
InDels	:	Intron Deletions
IPGRI	:	International Plant Genetic Resources Institute
IRGSP	:	International Rice Genome Sequencing Project
KLAC	:	Kernel Length After Cooking
MAS	:	Marker Assisted Selection
min	:	minutes
ml	:	milli liter
M	:	Molar
mM	:	milli Molar
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide
ng	:	nanogram
NILS	:	Near Isogenic Lines
No.	:	Number
PAGE	:	Poly Acrylamide Gel Electrophoresis
PCR	:	Polymerase Chain Reaction
pH	:	pH
PIC	:	Polymorphic Information Content
PVP	:	Poly Vinyl Pyrrolidone
QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism

RM	:	Rice Microsatellite
rpm	:	revolutions per minute
SAHN	:	Sequential Agglomerative Hierarchial Non overlapping
sec	:	Second
SSLP	:	Simple Sequence Length Polymorphism
SSR	:	Simple Sequence Repeats
STS	:	Sequence Tagged Site
t ha ⁻¹	:	Tonnes per hectare
TAE	:	Tris Acetate EDTA buffer
TBE	:	Tris Borate EDTA buffer
TE	:	Tris EDTA Buffer
Tris. HCl	:	Tris (hydroxyl methyl) aminomethane hydro chloride
UPGMA	:	Unweighted Pair Group Method Arithmetic Averages
U	:	Unit
UPOV	:	International Union for Protection of New Plant Varieties
UV	:	Ultra violet
V	:	Volts
<i>viz.</i>	:	Namely
WPH	:	White Plant Hopper

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ABSTRACT

In the present investigation, 76 rice genotypes were evaluated for diversity to cold tolerance by employing morphological traits and SSR markers. The experiment was laid out in RBD with three replications at college farm, as well as in CRD at the laboratory, Institute of Biotechnology, College of Agriculture, Rajendranagar, Hyderabad during *rabi* 2011.

In the study the genotypes showed significant differences for days to germination, germination percentage, growth rate and seedling vigour at seedling stage. The highest germination percentage was observed with genotype Pusa superfast followed by IR-83222-174 indicating their tolerance to cold. Gedanzipeton has the lowest germination percentage.

Among 76 genotypes tested for various parameters the genotypes Pusa superfast, Emergency, IR-83222-174 and Tellahamsa exhibited tolerance to cold temperature prevailing at seedling stage in terms of leaf yellowing score. The seedlings of these genotypes remained green with higher growth rate.

Analysis of variance indicated the existence of significant difference among genotypes for all the characters observed. Studies of genetic variability revealed high phenotypic and genotypic coefficients of variation, heritability and genetic advance as per cent of mean for the traits viz., number of tillers per plant, grain yield per plant, number of filled grains per panicle and total number of grains per panicle indicating simple selection can be practiced for improvement of these characters. Detection of minor differences

between GCV and PCV for all the characters indicated less influence of environment on these characters.

Morphological divergence by Euclidian² method indicated the existence of significant diversity among genotypes which were grouped into nine clusters. Days to 50 percent flowering, unfilled grains per panicle, filled grains per panicle and panicle length contributed maximum towards genetic diversity. Maximum difference among the genotypes within the same cluster was shown by cluster VII. The maximum inter cluster distance was noticed between the cluster VI and IX. From the inter cluster D² values of nine clusters the highest divergence occurred between cluster VI and IX indicating their importance to contributing genetic diversity. Cluster VI and IX were represented by single genotype indicating high degree of heterogeneity among the genotypes. The genotypes showing cold tolerance in terms of leaf yellowing score, germination percentage, days to germination and growth rate at seedling stage were grouped in cluster I, II, III and V indicating their divergence to cold tolerance from other clusters.

Character association studies revealed that the character grain yield per plant showed significant positive association with filled grains per panicle, number of tillers per plant, total number of grains per panicle and panicle length. This indicated that simultaneous selection of all these characters were important for yield improvement.

A critical analysis of the results by path analysis revealed that the traits number of filled grains per panicle, number of tillers per plant, panicle length and plant height were directly influencing the grain yield per plant. Hence, these traits were considered as important attributes in formulating selection criterion for achieving desired targets.

Diversity analysis for cold tolerance through molecular markers was carried out employing 12 SSR markers. The number of alleles ranged from 2 to 8 with a mean of 4.08 indicating greater magnitude of diversity among the genotypes. The PIC value ranged from 0.688 to 0.943 with an average of 0.855, conformed the markers used were highly informative. Cluster analysis using SSR markers revealed a dendrogram with range of 0.51 to 0.96 similarity indicating considerable amount of diversity among the genotypes. Cluster analysis was done with cold tolerant QTL linked markers. Cluster analysis using SSR markers revealed a dendrogram with range of 0.51 to 0.96 similarity index. The genotypes were grouped into two main clusters. Cluster I consisted of single genotype while the remaining 75 genotypes were accommodated in the cluster II, which was the major cluster. Cluster II was classified into two sub-clusters IIA and IIB. Cluster IIA consisted of single genotype while IIB had 74 genotypes. Cluster IIB sub grouped into IIB1 and IIB2. Cluster IIB1 consisted of single genotype while IIB2 had 73. IIB2 was a major cluster sub grouped into IIB2a and IIB2b. Cluster IIB2a contains 31 genotypes while cluster IIB2b had 42.

Molecular and morphological analysis did not give similar results, but molecular markers (SSR) were more informative and could group the genotypes into different clusters based on their genetic constituency. But both can be complementary for assessment of diversity for cold tolerance and genotype identification in breeding programme.

Based on the present study, cold tolerant (Tellahamsa, IR-83222-174, Emergency and Pusa superfast) and susceptible genotypes were identified. The findings of this study can be utilised in Breeding programmes and marker assisted Breeding for development of cold tolerant varieties for the benefit of the farmers.

Chapter I

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop for more than 60% of the global population and forms the cheapest source of food and energy. In India rice cultivation is very closely inter woven with livelihood and culture of millions of people and is not only the major staple food crop but gained the status of major export commodity in the last ten years.

Rice occupies 44.80 million ha area with a production of 99.18 million tones, which is 42.4% of total food grains and productivity of 2214 kg/ha in India. In Andhra Pradesh it is grown in an area of 43.75 lakh ha with a production of 142.1 lakh tones and productivity of 3248 kg/ha (CMIE Report, 2010). Inorder to meet the food demands of the growing population and to achieve food security in the country, the present production levels need to be increased by 2 m. tones every year.

Rice is cultivated during *kharif* (July-October), *rabi* (November-March) and summer (April-June). Rice nurseries during *Rabi* are raised from second fortnight of November to the end of the December in Telangana region of Andhra pradesh, The nurseries sown in December experience low temperature (daily minimum temperature of of 8-10⁰C) which severely restricts seedling growth and sometimes death of the seedlings. This type of cold injury causes delayed growth of rice plant (Shibata, 1970). Stunted growth of rice seelings under cold temperature is a sign of low cold tolerance (Chang and Vergara,1972). Low temperature is a major climatic problem for growing rice in 25 countries (Kaneda and Beachell, 1974). Temperature is one of the most important limiting factors affecting rice production, and during the early growth stages of rice. The occurrence of low temperature stress inhibits seedling establishment and

eventually leads to non-uniform crop maturation. Even though temperature does not prevent rice germination, it delays its beginning and consequently, plant emergence.

Rice cultivars vary greatly in their tolerance to low temperature. The indica rice subspecies, associated with tropical environments, is more sensitive to low temperature. The more tolerant japonica subspecies is divided into tropical and temperate groups (Glaszmann, 1987; Glaszmann *et al.*, 1990; Oka, 1988).

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species (Chakravarthi and Naraveneni, 2006). Analysis of genetic diversity at molecular level could be used to identify desirable traits and develop genetically unique germplasm that compliments existing cultivars. DNA markers are the most widely used and are predominant due to their abundance and repeatability; they remain unaffected across different stages, seasons, locations and agronomic practices (McCouch *et al.*, 1997). Of the several classes of DNA markers available, microsatellite or simple sequence repeat (SSR) markers are considered as most amenable for several applications including genetic diversity studies due to their multiallelic nature, high reproducibility, co-dominant inheritance, abundance and extensive genome coverage (McCouch *et al.*, 2002).

In order to develop cold tolerant rice varieties and to increase the present levels of heterosis for yield, there is a need to identify and utilize genetically divergent parents for inter and intra sub-specific crosses in rice. A systematic evaluation of germplasm helps in identification of superior and genetically divergent genotypes. Quantification of degree of divergence in a given experimental material is of immense value in identification of diverse genotypes for further use in hybridization.

In view of the above, the present study on genetic diversity for cold tolerance in rice cultivars was carried out with the following objectives:

1. To study the genetic variation for cold tolerance among selected rice genotypes.
2. To assess the genetic variation for cold tolerance among the accessions of rice germplasm using molecular markers.

Chapter II

REVIEW OF LITERATURE

A brief review of available literature in consonance with the objectives of the present investigation in respect of rice crop are reviewed and presented under the following heads.

2.1 Genetic diversity in rice based on morphological traits.

2.2 Assessment of Genetic diversity using molecular markers.

2.1 GENETIC DIVERSITY IN RICE

Rice has the most diversified crop species due to its adaption to a wide range of geographical, ecological and climatic regions (Chang, 1976). The quality preferences of rice consumers have also resulted in a wide diversity of varieties specific to different localities. Although the exact diversity cannot be gauged, it is estimated to be around 140 000 different genotypes. The IRRI gene bank preserves nearly 100000 accessions. India alone has about 86330 accessions, of which 42004 are in the national gene bank (Rai, 1999), which is enriched by further explorations, collections and conservation.

The cultivated Asian rice species (*O.sativa*) is composed of two subspecies, *indica* and *japonica* (Oka, 1988). The genetic diversity of *japonica* is thought to be lower than *indica* (Glaszmann, 1987; Zhang *et al.*, 1992). North East India has been the heartland of rice cultivation since long back. Therefore, the indigenous rice germplasm of Northeast India, including Assam (known as 'Assam rice collection') is enriched with wide genetic diversity and valuable gene system for yield attributes and adaptability (Shastri *et al.*, 1971; Vairavan *et al.*, 1973 and Sharma *et al.*, 1982).

Rice has one of the largest *ex situ* germplasm collections in the world (Jackson and Juggan, 1993). Its rich genetic diversity is in the form of thousands of landraces and progenitor species (Nagaraju *et al.*, 2002). Indian sub-continent has rich diversity of short grain aromatic rices (Sarawgi and Bisne, 2006). South India has been the heartland of rice cultivation since long and the indigenous rice germplasm of South India is enriched with wide genetic diversity (Vanaja *et al.*, 2006). These land races harbour a great genetic potential for rice improvement (Ram *et al.*, 2007).

2.1.1 Approaches to genetic diversity assessment in rice

2.1.1.1 Morphological Methods

The earliest noteworthy attempt on a detailed classification of rice varieties was carried out (Kikkawa, 1912) based on agronomical and physiological characters. Grain size and shape were employed in the classification of rice varieties (Kikkawa, 1912; Graham, 1913) despite minor environmental fluctuations. Graham (1913) stressed the need based system of classification on morphological characters that do not vary with environment and classified the Indian rice varieties based on leaf sheath colour and then on grain dimensions. Beale (1927) distinguished five groups among the rices from lower Burma on the basis of grain length and length/breadth ratio.

Kato *et al.* (1928) were the first to divide *O. sativa* into two types, *japonica* and *indica* based on morphology and sexual affinity. Hector (1930) used anthocyanin pigmentation of leaf sheath, apiculus and stigma colour for classification of Bengal rice varieties. Based on this, anthocyanin pigmentation along with grain characteristics have been used in classification of rice varieties (Sethi and Saxena, 1930; Mitra and Ganguli, 1932; Ram and Chetty, 1934; Ram and Ekbote, 1936). Gustchin (1934) identified two groups within subspecies on the basis of length/ breadth ratio of unhulled grain and divided the species into *indica* and *japonica*.

International Plant Genetic Resources Institute (IPGRI) promotes a minimal set of morphological characters that entails genetic diversity at particular point of genome corresponding to the observed characters or descriptors recognized internationally and thought to be satisfactory for custodial management of crop germplasm collection which covers most of the important crop genera (Erksine and Williams, 1980).

Rice varieties were classified into slender, medium and bold classes based on grain length to breadth ratio (Naidu *et al.*, 1986). Vanangamudi *et al.* (1988) grouped 85 rice varieties based on colour of hulled grain, length, shape, 1000 grain weight, presence or absence of pearl spot and shape of pearl spot. Geetha *et al.* (1994) suggested that various grain characters such as number of panicles, grains per panicle, grain yield, weight and seed shape are useful in identification of rice hybrids.

The rice varieties were differentiated from others by different characters such as 50 per cent flowering, leaf width, plant height, 1000 grain weight, grain length and width and phenol test (Aidy *et al.*, 2000). Thimmanna *et al.* (2000) studied different characters including 1000 grain weight and suggested the usefulness in differentiating the parental lines of rice hybrids. Sarma *et al.* (2004) characterized a set of 142 *ahu*

rice genotypes of Assam based on 21 morph-agronomic traits. According to (Suman *et al.*, 2005) harvest index contributed maximum to the divergence followed by seed density and total number of tillers per plant. Bose and Pradhan (2005) studied the genetic divergence of rice genotypes and suggested that traits like plant height and days to 50 per cent flowering were the major contributors to genetic divergence. Further, these characters are important in the choice of parents for hybridization programme (Le and Yang, 2005; Madhavilatha and Suneetha, 2005; Singh *et al.*, 2006).

The genetic divergence of 50 indigenous scented rice accessions was estimated using 11 quantitative traits (Naik *et al.*, 2006). These traits were effectively used for clustering of rice genotypes (Behla and Rang, 2007).

Joshi *et al.* (2007) characterized 19 varieties of basmati and non-basmati groups based on 52 morphological characters and suggested that most of the morphological characters were significant in varietal identification. To characterize genetic variability among South American wild rices, 20 morph-agronomic traits were assessed and significant differences between species were observed for all the traits (Veasey *et al.*, 2008).

The assessment of genetic diversity using quantitative traits is of prime importance, particularly in discriminating well defined populations. Several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives, of which Mahalanobis's generalized distance occupy a unique place. It is an efficient method to gauge the extent of diversity among genotypes, which quantify the differences among several quantitative traits. For successful hybridization programme, selection of diverse parents is essential since the crosses involving genetically divergent parents are likely to produce good heterotic effects and also help in the recovery of better segregants in the following segregating generations through precise genetic recombination.

The importance of genetic diversity in crop plants was first reported by Darwin (1859) and then the term "morphism" employing genetic morphs was given by Huxely (1955), which means the existence of distinct genetic forms in balance within the population. Pioneering workers on genetic diversity regarded geographical isolation as a reasonable index of genetic diversity (Vavilov, 1926 and Moll *et al.*, 1962). However, scientists working with different crops observed that there was no correlation between geographical distribution and genetic diversity (Murty *et al.*, 1965 in linseed; Arunachalam and Ram, 1967 in sorghum; Bhat, 1970 in green gram; Joshi *et al.* 1988

in bajra). Genetic improvement in any crop mainly depends upon the amount of genetic variability present in the population.

2.1.1.2 Low Temperature-Plant growth

Chilling injury limits both the rice producing area and the length of the growing season (Sthapit, 1991). Above 1000m altitude, cool weather and cold irrigation water cause delayed heading, leaf yellowing, partial panicle exertion, spikelet degeneration, spikelet sterility and low yields. Poor germination and slow seedling growth are additional symptoms in irrigated short duration rice seeded in February below 900 m in Nepal. He has evaluated both indigenous and exotic genotypes in four different National Rice Cold Tolerance Nursery at Yampaphant, Tapy, Lumle and Chhomro. Leaf color, panicle exertion, spikelet sterility and yield parameters were scored at seedling, booting, anthesis and maturity stages. Local variety Chhomro outyielded all other varieties by having an ability to tolerate chilling at different growth stages at all altitudes.

Alioune Coly and Richard-Toll. (1979) reported that rice plant is affected during its stages of growth by low temperature. Only water temperature has an effect on rice growth in early stage. During this phase of growth, all growing points of the rice plant are below the surface of the water. From flag leaf differentiation upto final stage of reduction division of the pollen mother cell, both air and water temperature have serious effect on rice growth and yield.

Reddy *et al.* (1987) reported that spikelet sterility appeared at epidemic levels in farmers' field of the North Telangana Zone of Andhra Pradesh in winter. They screened Twenty-two rice varieties sown in the winter season for spikelet sterility and counted Sterile and healthy spikelets on 10 randomly selected panicles in each plot. They found Genotypic differences for spikelet sterility were significant. Sterility varied from 2.7 to 18%. The lowest was in NLR 26706 and the highest in WGL33343.

Vishwambaran *et al.* (1989) conducted field experiments to identify the factors responsible for the low productivity of high yielding varieties in winter season. The rice varieties showed the variation in rice yield between the different planting dates were primarily due to spikelet sterility and concluded that the delayed planting will reduce the yield.

Umesh (1990) screened 100 rice lines for cold tolerance during 1987-88 in Nigeria. Eight lines failed to germinate. Seedlings in the nursery exhibited stunted growth, leaf yellowing, scorched leaf tips, retarded tillering and poor root

development. Eight entries were completely sterile (100%) and in remaining 64 entries sterility ranged from 18.0 to 60.0 per cent. This was due to low temperature at anthesis in late March to early April. Days to 50 per cent flowering and plant height were also affected.

Pandey and Gupta (1993) crossed four cold-tolerant rice varieties with two cold susceptible ones in all possible combinations to study the inheritance of genes governing panicle exertion. He reported that single dominant gene designed as Ctr-1 is responsible for panicle exertion.

Bertin *et al.* (1995) screened rice varieties for chilling tolerance during germination and vegetative growth using different techniques. The effects of temperature during germination were investigated from 10-25⁰C. The screening was most effective at 10⁰C. Time of data collection has to be considered in order to discriminate slow germinating from chilling sensitive varieties. Chilling survival tests applied at 2-leaf stage revealed that different chilling durations brought complementary results. The longer treatments allowed to identify the most tolerant varieties, while shorter treatments allowed distinguishing intermediate from most sensitive rice.

Williams and Wensing (1997) reported that the periods during which temperature treatments were applied are referred to as microspore, flowering or the entire reproductive stage (EAS) respectively. The medium grain cultivar Millin was most tolerant to low temperatures at microspore, flowering and the entire reproductive stage, producing the greatest per cent filled grains and harvesting index. The long grain cultivar Langi was most sensitive to low temperatures when exposed for the entire reproductive stage.

Qian *et al.* (2000) investigated the seedling cold tolerance (SCT) by using a doubled haploid population derived from anther culture of F1 hybrid between a typical *indica* cv. and a *japonica* cv.. By dynamically analyzing every day's survival percentages of the parents and DH lines under 7-d cold plus 9-d normal temperature condition, the quantitative trait loci (QTLs) for SCT have been mapped based on a molecular linkage map constructed from this population. The results show that two parents had significant differences in SCT and the segregation of SCT in DH lines was basically a continuous distribution with most serious injury on the 6th d of the cold treatment. A total of 4 QTLs for SCT have been identified on chromosomes 1, 2, 3 and 4 respectively and the additive effects of qSCT-1, qSCT-2 and qSCT-3 have been

contributed by the *japonica* cv JX17, but that of qSCT-4 has been contributed by the *indica* cv ZYQ8. The mechanism of SCT seems complicated since the above 4 QTLs detected at different stages during the treatment and further study on the genotypes for these SCT QTLs in the DH lines shows transgressive segregation.

Kumari Priyanka and Mishra (2001) observed that out of several biochemical parameters, non-reducing sugars and phenols were found to be most efficient markers for screening of cold tolerant genotypes at seedling stage as these traits exhibited highly significant correlation with cold tolerance.

Kim *et al.* (2003) confirmed OPT8₅₁₁ marker association with cold tolerance of rice through RAPD analysis for the cold tolerance of 94 F₂ plants from a cross between Dular (*indica*, cold-sensitive cultivar) and Toyohatamochi (*japonica*, cold tolerant cultivar) and this marker could be of use in marker-assisted selection for cold tolerance in rice. The clone OPT8₅₁₁ was amplified from polymerase chain reaction of genomic DNA in 21 rice cultivars by custom-made primer.

Andaya *et al.* (2003) constructed a genetic map using 191 recombinant inbred lines derived from a cross between a temperate *japonica*, M-202, and a tropical *indica*, IR50, in order to locate quantitative trait loci (QTLs) conferring cold tolerance. The map with a total length of 1,276.8 cM and an average density of one marker every 7.1 cM was developed from 181 loci produced by 175 microsatellite markers. QTLs on chromosomes 1, 2, 3, 5, 6, 7, 9 and 12 were identified to confer cold tolerance at the booting stage.

Ashok Kumar and Reddy (2003) evaluated the effect of low temperature and phosphorus fertilization on seedling growth of rice variety IR-64. The treatments consisted of three dates of wet nursery sowing (20 Nov, 1Dec, and 10 Dec) and four levels of P fertilization (0.5, 1.0, and 2.0 kg P₂O₅ 100 m⁻²). They found a positive relation between minimum temperature and root length and between seedling height and seedling weight, confirming that a temperature decline resulted in decreased growth of rice seedlings.

Yong *et al.* (2003) observed that cold temperature damage is a common problem for early planted rice in temperate countries and they exposed the plants to 15⁰C (cold acclimated) or 25⁰C (non-acclimated) for 3 days. All plants were then

exposed to chilling temperature at 5⁰C for 3 days and allowed recover at 25⁰C for 5days. Leaf fresh weight, relative water content, chlorophyll a fluorescence and quantum yield showed that cold acclimated leaves were less affected by chilling compared to non-acclimated.

Guo *et al.* (2006) studied responses of antioxidative defense systems to chilling and drought stresses in four cultivars of rice (*Oryza sativa* L.) differing in sensitivity, two of them (Xiangnuo no. 1 and Zimanuo) are tolerant to chilling but sensitive to drought and the other two (Xiangzhongxian no. 2 and IR50) are tolerant to drought but sensitive to chilling and the results indicated that tolerance to drought or chilling in rice is well associated with the enhanced capacity of antioxidative system under drought or chilling condition, and that the sensitivity of rice to drought or chilling is linear correlated to the decreased capacity of antioxidative system.

Renata *et al.* (2006) reported that evaluation of cold tolerance under field conditions is limited by environmental variation, which makes it difficult to identify genetically superior lines. Evaluation of cold tolerance under controlled temperature conditions may be performed by assessing percentage of reduction in coleoptile length and coleoptile growth.

Shahi and khush (2008) examined One hundred thirty-four rice varieties for cold tolerance. Seedling tolerance was scored by growing seedlings in the phytotron in Yoshida's culture medium at 10⁰ C for 30 days and applying IRRI's Standard Evaluation System of Rice. Similarly, adventitious root regeneration was scored by growing seedlings in Yoshida's culture medium at 10⁰ C for 10 days and Rice varieties could be grouped into four categories according to reactions for cold tolerance at two stages of growth. Inheritance of cold tolerance was examined in seven hybrids between tolerant and nontolerant parents. Cold tolerance as measured by leaf yellowing is governed by a single dominant gene. A single dominant gene also controls cold tolerance as measured by root regeneration ability.

Angelica Lindlof (2008) reported the use of biotechnological methods to know the cold tolerance mechanisms. The result showed that both oat and rice are cold responsive, with many of the previously identified cold regulated genes having a counterpart in these species. In rice, however, the response is less dynamic than in the model organism *Arabidopsis thaliana* and this may explain its inability to fully cold acclimate.

Sharifi (2008) studied the inheritance and heritability of cold tolerance at the germination stage in crosses between seven rice genotypes by partial diallel crosses between them. Analysis of variance indicated that there were highly significant differences among the replications, genotypes, General Combining Ability (GCA) and Specific Combining Ability (SCA) for percentage of reduction in Radicle Length (RL), percentage of reduction in plumule length (PR) and percentage of reduction in germination measure at percentage (GP).

Akhil *et al.* (2009) analysed the pattern of variation and the genetic base for cold tolerance at early growth stages among fifty seven strains of Asian rice including cultivated rice (*Oryza sativa* ssp. *indica* and ssp. *japonica*) and its wild progenitor (*Oryza rufipogon*). It is found that at germination stage, both *indica* and *japonica* subspecies tended to be more tolerant than *O. rufipogon*.

Changrong *et al.* (2009) reported that the seed from varieties B55, Banjiemang, HSC55, and Lijiangheigu could germinate quickly at low temperature. At seedling stage, the seedling growth was severely delayed by low temperature stress. Changes in leaf number, leaf elongation rate, tiller number, days to 1st tiller, plant height, and shoot dry weight were useful measurements for evaluating the cold tolerance of rice seedlings.

Sujoita and Majumder (2009) reported that early heading coupled with high yielding potentiality is a desired objective in rice breeding programme for boro culture in eastern India. Spikelet number per panicle is a major target trait for improving rice. Development of new plant types and hybrids are two major approaches for improving yield potential of irrigated rice.

Ye *et al.* (2010) attempted to identify the QTL controlling low temperature induced spikelet sterility in rice. The progeny of F₂, BC₁F₁ × BC₂F₁ population were exposed to 21/15⁰ C for 15 days at the booting stage, and spikelet sterility was assed. For genotyping, 92 polymorphic markers from 373 SSR and 325 STS primer pairs were used. A major QTL was initially identified on the short arm of chromosome 10 by selective genotyping using highly tolerant and susceptible progeny from F₂ and BC₁F₁ populations.

Cheng *et al.* (2012) identified four QTLs (*qSRS1*, *qSRS7*, *qSRS11a*, and *qSRS11b*) for SRS (survival rate of seedlings) at seedling stage under cold stress. using survival rate of seedlings (SRS) and spikelet fertility (SF) as the index traits of cold

tolerance (CT) and heat tolerance (HT) they identified four QTLs for SF (spikelet fertility) on chromosomes 4, 5, 6, and 11 at anthesis under heat stress. Among them, two QTLs (*qSF4* and *qSF6*) contributed to HT because they reduced the trait difference between heat stress and normal conditions.

2.1.1.3 Limitation of Morphological Method

According to Payne (1987) a major limitation of morphological characters lied in the fact that majority of the newer varieties had similar physical characters, particularly with respect to seed and seedling characters. This view was also supported by the work of Singhal and Prakash (1992) who observed a high degree of morphological similarity among the released wheat varieties. To obtain precise and repeatable morphological descriptors of a variety, extensive replicated trials were to be conducted (Lin and Binns, 1984; Zeven, 1990).

In addition, morphological characterization is time consuming, requires large land area and highly skilled personnel (Luchese *et al.*, 1999). Moreover, most of these descriptors are influenced by environment and are under multigenic control and require complex statistical treatments to determine the genetic distinctiveness (Cooke, 1999).

Thus, direct and unambiguous correspondence between genotypes and phenotypes were often not provided by morphological data. This led to consider the use of molecular markers to supplement morphological characters.

2.1.1.4 Mahalanobis's D^2 analysis

Mahalanobis's D^2 analysis is a powerful tool in quantifying the degree of divergence between biological populations at genetic level and provides a quantitative measure of association between geographic and genetic diversity based on generalized distance (Mahalanobis, 1936).

Murthy and Arunachalam (1966) emphasized the importance of genetic diversity in selection of parents for hybridization in different crops and reported that the greatest contributing characters to genetic diversity in grain crops were flowering time, plant height and tiller number per plant.

Vivekanandan and Sukanya Subramanian (1993) carried out genetic divergence using D^2 analysis in 28 genotypes of rainfed rice and grouped them into five clusters. Plant height and grain yield contributed considerably (85 %) to the total divergence. The geographical diversity was not found to be related to genetic diversity.

Ushakumari and Rangaswamy (1997) carried out genetic divergence among 74 rice genotypes and grouped them into six clusters by multivariate analysis using D^2 statistic. The characters grain yield/plant and plant height contributed maximum towards divergence. Relation between geographic diversity and genetic diversity was not observed.

Pandey *et al.* (1999) reported that the studies on fifty rice genotypes by using Mahalanobis's D-statistic assigned the genotypes into six clusters and revealed no correlation between genetic diversity and geographical diversity. The characters days to 50 per cent flowering, plant height, 100-seed weight contributed maximum to the genetic diversity.

Madhavi Latha, (2002) studied 54 rice genotypes for their genetic divergence which were grouped into nine clusters. The mode of distribution of genotypes from different eco- geographical regions into various clusters was at random indicating that geographical diversity and genetic diversity were not related. The characters, plant height and days to 50 per cent flowering were contributed maximum towards genetic divergence

Surender Raju (2002) assessed the genetic divergence among 42 genotypes by Mahalanobis's D^2 statistic and grouped them into 12 clusters. The characters 100-grain weight, volume expansion ratio, kernel length and days to 50per cent flowering have contributed maximum to the genetic divergence.

Vanaja *et al.* (2003) carried out genetic divergence among 56 high yielding rice genotypes representing different eco-geographical regions and grouped them into nine clusters. It was concluded that there was no association between geographical distribution and genetic diversity. At the same time the relationship between genetic divergence and geographical origin also exists to some extent.

Madhavalatha and Suneetha (2005) evaluated 54 elite rice germplasm lines for their genetic diversity with regard to yield, yield components and quality traits. The genotype were grouped into nine clusters, a perusal of the results on cluster means revealed plant height and days to 50 per cent flowering, together accounted for 82.04 per cent of the total genetic divergence, indicating their importance in the choice of parents for hybridization programmes..

Ramya and Senthil Kumar (2008) carried out an investigation with fifty genotypes for studying genetic diversity and these genotypes formed into eleven

clusters. Number of grains per panicle, number of productive tillers per plant and grain yield per plant showed high per cent contribution towards genetic divergence.

Subudhi. *et al.* (2009) studied the divergence analysis for quality characters in 41 rice genotypes. The genotype grouped into 10 clusters. The cluster I is having highest cluster mean for head rice recovery and cluster IX is highest L/B ratio and lowest KB. The genotypes of these clusters may be used as parents. Selected for future breeding programme.

Banumathy *et al.* (2010) evaluated 53 rice genotypes for yield and yield attributing characters using D^2 analysis, based on the analysis the genotype were grouped into 11 clusters. Among the eight traits studied, grain yield, days to 50 per cent flowering, total grains per panicle and plant height together contribute 86.62% towards total divergence. Therefore, these characters may be given importance during hybridization programme.

Baradhan *et al.* (2010) used Mahalanobis D^2 statistics to study the nature and magnitude of genetic diversity among 45 genotypes of rice. The genotypes were grouped into six clusters. In the present study, parental lines selected from cluster VI for grain yield, number of productive tillers per hill, panicle length and plant height and cluster I for number of grains per panicle exhibited high mean values. It is suggested that varietal improvement through hybridization, should be done among the genotypes more of divergent clusters.

Dushyantha Kumar *et al.* (2010) evaluated 39 rice genotypes using Mahalanobis D^2 statistic. On the basis of genetic distance, these genotypes were grouped into eight clusters. Based on high mean performance of the traits studied, may be used as parents in future breeding programme to achieve desirable segregants for tall plant stature, number of panicles per plant, panicle length and high grain yield.

Hosan *et al.* (2010) evaluated twenty rice landraces to assess the nature and magnitude of genetic divergence among them. Based on twelve characters, the genotypes were grouped into five clusters. Number of filled grains number /panicle, number of panicles/plant and grain yield contributed considerably towards total divergence.

Nibedita Mohanty *et al.* (2010) analysed 40 rice genotypes using Mahalanobis's D^2 analysis. The 40 rice genotypes were grouped into five clusters. The pattern of distribution of genotypes from different eco-geographical regions into various clusters was at random indicating that geographical diversity and genetic diversity were not

related. The characters viz. leaf area at 90 DAS, Number of grains panicle-1 and days to 50% flowering contributed maximum towards genetic divergence among the genotypes.

Ubarhande *et al.* (2010) evaluated Forty four indica rice cultivars for sixteen quantitative traits to examine the nature and magnitude of variability and genetic divergence. On the basis of D^2 analysis 44 rice genotypes were grouped into nine clusters and three ungrouped clusters. The traits viz. days to maturity, effective tillers/plant, grain yield/plant and chlorophyll content. Contribution (%) towards the total divergence.

Anandan *et al.* (2011) evaluated forty four salt tolerant rice genotypes from different geographic regions were assessed using Mahalanobis D^2 and principal component analysis (PCA). The D^2 statistics grouped the genotypes into 12 distinct clusters. The number of grains per panicle (42.71%), followed by the grain yield per plot (29.81%), was the major contributor to the total divergence. Hybridization among the genotypes which had the maximum inter-cluster distances could produce heterotic combinations and wide variability in segregating generations for many beneficial traits.

Mall *et al.* (2011) studied the grain quality characteristics of 24 drought promising genotypes were evaluated under irrigated and drought conditions for studying variability and genetic divergence. Genotypes were grouped into seven clusters, where cluster I was the largest and contained 18 genotypes while, rest of the clusters contained single genotypes. Based on clustering pattern and per se performance five genotypes were identified, which may be used in breeding programme to obtain improved genotypes with desirable attributes for stress environments.

Narendra Pratap *et al.* (2011) assessed the genetic divergence in 104 genotypes of rice comprising aromatic as well as non-aromatic germplasm lines by using Non-hierarchical Euclidean cluster analysis. The 104 genotypes were grouped into 11 distinct clusters. Clustering pattern of genotypes showed lack of any relationship between geographic origin and genetic diversity. The crossing between superior genotypes of above diverse cluster pairs may provide desirable transgressive segregants for developing high yielding varieties of aromatic and non-aromatic rice is recommended.

Parikh *et al.* (2011) evaluated the Seventy-one rice accessions to study the diversity pattern among the genotypes. The genotypes were grouped into eight clusters. The mode of distribution of genotypes from different geographic regions into various

clusters was at random, indicating no association between geographical distribution of genotypes and genetic divergence. The inter cluster distance in most of the cases was higher than the intra-cluster distance, indicating wider genetic diversity among the accessions of different groups.

Vennila *et al.* (2011) evaluated 41 rice genotypes to identify diverse genotype for nine yield and yield attributing characters using Mahalanobis D^2 statistics. Based on the genetic distance all the 41 genotypes were grouped under thirteen different clusters. The characters like number of grains per panicle, plant height, grain length and grain breadth contributed maximum towards genetic diversity. Hence these characters could be given due importance for selection of genotypes for further crop improvement programme.

Yadav *et al.* (2011) evaluated nine genotypes for genetic divergence among them based on nine characters grouped and arranged in to 3 non-overlapping clusters by Tocher's method and Mahalanobis (1936) D^2 statistics. Clustering pattern of genotypes showed no definite relationship between genetic divergence and geographical distribution of genotypes. On the basis of analysis result of genetic divergence and mean performance of four diverse and superior genotypes viz., CSR-27, CSR-36, CSR-839-3 and USAR-3 being exceptionally better for one or more traits and reasonable for other, as compared to checks were selected.

2.1.2 Genetic Parameters

2.1.2.1 Variability

The nature and extent of variability forms the basis for all crop improvement programmes. According to Allard (1960), yield is polygenically controlled quantitative character and is highly influenced by environment.

Partitioning of observed variability into heritable and non-heritable components is very much essential to get a true indication of the genetic coefficient of variability as a useful measure of the magnitude of genetic variance present in the population.

A brief review of studies on genotypic and phenotypic coefficients of variation in rice is presented hereunder in tabular form.

S.No.	Character	Range	Reference
1.	Days to 50 per cent flowering	High	Awasthi and Pandey (2000) Rita Bisne <i>et al.</i> (2011) Mulugeta Seyoum <i>et al.</i> (2012).
		Low	Patil <i>et al.</i> (2003) Sinha <i>et al.</i> (2004) Vijayalakshmi <i>et al.</i> (2008) Manoj Kumar Prajapate (2011).
2.	Plant height(cm)	High	Sinha <i>et al.</i> (2004) Akhtar <i>et al.</i> (2011) Mulugeta Seyoum <i>et al.</i> (2012).
		Moderate	Tara Satyavathi <i>et al.</i> (2001) Nayak <i>et al.</i> (2002) Krishna <i>et al.</i> (2008).
		Low	Chikkalingaiah <i>et al.</i> (1999) Patil <i>et al.</i> (2003) Kole <i>et al.</i> (2008)
3.	Number of tillers per plant	High	Nayudu <i>et al.</i> (2007) Chandra <i>et al.</i> (2009) Akhtar <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011)
		Moderate	Vange and Ojo (1997) Nagajyothi (2001) Krishna <i>et al.</i> (2008)
		Low	Niranjana Murthy <i>et al.</i> (1999) Tara Satyavathi <i>et al.</i> (2001), Surender Raju (2002) Pankaj Garg <i>et al.</i> (2010).
4.	Panicle length (cm)	High	Tripathi <i>et al.</i> (1999) Nayudu <i>et al.</i> (2007)
		Low	Tara Satyavathi <i>et al.</i> (2001)

S.No.	Character	Range	Reference
			Patil <i>et al.</i> (2003) Kole <i>et al.</i> (2008) Manoj kumar prajapate (2011)
5.	Number of filled grains per panicle.	High	Nayak <i>et al.</i> (2002) Patil and Sarawgi (2005) Nayudu <i>et al.</i> (2007) Pankaj Garg <i>et al.</i> (2010) Mulugeta seyoum <i>et al.</i> (2012)
		Moderate	Sarma and Roy (1993) Tara Satyavathi <i>et al.</i> (2001) Kole <i>et al.</i> (2008)
		Low	Nath and Talukdar (1997)
6.	1000 grain weight (g)	High	Patil and Sarawgi (2005) Nayudu <i>et al.</i> (2007) Chandra <i>et al.</i> (2009) Mulugeta seyoum <i>et al.</i> (2012)
		Low	Chikkalingaiah <i>et al.</i> (1999) Vange <i>et al.</i> (1999) Tara Satyavathi <i>et al.</i> (2001)
7.	Grain yield per plant (g)	High	Sinha <i>et al.</i> (2004) Nayudu <i>et al.</i> (2007) Anbanandan <i>et al.</i> (2009) Pankaj Garg <i>et al.</i> (2010) Mulugeta seyoum <i>et al.</i> (2012)
		Moderate	Chauhan <i>et al.</i> (1993) Pradyumna Rao <i>et al.</i> (1996) Nagajyothi (2001) Tara Satyavathi <i>et al.</i> (2001)
		Low	Supriyo Chakraborty and Hazarika (1994)

2.1.2.2 Heritability

Heritability in broad sense refers to the genetic variation present in the population in relation to the total observed variance. Consistency in the performance of selection in succeeding generations depends on the magnitude of heritable variation present in relation to observed variation. Basic information on heritability is a prerequisite for planning any breeding programme. High heritability indicates that it should be easy to conduct effective selection for the trait

A brief review of studies on heritability in rice is presented hereunder in tabular form.

S.No.	Character	Range	Reference
1.	Days to 50 per cent flowering	High	Nayak <i>et al.</i> (2002) Sankar <i>et al.</i> (2006) Bharadwaj <i>et al.</i> (2007) Kishore <i>et al.</i> (2008)
		Medium	Chauhan <i>et al.</i> (1993) Sahdev Singh <i>et al.</i> (1996) Singh and Choudhary (1996)
2.	Plant height (cm)	High	Akhtar <i>et al</i> (2011) Ravindra Babu <i>et al.</i> (2011) Mulugeta seyoun <i>et al</i> (2012)
		Moderate	Akinwale <i>et al.</i> (2008)
		Low	Vijayalakshmi <i>et al.</i> (2008)
3.	Number of tillers per plant	High	Sankar <i>et al.</i> (2006) Nayudu <i>et al.</i> (2007) Anbanandan <i>et al.</i> (2009) Nandan and Sweta Singh (2010) Immanuel selvaraj <i>et al.</i> (2011)
		Moderate	Satya Priya Lalitha and Sreedhar (1998) Venkata Suresh (2001)

S.No.	Character	Range	Reference
		Low	Ramesh Kumar (1989) Niranjana Murthy <i>et al.</i> (1999)
4.	Panicle length (cm)	High	Chikkalingaiah <i>et al.</i> (1999) Nayak <i>et al.</i> (2002) Nayudu <i>et al.</i> (2007) Mohan and Devendra (2011)
		Moderate	Satya Priya Lalitha and Sreedhar (1996) Venkata Suresh (2001)
		Low	Singh and Chaudhary (1996)
5.	Number of filled grains per panicle.	High	Nagajyothi (2001) Patil <i>et al.</i> (2003) Sankar <i>et al.</i> (2006) Nayudu <i>et al.</i> (2007) Krishna <i>et al.</i> (2008) Pankaj Garg <i>et al.</i> (2010)
		Moderate	Satya Priya Lalitha and Sreedhar (1996) Mulugeta <i>et al.</i> (2012)
6.	1000 grain weight (g)	High	Bharadwaj <i>et al.</i> (2007) Nandan and Sweta Singh (2010) Abdul Fiyaz <i>et al.</i> (2011) Mulugeta seyoun <i>et al.</i> (2012)
		Moderate	Ramesh kumar (1989)
7.	Grain yield per plant (g)	High	Nayudu <i>et al.</i> (2007) Anbanandan <i>et al.</i> (2009) Pankaj Garg <i>et al.</i> (2010)
		Moderate	Satya Priya Lalitha and Sreedhar (1999)
		Low	Bharadwaj <i>et al.</i> (2007)

2.1.3 CHARACTER ASSOCIATION

Study of character association helps the breeder in fixing a selection criteria for grain yield in parental lines, such that selections will be effective in isolating the plants with desired combination of characters. Phenotypic correlation is the correlation of phenotypic values and is subjected to changes in the environment. It measures the environment deviation together with non-additive gene action. Genotypic correlation is the correlation of breeding value. Hence, knowledge of association between different characters is highly essential for planning a sound breeding programme.

Several workers have studied the correlation coefficients in rice and contradictory association have been reported for almost all the character pairs which may be due to the different experimental material handled by them.

A brief review of studies on the association of characters in rice is presented here under:

2.1.3.1 Association of yield component characters with grain yield/plant in rice

Character	Nature of association	Reference
Days to 50per cent flowering	Positive significant	Suman (2003) Kuldeep <i>et al.</i> (2004) Sankar <i>et al.</i> (2006) Krishna <i>et al.</i> (2008) Abdul Fiyaz <i>et al.</i> (2011)
	Positive non-significant	Rao and Shrivastav (1999) Madhavi Latha <i>et al.</i> (2005) Satish Chandra <i>et al.</i> (2009) Nandan and Sweta Singh (2010)
	Negative significant	Eradasappa <i>et al.</i> (2007) Pankaj Garge <i>et al.</i> (2010) Ravindra Babu <i>et al.</i> (2012)
	Negative non-significant	Sarma and Roy (1993) Yolanda and Vijendra Das (1995) Meenakshi <i>et al.</i> (1999) Muluget <i>et al.</i> (2012)
		Nayak <i>et al.</i> (2001) Madhavi Latha (2002)

Character	Nature of association	Reference
Plant height	Positive significant	Eradasappa <i>et al.</i> (2007) Krishna <i>et al.</i> (2008) Abdul Fiyaz <i>et al.</i> (2011)
	Positive non-significant	Suman (2003) Madhavi Latha <i>et al.</i> (2005) Karad and Pol (2008) Nandan and Sweta Singh (2010)
	Negative significant	Yolanda and Vijendra Das (1995) Reddy <i>et al.</i> (1997) Rao and Shrivastava (1999) Tara Satyavathi <i>et al.</i> (2001) Mahto <i>et al.</i> (2003)
	Negative non-significant	Gupta <i>et al.</i> (1998) Meenakshi <i>et al.</i> (1999) Satish Chandra <i>et al.</i> (2009) Muluget seyoum <i>et al.</i> (2012)
No. of tillers/plant	Positive significant	Suman (2003) Sankar <i>et al.</i> (2006) Eradasappa <i>et al.</i> (2007) Krishna <i>et al.</i> (2008) Satish Chandra <i>et al.</i> (2009) Abdul Fiyaz <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
	Positive non-significant	Chaubey and Singh (1994) Yolanda and Vijendra Das (1995) Reddy <i>et al.</i> (1997) Madhavi Latha <i>et al.</i> (2005) Muluget seyoum <i>et al.</i> (2012)
	Negative non-significant	Haque <i>et al.</i> (1991)

Character	Nature of association	Reference
Panicle length	Positive significant	Madhavi Latha (2002) Kuldeep <i>et al.</i> (2004) Sankar <i>et al.</i> (2006) Eradasappa <i>et al.</i> (2007) Satish Chandra <i>et al.</i> (2009) Basavaraja <i>et al.</i> (2011)
	Positive non-significant	Vange <i>et al.</i> (1999) Tara Satyavathi <i>et al.</i> (2001) Madhavi Latha <i>et al.</i> (2005) Krishna <i>et al.</i> (2008) Muluget seyoum <i>et al.</i> (2012)
	Negative non-significant	Chauhan <i>et al.</i> (1993) Ramesh Babu (1999)
Number of filled grains/panicle	Positive significant	Sankar <i>et al.</i> (2006) Eradasappa <i>et al.</i> (2007) Krishna <i>et al.</i> (2008) Satish Chandra <i>et al.</i> (2009) Pankaj Garg <i>et al.</i> (2010) Padmaja <i>et al.</i> (2011) Zulqarnain <i>et al.</i> (2012)
	Positive non-significant	Sarma and Roy (1993) Chaubey and Singh (1994) Paul and Sarmah (1997) Madhavi Latha <i>et al.</i> (2005) Nandan and Sweta Singh (2010) Akinwale <i>et al.</i> (2011)
1000-grain weight	Positive significant	Satish Chandra <i>et al.</i> (2009) Yadav <i>et al.</i> (2011) Basavaraja <i>et al.</i> (2011) Zulqarnain <i>et al.</i> (2012)
	Positive non-significant	Supriyo Chakraborty and Hazarika (1994) Gupta <i>et al.</i> (1998)

Character	Nature of association	Reference
		Vange <i>et al.</i> (1999) Madhavi Latha <i>et al.</i> (2005)
	Negative non-significant	Suman (2003)
	Negative non-significant	Wilfred Manuel and Rangaswamy (1993) Geeta <i>et al.</i> (1994) Muluget seyoum <i>et al.</i> (2012)

2.1.3.2 Association among the yield component traits in rice, association of days to 50 per cent flowering with

Character	Nature of association	Reference
Plant height	Positive significant	Kavitha and Sree Rama Reddi (2001) Sakthivel (2001) Madhavi Latha (2002) Ravindra Babu <i>et al</i> (2012)
	Positive non-significant	Chauhan <i>et al.</i> (1993) Ramesh Babu (1999)
	Negative significant	Satish Chandra <i>et al.</i> (2009)
	Negative non-significant	Debchoudhury and Das (1998) Rao and Shrivastav (1999) Nandan <i>et al</i> (2010)
Number of tillers/plant	Positive significant	Sawant <i>et al.</i> (1995) Sakthivel (2001)
	Positive non-significant	Kavitha and Sree Rama Reddy (2001) Surender Raju (2002) Satish Chandra <i>et al.</i> (2009)
	Negative non-significant	Yolanda and Vijendra Das (1995)
	Negative and significant	Meenakshi <i>et al.</i> (1999) Ravindra Babu <i>et al.</i> (2012)
Panicle length	Positive significant	Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)

Character	Nature of association	Reference
	Positive non-significant	Sarma and Roy (1993) Sawant <i>et al.</i> (1995) Kavitha and Sree Rama Reddi (2001)
	Negative non-significant	Chauhan <i>et al.</i> (1993) Ramesh Babu (1999) Nandan <i>et al.</i> (2010)
Number of filled grains/panicle	Positive significant.	Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Mulugeta seyoum <i>et al.</i> (2012)
	Positive non-significant	Satish Chandra <i>et al.</i> (2009) Vange <i>et al.</i> (1999) Nandan <i>et al.</i> (2010)
	Negative non-significant	Sawant <i>et al.</i> (1995) Meenakshi <i>et al.</i> (1999)
1000-grain weight	Positive significant	Ravindranath <i>et al.</i> (1982) Yadav <i>et al.</i> (2011)
	Positive non-significant	Roy <i>et al.</i> (1995)
	Negative significant	Ramesh Babu (1999) Kavitha and Sree Rama Reddy (2001) Nayak <i>et al.</i> (2001) Sakthivel (2002)
	Negative non-significant	Vange <i>et al.</i> (1999) Satish Chandra <i>et al.</i> (2009) Nandan <i>et al.</i> (2010)

2.1.3.3 Association among the yield component traits in rice, association of plant height with

Character	Nature of association	Reference
Number of tillers/plant	Positive significant	Janardhanam <i>et al.</i> (2001) Kavitha and Sree Rama Reddy (2001) Surender Raju (2002) Satish Chandra <i>et al.</i> (2009)
	Positive Non-significant	Roy <i>et al.</i> (1995) Yolanda and Vijendra Das (1995)
	Negative significant	Satya Priya Lalitha and Sreedhar (1997) Meenakshi <i>et al.</i> (1999) Nayak <i>et al.</i> (2001) Nayak (2008)
Panicle length	Positive significant	Tara Satyavathi <i>et al.</i> (2001) Madhavi Latha (2002) Sinha <i>et al.</i> (2004) Satish Chandra <i>et al.</i> (2009)
	Positive non-significant	Chaubeyand Singh (1994) Yolanda and Vijendra Das (1995) Reddy <i>et al.</i> (1997) Rao and Shrivastav (1999)
Number of grains/panicle	Positive significant	Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009)
	Positive non-significant	Geeta <i>et al.</i> (1994) Meenakshi <i>et al.</i> (1999) Kavitha and Sree Rama Reddy (2001) Abdus salman khan <i>et al.</i> (2009)
	Negative significant	Debchoudhury and Das (1998) Surender Raju (2002)

Character	Nature of association	Reference
	Negative non-significant	Chauhan <i>et al.</i> (1993) Sawant <i>et al.</i> (1995) Reddy <i>et al.</i> (1997)
1000-grain weight	Positive significant	Reddy <i>et al.</i> (1997) Suman (2003) Yogameenakshi <i>et al.</i> (2004)
	Positive non-significant	Ramesh Babu (1999) Satish Chandra <i>et al.</i> (2009)
	Negative significant	Sukanya Subramanian and Rathinam (1984)
	Negative non-significant	Roy <i>et al.</i> (1995) Meenakshi <i>et al.</i> (1999) Nayak <i>et al.</i> (2001)

2.1.3.4 Association among the yield component traits in rice association of number of tillers/plant with

Character	Nature of association	Reference
Panicle length	Positive significant	Janardhanam <i>et al.</i> (2001) Sakthivel (2001) Padmaja <i>et al.</i> (2011)
	Positive non-significant	Yolanda and Vijendra Das (1996) Kavitha and Sree Rama Reddy (2001) Satish Chandra <i>et al.</i> (2009)
	Negative significant	Chaubey and Richharia (1993) Tara Satyavathi <i>et al.</i> (2001) Suman (2003)
	Negative non-significant	Reddy <i>et al.</i> (1997) Satya Priya Lalitha and Sreedhar (1997) Abdus salman kahan <i>et al.</i> (2009)
Plant height	Positive significant	Meenakshi <i>et al.</i> (1999) Janardhanam <i>et al.</i> (2001) Sakthivel (2001)

Character	Nature of association	Reference
Number of filled grains per panicle	Positive non-significant	Yolanda and Vijendra Dos (1995) Satya Priya Lalitha and Sreedhar (1996) Abdus salman kahan <i>et al.</i> (2009)
	Negative significant	Chaubey and Richharia (1993)
	Negative non-significant	Reddy <i>et al.</i> (1997) Tara Satyavathi <i>etal.</i> (2001) Nayak <i>et al.</i> (2001) Satish Chandra <i>et al.</i> (2009)
1000-grain weight	Positive and significant	Suryanarayana (2000) Sakthivel (2001)
	Positive non-significant	Meenakshi <i>et al.</i> (1999) Kavitha and Sree Rama Reddy (2001) Tara Satyavathi <i>et al.</i> (2001)
	Negative significant	Gopinath <i>et al.</i> (1984) Nayak <i>et al.</i> (2001) Padmaja <i>et al.</i> (2011)
	Negative non-significant	Reddy <i>et al.</i> (1997) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)

2.1.3.5 Association among the yield component traits in rice association of panicle length with

Character	Nature of association	Reference
Number of tillers/plant	Positive significant	Bala (2001)
	Negative and significant	Ravindra Babu et al (2012)
Number of grains/panicle	Positive significant	Satish Chandra <i>et al.</i> (2009) Abdus salman khan <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011)
	Positive non-significant	Reddy <i>et al.</i> (1997) Debchoudhury and Das (1998) Tara Satyavathi <i>el al.</i> (2001)
	Negative significant	Gopinath <i>et al.</i> (1984)

Character	Nature of association	Reference
	Negative non-significant	Sawant <i>et al.</i> (1995) Nandan <i>et al.</i> (2010)
1000-grain weight	Positive significant	Gopinath <i>et al.</i> (1984) Yogameenakshi <i>et al.</i> (2004) Ravindra Babu <i>et al.</i> (2012)
	Positive non-significant	Kavitha and Sree Rama Reddy (2001) Satish Chandra <i>et al.</i> (2009) Nandan <i>et al.</i> (2010)
	Negative significant	Sukanya Subramanian and Rathinam (1984)
	Negative non-significant	Roy <i>et al.</i> (1995) Vange <i>et al.</i> (1999) Nayak <i>et al.</i> (2001)

2.1.3.6 Association among the yield component traits in rice association of number of grains per panicle with

Character	Nature of association	Reference
1000-grain weight	Positive significant	Ravindranath <i>et al.</i> (1982)
	Positive non-significant	Nanja Reddy <i>et al.</i> (1995) Meenakshi <i>et al.</i> (1999) Satish Chandra <i>et al.</i> (2009)
	Negative significant	Geetha <i>et al.</i> (1994) Nayak <i>et al.</i> (2001)
	Negative non-significant	Kavitha and Sree Rama Reddy (2001) Reddy <i>et al.</i> (1997) Nandan <i>et al.</i> (2010)

2.1.4 PATH COEFFICIENT ANALYSIS

Path coefficient analysis, a statistical device developed by Wright (1921) helps in partitioning of the correlation coefficients into direct and indirect effects of independent variable on dependent variable. As grain yield is a complex character influenced by several factors, selection based on simple correlation without taking into consideration between the component characters is not effective. Hence, path analysis

is of much importance in any plant breeding programme. Correlation in combination with path analysis would give a better insight into cause and effect relationship between different pairs of characters. Dewey and Lu (1959) and Frakes *et al.* (1961) demonstrated the utility of path coefficient analysis in plant selection and since then its application has been extended to almost to every crop.

The findings of earlier workers on the relative contribution of different characters to grain yield in rice are furnished here under in a tabular form:

2.1.4.1 Direct effects

Character	Positive direct effect on grain yield	Negative direct effect on grain yield
Days to 50 per cent flowering	Khedikar <i>et al.</i> (2004) Sankar <i>et al.</i> (2006) Satish Chandra <i>et al.</i> (2009) Basavaraja <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Mulugeta <i>et al.</i> (2012)	Debchoudhury and Das (1998) Gupta <i>et al.</i> (1998) Nayak <i>et al.</i> (2001) Pankaj <i>et al.</i> (2010) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
Plant height	Madhavi Latha (2002) Sankar <i>et al.</i> (2006) Basavaraja <i>et al.</i> (2011) Yadav <i>et al.</i> (2011) Mulugeta <i>et al.</i> (2012) Ravindra Babu <i>et al.</i> (2012)	Saravanan <i>et al.</i> (1996) Tara Satyavathi <i>et al.</i> (2001) Suman (2003) Karad and Pol (2008) Satish Chandra <i>et al.</i> (2009) Akhtar <i>et al.</i> (2011)
Number of tillers/plant	Sankar <i>et al.</i> (2006) Karad and Pod (2008) Satish Chandra <i>et al.</i> (2009) Basavaraja <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Valarmathi and Leenakumari (1998) Kavitha and Sree Rama Reddy (2001) Mulugeta <i>et al.</i> (2012)
Panicle length (cm)	Khedikar <i>et al.</i> (2004) Karad and Pol (2008) Basavaraja <i>et al.</i> (2011) Mulugeta <i>et al.</i> (2012) Ravindra Babu <i>et al.</i> (2012)	Ganesan <i>et al.</i> (1997) Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Pankaj <i>et al.</i> (2010)

Character	Positive direct effect on grain yield	Negative direct effect on grain yield
Number of grains/panicle	Sankar <i>et al.</i> (2006) Satish Chandra <i>et al.</i> (2009) Nandan and Sweta Singh (2010) Mulugeta <i>et al.</i> (2012)	Amirthadevarathinam (1990) Gupta <i>et al.</i> (1998) Akhtar <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
1000-grain weight	Suman (2003) Khedikar <i>et al.</i> (2004) Satish Chandra <i>et al.</i> (2009) Yadav <i>et al.</i> (2011) Akhtar <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011)	Karad and Pol (2008) Pankaj Garge <i>et al.</i> (2010) Basavaraja <i>et al.</i> (2011) Mulugeta seyoum <i>et al.</i> (2012) Ravindra Babu <i>et al.</i> (2012)

2.1.4.2 Indirect effects

Indirect effects of days to 50 per cent flowering on grain yield through

Character	Positive indirect effect	Negative indirect effect
Plant height	Basavaraja <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Debchoudhury and Das (1998) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)
Number of tillers/plant	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Meenakshi <i>et al.</i> (1999) Kavitha and Sree Rama Reddy (2001) Padmaja <i>et al.</i> (2011) Basavaraja <i>et al.</i> (2011)
Panicle length	Basavaraja <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)
Number of filled grains/ panicle	Debchoudhury and Das (1998) Nayak <i>et al.</i> (2001) Madhavi Latha (2002)	Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)

1000-grain weight	Kavitha and Sree Rama Reddy (2001) Madhavi Latha (2002) Basavaraja <i>et al.</i> (2011)	Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
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Indirect effects of plant height on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 per cent flowering	Gupta <i>et al.</i> (1998) Madhavi Latha (2002) Madhavi Latha <i>et al.</i> (2005) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Sinha <i>et al.</i> (1999) Kavitha and Sree Rama Reddy (2001) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011)
Number of tillers/plant	Nagajyothi (2001) Madhavi Latha <i>et al.</i> (2005) Satish Chandra <i>et al.</i> (2009) Basavaraja <i>et al.</i> (2011)	Nayak <i>et al.</i> (2001) Tara Satyavathi <i>et al.</i> (2001) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
Panicle length	Satish Chandra <i>et al.</i> (2009) Rajamadhan <i>et al.</i> (2011) Basavaraja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Padmaja <i>et al.</i> (2011)
Number of grains/panicle	Madhavi Latha <i>et al.</i> (2005) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Valarmathi and Leena Kumari (1998) Suryanarayana (2000)
1000-grain weight	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Rajamadhan <i>et al.</i> (2011)	Nayak <i>et al.</i> (2001) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)

Indirect effects of number of tillers/plant on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 per cent flowering	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Amirthadevarathinam (1998) Kavitha and Sree Rama Reddy (2001) Basavaraja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
Plant height	Madhavi Latha (2002) Basavaraja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Reddy <i>et al.</i> (1997) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)
Panicle length	Madhavi Latha (2002) Padmaja <i>et al.</i> (2011) Basavaraja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Kavitha and Sree Rama Reddy (2001) Nayak <i>et al.</i> (2001) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)
Number of filled grains/panicle	Tara Satyavathi <i>et al.</i> (2001) Satish Chandra <i>et al.</i> (2009) Rajamadhan <i>et al.</i> (2011) Mulugeta <i>et al.</i> (2012)	Valarmathi and Leena Kumari (1998) Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Ravindra Babu <i>et al.</i> (2012)
1000-grain weight	Reddy <i>et al.</i> (1997) Nagajyothi (2001) Tara Satyavathi <i>et al.</i> (2001)	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Akhtar <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)

Indirect effects of panicle length on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 per cent flowering	Satish Chandra <i>et al.</i> (2009) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Debchoudhury and Das (1998) Padmaja <i>et al.</i> (2011)

Character	Positive indirect effect	Negative indirect effect
Plant height	Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Janardhanam <i>et al.</i> (2001) Tara Satyavathi <i>et al.</i> (2001)
Number of tillers/plant	Basavaraja <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Tara Satyavathi <i>et al.</i> (2001) Madhavi Latha (2002) Ravindra Babu <i>et al.</i> (2012)
Number of grains/panicle	Nayak <i>et al.</i> (2001) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011) Mulugeta <i>et al.</i> (2012)	Amirthadevarathinam (1998) Satish Chandra <i>et al.</i> (2009)
1000-grain weight	Nagajyothi (2001) Madhavi Latha (2002) Basavaraja <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Nayak <i>et al.</i> (2001) Satish Chandra <i>et al.</i> (2009)

Indirect effects of number of grains per panicle on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 per cent flowering	Gupta <i>et al.</i> (1998) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011)	Kavitha and Sree Rama Reddy(2001) Nayak <i>et al.</i> (2001) Rajamadhan <i>et al.</i> (2011)
Plant height	Madhavi Latha (2002) Akhtar <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Reddy <i>et al.</i> (1997) Debchoudhury and Das (1998) Satish Chandra <i>et al.</i> (2009)
Number of tillers/plant	Amirthadevarathinam (1998) Valarmathi and Leena Kumari 1998) Akhtar <i>et al.</i> (2011)	Ganesan <i>et al.</i> (1997) Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011)

Character	Positive indirect effect	Negative indirect effect
	Rajamadhan <i>et al</i> (2011)	
Panicle length	Reddy <i>et al.</i> (1997) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Madhavi Latha (2002)
1000-grain weight	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Akhtar <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Tara Satyavathi <i>et al.</i> (2001)

Indirect effects of 1000-grain weight on grain yield through

Character	Positive indirect effect	Negative indirect effect
Days to 50 per cent flowering	Nayak <i>et al.</i> (2001) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009)	Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
Plant height	Nagajyothi (2001) Madhavi Latha (2002) Rajamadhan <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)	Ganesan <i>et al.</i> (1997) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011)
Number of tillers/plant	Nagajyothi (2001) Tara Satyavathi <i>et al.</i> (2001)	Satish Chandra <i>et al.</i> (2009) Rajamadhan <i>et al.</i> (2011) Padmaja <i>et al.</i> (2011) Ravindra Babu <i>et al.</i> (2012)
Panicle length	Nayak <i>et al.</i> (2001) Padmaja <i>et al.</i> (2011) Rajamadhan <i>et al.</i> (2011)	Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Ravindra Babu <i>et al.</i> (2012)
Number of filled grain/panicle	Tara Satyavathi <i>et al.</i> (2001) Madhavi Latha (2002) Satish Chandra <i>et al.</i> (2009) Padmaja <i>et al.</i> (2011)	Reddy <i>et al.</i> (1997) Nayak <i>et al.</i> (2001) Rajamadhan <i>et al.</i> (2011)

2.2 Assessment of Genetic Diversity Using Molecular Markers

Duwick (1984) stated that molecular marker based genetic diversity analysis has potential for assessing changes in genetic diversity over time and space. The need to characterize available landraces has therefore become important in modern crop improvement (Frey *et al.*, 1984; Dale *et al.*, 1985; Rezai and Frey, 1990).

Sebastian *et al.* (1998) studied molecular diversity of Philippine landraces and cultivars using SSR markers and reported that, the diversity was more in land races compared to modern cultivars.

Garland *et al.* (1999) determined the microsatellite polymorphisms for identifying Australian breeding lines of rice and found that most of the cultivars could be uniquely identified by at least one microsatellite marker.

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

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

Nagaraju *et al.* (2002) carried out genetic analysis of traditional and evolved basmati and semi dwarf non basmati rice varieties by using fluorescence based ISSR-PCR and SSR markers. The lowest genetic diversity was recorded among traditional basmati varieties, whereas evolved basmati showed the highest genetic diversity with both the markers. Traditional and semidwarf non basmati were also clearly delineated by both the markers.

Ni *et al.* (2002) evaluated the genetic diversity within a diverse collection of rice (*Oryza sativa* L.) accessions, and to determine differences in the patterns of diversity within the two rice subspecies *indica* and *japonica* by means of 111 microsatellites and reported that *japonica* group showed significantly higher genetic diversity on chromosomes 6 and 7, and considerably lower diversity on chromosome 2. The results suggested that a relatively small number of microsatellite markers could be used for the estimation of genetic diversity and the identification of rice cultivars.

Chaudhary and Motiramani (2003) evaluated 54 traditional aromatic rice accessions and recorded a wide range of variation for most of the characters.

Ravi *et al.* (2003) used RAPD and SSR markers to assess the genetic diversity of 40 cultivated varieties and five wild relatives of rice. The average PIC value obtained was 0.578 and reported that employment of RAPD and SSR markers in genetic diversity analysis helped in grouping the genotypes according to their subspecies level. It was concluded that SSR markers have higher efficiency for the accurate determination of relationship between accessions that were too close to be differentiated by RAPD markers.

Zhou *et al.* (2003) investigated the genetic diversity and the genetic structures of natural population of *O. rufipogon* in China using SSR markers and the information was found to be significant for the conservation.

Jain *et al.* (2004) assessed the genetic relationships among Indian aromatic and quality rice (*Oryza sativa*) germplasm using 30 fluorescently labelled rice microsatellite markers. The average number of alleles per locus obtained was 7.8 with an average PIC value of 0.6 and reported that Indian aromatic and quality rice genotypes could be distinguished from both *indica* and *japonica* cultivars, and crossbred varieties could be distinguished from traditional Basmati rices. The results indicated that Indian aromatic and quality germplasm was genetically distinct from other groups within *O. sativa* and was the product of a long, independent pattern of evolution.

Pal *et al.* (2004) evaluated 13 rice cultivars for allelic diversity using SSR markers. The average PIC value recorded was 0.447 and the varieties were clustered into three groups namely *indica*, *japonica* and *Basmati*.

Siwach *et al.* (2004) carried out microsatellite analysis to assess allelic diversity and prepare a DNA fingerprint database of 24 rice genotypes including three premium traditional Basmati, 9 cross-bred Basmati, a local scented selection, eight *indica* and three *japonica* rice varieties. A number of SSRs have been identified, which could be used to differentiate among traditional Basmati varieties and between traditional Basmati and other cross-bred Basmati or long grain, non-Basmati rice varieties with an average PIC value of 0.62. It was also observed that the three traditional Basmati rice varieties were closely related and have varying degree of similarity with other cross-bred Basmati rice varieties.

Traore *et al.* (2004) determined the genetic diversity of several West African rice accessions in terms of grain quality parameters and were able to identify genetic resources for novel quality traits using SSR markers.

Ukoskit (2004) comprehensively studied the genetic diversity of natural populations of common wild rice in the northern region of Thailand using 136 polymorphic AFLP markers. AFLP markers were used for characterization of red rice varieties and found that AFLP marker detected high level of variation within red rice when compared to morphological characters (Bertuso *et al.*, 2005). Malone *et al.* (2005) assessed the molecular diversity of 56 rice genotypes using six AFLP primers and obtained 249 bands out of which 205 (82.32%) bands were found polymorphic.

Zeng *et al.* (2004) studied the genetic diversity within a subset of rice germplasm with different adaptations to saline soils using microsatellite markers. The genetic diversity in the subset was found to be relatively low compared to other reports with the average of the PIC values (0.57) but was higher than that determined by Akagi *et al.*, (1997) in 59 *japonica* cultivars in Japan (0.37), it was lower than that determined by Panaud *et al.* (1996) in 24 rice cultivars and wild rice species (0.69) and Garland *et al.*, (1999) in 43 Australian cultivars (0.74).

Arif *et al.* (2005) assessed the genetic diversity among 19 rice genotypes of Pakistan including three commercial Basmati cultivars viz., Basmati 370, Basmati 385 and Super Basmati using random amplified polymorphic DNA (RAPD) markers. Cluster analysis using UPGMA analysis showed two major clusters with genetic similarity coefficients of 0.42 to 0.85 among 19 genotypes.

Collection and evaluation of landraces are an integral part of the pre-breeding process carried out by rice breeders (Vaughan, 1991 ; Siddiq *et al.*, 2005). Kojima *et al.* (2005) studied the diversity of a set of germplasm involving 322 accessions of cultivated rice using 179 RFLP markers and based on this analysis, the accessions were classified into three major groups, one consisting of *japonica* cultivars and other two consisting of *indica* cultivars.

Chakravarthi and Naravaneni (2006) studied genetic diversity and DNA fingerprinting of 15 elite rice genotypes using 30 SSR primers and concluded that the larger range of similarity values for related cultivars using microsatellites provided greater confidence for the assessment of genetic diversity and relationships.

Joshi and Behera (2006) used twelve microsatellite primer pairs for evaluating the genetic diversity among 38 traditional indigenous non-Basmati aromatic rice cultivars. The average number of alleles per locus obtained was 2.6 with average PIC

value of 0.54 and reported that microsatellite markers could efficiently identify indigenous non-Basmati aromatic rice genotypes which could help in genetic conservation management and support intellectual property protection.

A study among 39 traditional rice (*Oryza sativa* L.) varieties and 11 improved varieties from Cuba (Alvarez *et al.*, 2007) revealed the genetic diversity of traditional rice into two different origins using microsatellite markers.

Bhuyan *et al.* (2007) reported about the genetic variability of rice from Assam using two molecular markers (RAPD and ISSR). According to Tu *et al.* (2007) in the distribution of genetic diversity and relationship of Yunnan rice germplasm, the cultural habits of exchanging seeds among farm families, villages or regions might have contributed to the enhancing of rice genetic diversity within the region by adding different genotypes to the local rice gene pool.

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

Ram *et al.* (2007) investigated the genetic diversity among 35 rice accessions, consisting of 19 landraces, nine cultivars and seven wild relatives by using microsatellite (SSR) markers distributed across the rice genome. It was concluded that the allelic diversity revealed by 25 SSR primers was sufficient enough to distinguish between the cultivars, landraces and wild relatives. The allelic variation was lower within the cultivar group than among landraces and wild germplasm indicating the possibility to exploit distant relatives to broaden the genetic base of rice.

Thomson *et al.* (2007) analyzed the genetic diversity of traditional and improved Indonesian rice (*Oryza sativa*) germplasm using microsatellite markers and reported that the germplasm presents a valuable source of diversity for future breeding and association mapping efforts.

Chattopadhyay *et al.* (2008) studied the genetic diversity of 25 rice landraces of undivided Bengal which have suffixed *sail* or *shali* in their name using SSR markers. The study revealed that Bengal landraces of rice might serve as an important source of various unexplored desirable alleles for quality improvement programme of rice. The polymorphism identified at individual SSR loci and resultant diversity analysis might also serve in selecting parental combination as well as in marker assisted breeding programme.

Kibbria *et al.* (2009) employed both SSR and RAPD markers to assess the genetic diversity among aromatic rice genotypes and reported that SSR and RAPD markers in genetic diversity analysis helped in grouping the genotypes according to their subspecies level. The results of the genetic diversity would be useful for the selection of the parents for developing rice breeding varieties.

Pervaiz *et al.* (2009) determined the allelic diversity and relationship among 35 Asian cultivars including 19 aromatic, 13 non aromatic and 3 *japonica* type cultivars using SSR markers. An average PIC value of 0.603 was recorded. Cluster analysis with microsatellitemarkers grouped 35 rice cultivars into two major groups. It was concluded that microsatellite analysis could be efficiently utilized for diversity analysis and differentiation of aromatic and non-aromatic rice cultivars.

Seetharam *et al.* (2009) using SSR markers and morphological characters characterized 30 rice genotypes comprising land races, pure lines, somaclones, breeding lines and varieties specifically adapted to costal saline environments where, the PIC value of 0.064 (RM 274) to 0.72 (RM 580) with an average of 0.46 was recorded. The Jaccard's similarity coefficient ranged from 0.42 to 0.90. The genotypes were grouped into five clusters at a genetic similarity of 50%.

Wong *et al.* (2009) analyzed the genetic diversity of Sarawak Bario rice using SSR markers where 31 alleles were generated at 12 polymorphic microsatellite loci with an average of 2.6 alleles per locus and average PIC value recorded was 0.5204. Further, genetic diversity determination using cluster analysis showed differentiation of Sarawak rice cultivars into two major groups and several sub-groups. From the results observed it was suggested that SSR markers were useful for differentiating the genotypes and assessment of genetic relationships due to its reproducibility and to multiallelic, codominant and informative properties.

El-Malky *et al.* (2010) used fluorescent labeled microsatellite markers to evaluate the genetic diversity among eight rice varieties. The average PIC value obtained was 0.782 and the cluster analysis indicated that the classification was in agreement with their parentage.

Pervaiz *et al.* (2010) evaluated the pattern, extent of genetic variability and relatedness among some rice landraces of Pakistan based on important agronomic traits using SSR markers. A 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 types from the short, early and bold non-aromatic.

Rabbani *et al.* (2010) analyzed the genetic relationship among traditional and improved cultivars of Pakistani rice using 30 SSR markers and revealed the efficiency of microsatellite markers for diversity analysis and differentiation of basmati and non-basmati rice cultivars. The average number of alleles per locus and average PIC value recorded was 3.6 and 0.571 respectively.

Sanal *et al.* (2010) evaluated the genetic diversity of traditional medicinal rice cultivar (Njavara) of Kerala through morphological and different markers analysis and identified unique bands that could be used as a marker for identification of Njavara types from other rice cultivars.

Sivaranjani *et al.* (2010) analyzed genetic diversity among 16 aromatic short grain local land races collected from various parts of India and 30 basmati local land races collected from the traditional basmati growing areas, using 26 hyper variable simple sequence repeat markers and reported that both the aromatic groups could be discriminated by either single (RM28102) or combination of two (RM577 + RM30) markers. Further, the genotypes were classified into two major clusters with 70% dissimilarity revealing presence of high diversity.

Skaria *et al.* (2011) analyzed the genetic diversity and phylogenetic relationships of eight different varieties of rice (*Oryza sativa*) from Kerala using RAPD markers and reported that lower the gene flow, higher will be the gene diversity.

2.2.1 Different types of Molecular markers

Genetic markers represent the genetic difference between individual organism or species. Among three types of markers, viz., morphological, biochemical and DNA markers; the first two types are limited in number and are influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). DNA markers are ubiquitous, innumerable, discrete, non deleterious, inherited by Mendelian laws, unaffected by environment and free from epistatic interaction (Tranksley *et al.*, 1989).

Molecular markers have demonstrated a potential to detect genetic diversity and to aid management of plant genetic resources (Ford-Lloyd *et al.*, 1997; Virk *et al.*, 2000 and Song *et al.*, 2003). In rice, molecular markers have been used to identify accessions (Virk *et al.*, 1995; Olufowote *et al.*, 1997), to determine the genetic structure and pattern of diversity for cultivars of interest (Zhang *et al.*, 1992; Yang *et al.*, 1994; Mackill, 1995 and Akagi *et al.*, 1997). Several types of molecular markers are available for evaluating the extent of genetic diversity in rice. These include restriction fragment length polymorphism (RFLP) (Botstein *et al.*, 1980), random amplified polymorphic

DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al.*, 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995), and microsatellite or simple sequence repeat (SSR) (Tautz, 1989). Of these, RFLP and microsatellites are co-dominant markers and their map positions on the rice genome are well known, whereas, RAPD and AFLP markers involve the use of random, largely dominant markers.

DNA markers are the most widely used and are predominant due to their abundance and repeatability; they remain unaffected across different stages, seasons, locations and agronomic practices (McCouch *et al.*, 1997). These markers have been recently utilised for many purposes including genome mapping, gene tagging, estimation of genetic diversity, varieties differentiation, resolution of uncertain parentage and purity testing (McCouch *et al.*, 1997; Olufowote *et al.*, 1997; Coburn *et al.*, 2002; Ni *et al.*, 2002).

Of several classes of DNA markers available, microsatellite or simple sequence repeat (SSR) markers are considered as the most amenable for several applications including genetic diversity studies due to their multiallelic nature, high reproducibility, co-dominant inheritance, abundance and extensive genome coverage (McCouch *et al.*, 2002).

2.2.1.1 Simple Sequence Repeats (SSR) or Microsatellite markers

The term microsatellite was coined by Litt and Luty (1989). Among classes of repetitive DNA sequences that have showed acquiescent for PCR amplification, SSRs remain the unsurpassed choice of markers (Jacob *et al.*, 1991), they are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Giovannoni *et al.*, 1991).

SSRs markers embrace a variety of di-, tri-, tetra- and pent nucleotide tandem repeats (Hamada *et al.*, 1982; Tautz, 1989; Weber and May, 1989) which could perceived high levels of polymorphism at multiple loci and that can serve as the major source of genetic variation. For the first time (Candit and Hubbell, 1991) published study on microsatellite in plants with an estimation of total 5×10^3 to 3×10^5 microsatellites per plant genome. Thereafter, many workers reported for several plants genomes such Soybean (Akkaya *et al.*, 1992; Rongwen *et al.*, 1995.), grape (Thomas and Scott, 1993), Sorghum (Brown *et al.*, 1996), cucumber and melon (Katzir *et al.*, 1996), rice (Zhao and Kochert, 1992; Wu and Tanksley, 1993) and barley (Saghai-Marouf *et al.*, 1994; Russel *et al.*, 1997). The first application of microsatellites in plants was used

for the cultivar identification and they remain the markers of choice in genotyping studies of cultivars (Weising *et al.*, 1991; Beyermann *et al.*, 1992).

SSR markers are valuable as genetic markers because they detect high levels of allelic diversity, co-dominant, easy and economically assayed by PCR (Weber and May, 1989) and are easily automated (Reed *et al.*, 1994; Westman and Kresovich, 1997 and Smith, 1998).

The screening of microsatellites alleles in varieties would create a valuable database for variety identification and the development of molecular markers through marker assisted selection (Garland *et al.*, 1999). Based on studies in a broad range of organisms, the advantages of using SSR loci for varieties characterization comprise: their abundance and even genomic distribution (Weber and May, 1989), high level of polymorphism (Saghai – Maroof *et al.*, 1994).

McCouch *et al.* (1997) reported the significant superiority of microsatellites allelic diversity and higher number of alleles over RFLP markers. Chen *et al.* (1997) found that, genetically mapped microsatellite markers cover the whole rice genome with at least one microsatellite for every 16 to 20 Cm.

Rice microsatellites have been confirmed to be polymorphic between and within the rice varieties (Wu and Tanksley, 1993; Yang *et al.*, 1994; Panaud *et al.*, 1996; Akagi *et al.*, 1997; Chen *et al.*, 1997; Olufowote *et al.*, 1997). Microsatellites markers revealed nearly 5 to 10 alleles among 59 closely related *japonica* cultivars (Olufowote *et al.*, 1997).

Mackill *et al.* (1996) found that microsatellite have an average polymorphism of at least 1.5 time higher than AFLP and RAPD markers in comparison of 12 *japonica* cultivars. Using SSR, distinctive studies were made between intra and inter cultivar polymorphism among the cultivated and wild rices and the extent of chloroplast genomic differentiation was quantified (Provan *et al.*, 1997). SSR's are superior molecular markers for hybrid rice research (Liu and Wu, 1998). SSR have also been used for tagging and mapping of bacterial blight resistance gene from *O. rufipogon* (Zhang *et al.*, 1998), restorer gene for CMS (Huang *et al.*, 1999), dominant sheath blight resistance gene *Rsb1* (Che *et al.*, 2003), QTL associated with submergence tolerance (Xu *et al.*, 2004).

SSR's are highly polymorphic even between closely related lines, requires low amount of DNA for amplification, easily automated, allow high throughput screening, exchanged between laboratories and highly transferable between populations (Gupta *et*

al.,1999). These markers are useful for assessment of hybrid seed purity and for genotype identification (Temnykh *et al.*, 2000).

Thousands of microsatellites have been identified in rice, and over 2,500 have been developed as molecular markers (Temnykh *et al.*, 2001; Blair *et al.*, 2002; Coburn *et al.*, 2002 and McCouch *et al.*, 2002) assembled a subset of SSR markers into kits for semi-automated fluorescent detection.

SSR's also applicable in estimation of genetic diversity among inbreds and parental lines of rice (Enoki *et al.*, 2002; Naveenkumar, 2003). Yashitola *et al.* (2002) utilized SSR and STS (Sequence Tagged Sites) markers in genetic purity assessment of rice varieties with the objective of replacing GOT with DNA based assay.

The SSR markers involving di-nucleotide repeats motifs particularly those with GA repeats amplified relatively small number of bands as reported earlier in rice (Cho *et al.*, 2000; Saini *et al.*,2004). Markers with PIC values of 0.5 or higher were highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a specific locus (DeWoody *et al.*,1995; Akkaya and Buyukunal Bal, 2004).

Microsatellite markers were used in genetic diversity assessment of 18 African rice varieties (Semagn *et al.*, 2006) and colored rice varieties of Korea (Ji *et al.*, 2007). Similarly, the SSR's were used for fingerprinting of Bangladeshi rice cultivars for the first time and large differences were observed in the polymorphism between *indica* and *japonica* rice sub-species (Jalaluddin *et al.*, 2007).

According to Li *et al.* (2007), polymorphism in SSR could be due to change in SSR region itself caused by the expansion or contraction of SSR or interruption. SSR and ISSR marker showed higher level of polymorphism in diversity analysis of rice genotypes with different adaptations to salinity than physio- morphological characters (Sheetal *et al.*,2008).

The microsatellites or SSRs are amongst the most widely used DNA marker types for many purposes such as diversity, genome mapping and varietal identification (Pervaiz *et al.*, 2009). Recently SSR have been widely used for diversity analysis in rice (Ram *et al.*, 2007 and Thomson *et al.*, 2007 ; Chattopadhyaya *et al.*, 2008; Rabbani *et al.*, 2008; Kibria *et al.*, 2009; Seetharam *et al.*, 2009; Wong *et al.*, 2009 and Sivaranjeni *et al.*, 2010) and tagging of fragrance gene (Cordeiro *et al.*, 2002; Kibria *et al.*, 2008; Nguyen and Bui, 2008 and Madhav *et al.*, 2010).

Chapter III

MATERIAL AND METHODS

The present investigation was undertaken at Institute of Biotechnology and Department of Genetics and plant Breeding, college of agriculture, Rajendranagar, Hyderabad with the objectives, viz., (i) To study the genetic variation for cold tolerance among selected rice genotypes (ii) To assess the genetic variation for cold tolerance among the accessions of rice germplasm using SSR markers.

3.1 MATERIALS

3.1.1 Material for genetic diversity assessment to cold tolerance

3.1.1.1 Plant material

The experimental material consists of 76 rice genotypes collected from different research stations (Table 3.1).

3.1.1.2 SSR markers

A total of 12 SSR markers were selected from cold tolerance QTL regions. (Table 3.2)

3.2 METHODS

3.2.1 Genetic diversity assessment for cold tolerance using morphological traits.

The present investigation was performed in Randomized block design with three replications at college farm, as well as in CRD (completely randomized design) at laboratory.

Randomized block design experiment was conducted at college farm college of agriculture, Rajendranagar, Hyderabad. Genotypes that have been collected from different research stations (Table 3.1) were sown on December 9th, 2012 so that the experimental genotypes phase low temperatures at their seedling stage and vegetative stage. Leaf yellowing is one of the earliest symptom of low temperature damage, was recorded at the seedling stage. Thirty days old seedlings were transplanted at a spacing of 20×15cm. Intercultural

operations such as weeding, irrigation, pest monitoring and control with pesticides were done as and when necessary. Five plants of each genotype in each replication selected randomly from central rows, used to record data on ten quantitative characters. The mean values were considered for statistical analysis.

Completely randomized block design was carried out in the laboratory with three replications. The genotypes were exposed to the low temperatures prevailed during winter. Observations on four characters viz., days to germination, germination percentage, growth rate on dry weight basis and Seedling Vigour Index were recorded during the experiment.

3.2.2 Morphological characterization

In the present investigation, morphological data were recorded for diversity to the cold tolerance studies from five plants in each replication. Observations on the following characters were recorded at the different stages of crop growth period for assessment of genetic diversity to cold tolerance.

3.2.2.1 Germination percentage

Germination percentage was calculated on 14th Day from the sowing and the germination percentage (GP) was calculated as follows.

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

3.2.2.2 Number of days to germination:

Time taken by the seeds for germination was counted.

3.2.2.3 Seedling Growth:

Seedling growth in terms of dry weights was recorded. For dry weight measurement, seedlings were dried at 110 °C for 24 h. The growth rate was calculated according to the following formula

$$GR = \frac{W_2 - W_1}{T_2 - T_1} \times 100$$

Where,

W_1 = Dry weight of the 5 days old seedling

W_2 = Dry weight of the 20 days old seedling

$(T_2 - T_1)$ = time interval in days

3.2.2.4 Seedling Vigour Index:

The seeds were tested for germination using between paper method. The germination percentage was recorded on 14th day. In each sample, 10 seedlings were taken separately for measuring seedling length in centimeters to calculate the seedling vigour Index as per the formula suggested by Abdul Baki and Anderson (1973).

$SVI = \text{Average seedling length (cm)} \times \text{Germination percentage.}$

3.2.2.5 Yellowing of leaves:

Leaf yellowing is one of the earliest symptom of cold temperature damage in rice during vegetative stage (IRRI, 1982). In the present study, the rice seedlings were scored for leaf yellowing in the scale of 1-9.

Scale (for seedlings)

1- Seedlings dark green.

3- Seedlings light green

5- Seedlings yellow

7- Seedlings brown

9- Seedlings dead

3.2.2.6 Days to 50 per cent flowering

The number of days from sowing to the days when primary panicles in 50 per cent plants were in heading was recorded.

3.2.2.7 Plant height (cm)

Plant height is measured in centimeters from ground level to the tip of the tallest panicle in each plant at the time of harvest.

3.2.2.8 Number of tillers per plant

Number of ear bearing tillers per plant was counted at the time of harvest.

3.2.2.9 Panicle length (cm)

It was measured in centimeters at the time of plant maturity from the base of panicle to the tip of last spikelet prior to harvesting.

3.2.2.10 Number of filled grains per panicle

Number of filled grains were counted from five panicles in each selected plant.

3.2.2.11 Number of unfilled grains per panicle

Number of unfilled grains were counted from five panicles in each selected plant.

3.2.2.12 Total grains per panicle

Number of filled and unfilled grains were counted from five panicles in each selected plant.

3.2.2.13 1000-grain weight (g)

One thousand well filled grains were counted at random from each plant and weighed after thorough drying at 15 per cent moisture content and recorded in grams.

3.2.2.14 Spikelet sterility (%)

Number of filled and unfilled grains in each panicle were counted and sterility percentage was calculated by dividing the unfilled grains with total number of grains per panicle and expressed in percentage.

3.2.2.15 Grain yield per plant (g)

The weight of filled grains harvested from each plant was recorded in grams after bringing the grains to required moisture content.

3.2.3 Assessment of Genetic diversity for cold tolerance through SSR markers

All the rice genotypes (Table 3.1) were grown in the petri plates and the following methodology was adopted during the experimental study. The techniques adopted for DNA isolation, DNA quantification, DNA quality assessment, setting of PCR with SSR primers and agarose gel electrophoresis for resolution of SSR markers are presented below. The procedure followed for DNA isolation is given below

3.2.3.1 DNA Isolation from Seventy six rice genotypes

DNA was isolated following the modified CTAB method (Murray and Thompson 1980). Genomic DNA was isolated from the leaves of seventy six genotypes. The DNA samples isolated were checked for their quality and quantity by using 0.8% agarose gel electrophoresis and spectrophotometer. A total of twelve SSR primer pairs (i.e., RM markers) were used for PCR amplification.

The procedure followed for DNA isolation is given below:

1. A 2-3 cm leaf pieces from young plants of rice seedlings were taken.
2. Leaf pieces were further cut into small pieces in the well of a spot test plate (available from Thomas Scientific USA) and 400µl of extraction buffer (50 mM Tris HCl pH 8; 25mM EDTA, 300 Mm NaCl) was added.
3. The leaf tissue was crushed using an alcohol sterilized blunt ended glass rod for about 15-20 seconds. It was ensured that the leaf pieces were completely ground and 400 µl more of extraction buffer was added to the well containing the homogenized leaf sample.
4. Using a micropipette tip of 1 ml capacity (cut at the bottom using a sterile scissors), the entire content was transferred from the well of spot test plate into 1.5 ml capacity micro centrifuge tube.
5. About half volume (400-500 µl) of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added. It was ensured that the pH of phenol used was ~8.0. The contents were mixed well by inversion for about 10 min and centrifuged at 10,000 rpm for about 10 min at room temperature.
6. After centrifugation, the supernatant was aliquated from the micro centrifuge tube into another freshly labelled 1.5 ml micro centrifuge tube. Care was taken that the intermediate layer of insoluble proteins was not disturbed. If the supernatant was not clear, the phenol: chloroform: Isoamyl alcohol purification step was repeated once again. To the supernatant 10 µl of RNase (10 mg/ml) was added and incubated for 30 min at room temperature. One more treatment with equal volume of chloroform (400 µl) was given and centrifuged at 10,000 rpm for 10 min at room temperature.
7. To the clear aqueous supernatant, 1/8 volume of 3M sodium acetate (pH 5.2) and equal volume (500-600 µl) of chilled Isopropyl alcohol was added. The

contents were mixed gently and centrifuged at 10,000 rpm for 10 min at room temperature.

8. The supernatant was drained gently and about 150-200 µl of 70% ethanol was added to the pellet collected at the bottom of the micro centrifuge tube. The tube was tapped gently so that the pellet was disturbed. Centrifugation is done at 10,000 rpm for 10 min at room temperature. After this the supernatant was drained and the pellet was washed with 70% ethanol once again as described above.

Finally, the pellet was left for air-drying over night at room temperature with the tube cap open. After complete drying of pellet, depending on the size of the pellet, about 50-100 µl of sterile T.E. buffer (10mM Tris-HCl, pH 8.0 and 1mM EDTA) or sterile distilled water was added to the tube for dissolving the pellet.

3.2.3.2 Quality and quantity assessment of DNA

3.2.3.2a Genomic DNA quantification by agarose gel electrophoresis

To check the quantity of genomic DNA isolated by the above mentioned mini preparation method, gel electrophoresis was performed. For running the genomic DNA 0.8% agarose gel was used.

1. The edges of a clean, dry glass plate were sealed with tape to form a mold. Later the mold was settled on a horizontal section of the bench.
2. Sufficient electrophoresis buffer (1X) was prepared to fill the tank and to cast the gel.
3. A solution of Agarose in electrophoresis buffer was prepared at a concentration of 0.8%.
4. The required percentage of Agarose (for genomic DNA - 0.8%) in 1X TAE buffer was melted in a micro wave oven.
5. 2 µl of Ethidium bromide solution was added to the melted agarose solution and thoroughly mixed.
6. Agarose solution poured in to the gel cast and allowed it to solidify for 30-35 min.
7. While the Agarose solution was cooling, an appropriate comb for forming the sample slots in the gel was chosen. When the Agarose solution was added to the mold, the comb was positioned at 0.5-1.0 mm above the plate so that a complete well was formed.

8. The warm Agarose solution was poured into the mold and ensured that no air bubbles were left.
9. The gel was allowed to set completely (30-45 min at room temperature) followed by a small amount of electrophoresis buffer was poured on the top of the gel, and carefully comb was removed. Later the electrophoresis buffer was poured off and carefully removed the tape. The gel was mounted in the electrophoresis tank.
10. Enough electrophoresis buffer was added just to cover the gel to a depth of approx. 1 mm.
11. Slowly the sample mixture was loaded into the slots of the submerged gel using a disposable micropipette tips.
12. The lid of the gel tank was closed and the electrical leads were attached so that the DNA will migrate towards the positive Anode (Red lead).
13. When the DNA samples or dyes were migrated a sufficient distance through the gel, electric current was turned off. Later, the leads and lid from the gel tank was removed.

Loading and running the gel

The solidified gel was transferred into Hoefer Super Submarine Electrophoresis unit (Pharmacia Biotech) containing 1X TAE buffer solution. Later the comb was removed without damaging the wells. Subsequently the genomic DNA was loaded along with Bromophenol blue dye into the well of gel and run at a constant voltage of about 100 volt for about 3 hours.

3.2.3.2b Quantification of DNA by spectrophotometer

The purity and concentration of the isolated genomic DNA samples was estimated by UV-absorption spectrophotometer (Thermo scientific UV1 model) as per the procedure described by Sambrook (2001). The ratio of absorbance at 260 nm and 280 nm was used as an indicator of DNA purity. A ratio between 1.4 and 1.9 was considered as relatively pure DNA samples as it did not show any effect on PCR reaction. Further the genomic DNA concentration was measured by using the following formula

Genomic DNA concentration in $\mu\text{g}/\mu\text{l}$ = OD at 260 nm $\times$ 50 $\mu\text{g}/\mu\text{l}$ $\times$ dilution factor

Quantification of DNA was also done by analyzing the purified DNA on 0.8 per cent agarose gel with diluted uncut λ ladder DNA as standard. Based on the intensity and thickness of genomic DNA bands when compared to λ DNA, the concentration and quality of DNA in individual samples was determined.

3.2.3.3 SSR Markers used

Among the several molecular markers available, simple sequence repeats (SSR) (Tautz et al. 1986) markers have received more attention in polymorphism, molecular tagging and finger printing studies, as they are polyallelic, highly reproducible, hyperpolymorphic and co-dominant in their inheritance (Barrett and Kidwell, 1998; Hartl et al. 1999; Parker and Langridge, 2000; Prins et al. 2001). The following Markers were used in the present study (Table 3.2).

3.2.3.4 Setting of PCR

The PCR mixture contained 50 ng of template DNA, 5 picomoles of each primer, 0.05 Mm dNTPs, 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 Mm MgCl₂ and 0.01 mg/ml gelatin) and 1U Taq DNA polymerase in a reaction volume of 25 μ l. Template DNA was initially denatured at 94⁰C for 5 min, denaturation 94⁰C for 45 sec, primer annealing 55⁰C for 45 sec, extension 72⁰C for 1 min, final extension 72⁰C for 10 min, and cooling 4⁰C for α was included. These steps were repeated for 35 cycles for amplification of DNA. The amplified products were electrophoretically resolved on 3% agarose gels (based on the distances between resistance and susceptible alleles) and visualized under UV. The master mix consisting of 0.2 μ l forward primer, 0.2 μ l reverse primer, 1.0 μ l dNTP's, 0.1 μ l of Taq DNA polymerase (1 U/ μ l), 1.0 μ l of 10x PCR buffer (Tris with 1.5 mM MgCl₂) and 5.5 μ l of sterile distilled water was added to make up the volume to 10 μ l. Then the master mix (~ 8.0 μ l) was dispensed to the PCR series tubes containing 2 μ l template DNA. The PCR tubes were covered with caps and then placed in a programmable thermal cycler for DNA amplification.

3.2.3.5 Primer annealing temperature: It is an important parameter in the success of the PCR experiment; the number and type of nucleotides (purine or pyrimidine bases) that are present in each primer pair will determine the annealing temperature. Primer annealing temperature is determined by using the formula, $T_m = 2 (\text{Adenine} + \text{Thymine}) + 4 (\text{Guanine} + \text{Cytosine})$ as suggested by Thein and Wallace, 1986. However, this annealing temperature for each primer

pair was standardized by running the gradient PCR. The temperature specifications that were followed during the PCR setting were given in the table 3.3.

Table 3.3 Steps of PCR programming

S.No.	Steps	Temperature	Time
1	Initial denaturation	94 ⁰ C	5 min
2	Denaturation	94 ⁰ C	45 s
3	Primer annealing	55.0 - 62 ⁰ C	45 s
4	Extension	72 ⁰ C	1 min
5	Final extension	72 ⁰ C	10 min
6	Cooling	4 ⁰ C	α

The above steps were repeated for 35 cycles for amplification of DNA and annealing temperatures were standardized for SSR primers.

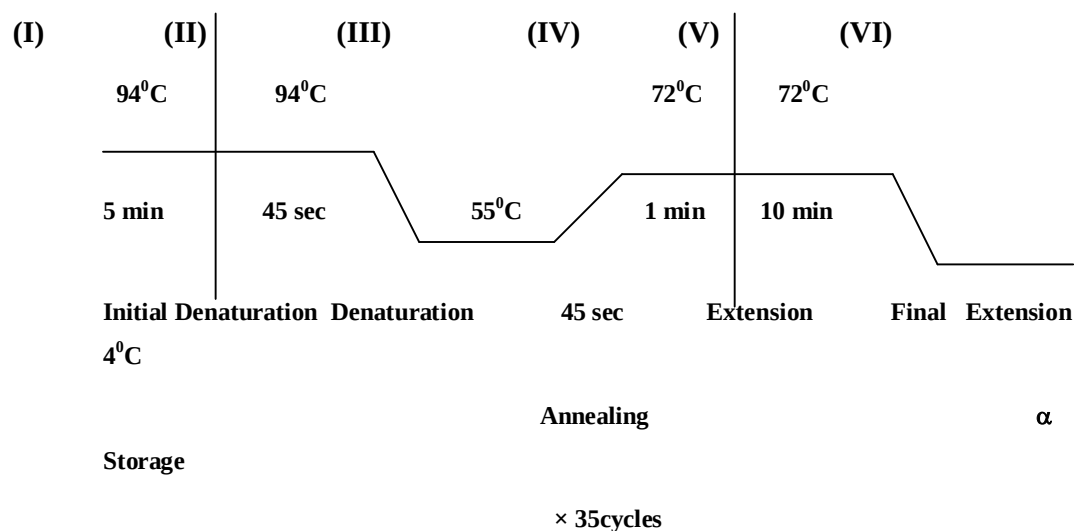


Fig. 3.1 Thermal profile of a PCR reaction.

The PCR profile starts with initial denaturation 94⁰ C for 5 min, denaturation 94⁰ C for 45 sec, primer annealing 55⁰ C – 62⁰ C for 45 sec,

extension 72⁰ C for 1 min, final extension 72⁰ C for 10 min, and cooling 4⁰ C for α was included. These steps were repeated for 35 cycles for amplification of DNA. After completion of amplification, PCR products were stored at -20⁰ C and the amplified products were analyzed by electrophoresis using 2% agarose gels.

3.2.3.6 Agarose gel electrophoresis for resolution of SSR markers

The PCR products were analyzed by electrophoresis using a 3% agarose gel using a Submarine Horizontal gel Electrophoresis Unit (CBS Scientific, USA). Depending on the percent gel to be used, 3.0 g of agarose was weighed and transferred to a reagent bottle containing 100 ml of 1X TAE buffer and mixed well. The contents were then boiled gently in a microwave oven with intermittent mixing. The process was followed until complete melting of agarose was achieved and the solution became crystal clear. The gel-casting tray was washed with water and wiped with ethanol. When the boiled agarose had cooled down substantially, 2 μ l of Ethidium bromide (10 mg/ml) was added to the melted agarose mixed thoroughly and poured in to the gel cast tray and the combs were arranged in the slots on the gel-casting tray and allowed to solidify at room temperature for 20-30 min. Care was taken so that no air bubbles were present. Later the gel was transferred to Submarine Horizontal Electrophoresis Unit containing 1X TAE buffer. Before loading on to the gel, PCR amplified products were mixed with 1/6th volume of gel loading dye (40% sucrose and 0.25% Bromophenol blue) and loaded into the wells. Then the electrodes were connected to power pack. The samples were run at 120 V for 1 to 1 ½ hours. During electrophoresis, 50 bp or 100 bp ladder was added in one well along with the samples for each primer pair to determine the size of amplified fragments.

3.2.3.7 Gel documentation

Ethidium bromide is added while pouring the gel so that the DNA fluoresces when gel was exposed to UV light. The DNA fragments were then visualized under UV Transilluminator and the banding pattern was observed and recorded using gel documentation unit. Documentation was done using Bio Rad gel documentation system which was stored for further scoring and permanent records.

3.2.3 STATISTICAL ANALYSIS

3.2.3.1 Statistical analysis of morphological data

The data with respect to the above characters were subjected to the following analysis with the help of standard statistical procedures.

Observations on Germination percentage, Number of days to germination, Seedling Growth rate and Seedling vigour index were analysed in Simple CRD and the remaining characters were analyzed in RBD.

3.2.3.1.1 Analysis of variance

3.2.3.1.2 Genotypic and phenotypic coefficients of variation

3.2.3.1.3 Heritability and genetic advance

3.2.3.1.4 Estimation of genetic divergence using Mahalanobis's generalized distances (D^2)

3.2.3.1.5 Estimation of correlation coefficients

3.2.3.1.6 Path coefficient analysis

3.2.3.1.1 Analysis of variance

Analysis of variance was computed based on Randomized Block design for days to 50 per cent flowering, Plant height, number of tillers per plant, Panicle length, number of filled grains per panicle, number of unfilled grains per panicle, total grains per panicle, 1000-grain weight, spikelet sterility percentage and grain yield per plant characters separately as per standard statistical procedure (Panse and Sukhatme, 1978). The significance was tested by referring to the values of 'F' table (Fischer and Yates, 1967).

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

Where,

Y_{ij} = Phenotypic observation of 'ith' genotype and 'jth' replication
 μ = General mean
 g_i = True effect of 'ith' genotype
 r_j = True effect of 'jth' replication
 e_{ij} = Random error association with 'ith' genotype and 'jth' replication.

The structure of analysis of variance:

Source	Degrees of freedom	Mean sum of squares	F-ratio
Replication	(r-1)	M'r	M'r/M'e
Treatment	(t-1)	M't	M't/M'e
Error (e)	(r-1)(t-1)	M'e	
Total	(tr-1)	TMSS	

Where,

r and t = number of replications and treatments, respectively

M'r, M't and M'e = mean sum of squares due to replications, treatments and error respectively.

Analysis of variance was computed based on Completely Randomized Block design for days to germination, germination percentage, growth rate and seedling vigour Index separately as per standard statistical procedure (Panse and Sukhatme, 1978). The significance was tested by referring to the values of 'F' table (Fischer and Yates, 1967).

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

Where,

Y_{ij} = Phenotypic observation of 'ith' genotype and 'jth' replication
 μ = General mean
 g_i = True effect of 'ith' genotype
 e_{ij} = Random error association with 'ith' genotype and 'jth' replication.

The structure of analysis of variance:

Source	Degrees of freedom	Mean sum of squares	F-ratio
Treatment	(t-1)	M't	M't/M'e
Error (e)	(tr-t)	M'e	
Total	(tr-1)	TMSS	

Where,

r and t = number of replications and treatments, respectively

M't and M'e = mean sum of squares due to treatments and error respectively.

Variance

The genotypic and phenotypic variance was calculated as per the formulae (Burton and Devane, 1953).

$$\text{Genotypic variance } (\sigma_g^2) = \frac{(\text{Mean sum of squares due to treatments} - \text{Mean sum of squares due to error})}{\text{Number of replications}}$$

$$\text{Phenotypic variance } (\sigma_p^2) = (\sigma_g^2) + (\sigma_e^2)$$

$$(\sigma_e^2) = \text{Error variance}$$

3.2.3.1.2 Genotypic and phenotypic coefficients of variation

The genotypic and phenotypic coefficients of variation were calculated according to the formula given by falconer (1981).

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\text{Genotypic standard deviation}}{\text{Mean}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\text{Phenotypic standard deviation}}{\text{Mean}} \times 100$$

Categorization of the range of variation was effected as proposed by Sivasubramanian and Madhavamenon (1973).

<10%	:	low
10-20%	:	moderate
>20%	:	high

3.2.3.1.3 Heritability and genetic advance

3.2.3.1.3.1 Heritability

Heritability in the broad sense refers to the proportion of genotypic variance to the total observed variance in the total population. Heritability (h^2) in the broad sense was calculated according to the formula given by Allard (1960).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

h^2 = heritability in broad sense

σ_g^2 = genotypic variance

σ_p^2 = phenotypic variance (σ_g^2) + (σ_e^2)

σ_e^2 = environmental variance

As suggested by Johnson *et al.* (1955) heritability (h^2) estimates were categorized as:

Low	:	0-30%
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Medium : 30-60%

High : above 60%

3.2.3.1.3.2 Genetic advance

Genetic advance refers to the expected gain or improvement in the next generation by selecting superior individuals under certain amount of selection pressure. From the heritability estimates the genetic advance was estimated by the following formula given by Burton (1952).

$$GA = K \cdot h^2(b) \cdot \sigma_p$$

Where,

GA = expected genetic advance

K = Selection differential, the value of which is 2.06 at 5% selection intensity

σ_p = phenotypic standard deviation

$h^2(b)$ = heritability in broad sense

In order to visualize the relative utility of genetic advance among the characters, genetic advance as per cent for mean was computed.

$$\text{Genetic advance as per cent of mean} = \frac{GA}{\text{Grand mean}} \times 100$$

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955).

Low : less than 10 %

Moderate : 10-20 %

High : more than 20 %

3.2.3.1.4 Estimation of genetic divergence using Mahalanobis's generalized distance (D^2)

A measure of group distance based on multiple characters was given by Mahalanobis (1936) using D^2 statistic. With the help of this, genetic divergence between genotypes was estimated.

D^2 values between i^{th} and j^{th} genotypes for 'p' characters were calculated as.

$$D_{ij}^2 = \sum_{t=1}^p (Y_{it} - Y_{jt})^2$$

Where

Y_{it} = Uncorrelated mean values of i^{th} genotype for 't' character,

Y_{jt} = Uncorrelated mean values of j^{th} genotype for 't' character,

D_{ij}^2 = D^2 between i^{th} and j^{th} genotypes

3.2.3.1.4.1 Test of significance

After testing the differences between genotypes for each of the characters, a simultaneous test for significance for differences in the mean values of a number of correlated variations with regard to the pooled effect of characters was carried out by using 'V' statistic which in turn utilizes Wilk's 'Λ' criterion. The sum of squares and sum of product of *error* + *genotype* were used for this purpose. The estimation of 'Λ' (Wilk's criterion) was done using the following relationship.

$$'Λ' = W/S$$

Where,

'Λ' = Wilk's criterion

W = determination of error matrix

S = determination of error variety matrix

The significance of 'Λ' was tested by:

$$X^2_{pq} = V(\text{stat}) = -m \log_e 'Λ' = -[n - \frac{P+Q+1}{2}] \log_e 'Λ'$$

Where,

$$m = n - (P+Q+1)/2$$

$$P = \text{Number of variables or characters}$$

$$Q = \text{Number of variables}-1(\text{or d.f. for population})$$

$$n = \text{Degrees of freedom for error + varieties}$$

$$\text{Log}_e 'A' = 2.3026 \log_{10} 'Λ'$$

V (stat) can be approximately considered to be distributed as X^2_{pq} and if the calculated 'V' value from the formula exceeds $X^2_{pq}(K)$, the hypothesis is rejected at K level of significance; otherwise not.

3.2.3.1.4.2 Transformation of correlated variables

In the present model, computation of D^2 values were reduced to simple summation values of the differences in mean values of various characters of the two genotypes i.e., d_i^2 . Therefore, transformation of correlated variables to uncorrelated ones was done before working out the D^2 values. Transformation was done by using pivotal consideration method.

3.2.3.1.4.3 Computation of D^2 values

For a given combination of 'i' and 'j' genotype, the mean deviation i.e., $Y_{it} - Y_{jt}$

for $t = 1, 2, \dots, P$ variables were computed and D^2 values were calculated as sum of square deviations:

$$D^2_{ij} = \sum_{t=1}^P (Y_{it} - Y_{jt})^2$$

Where,

Y_{it} = Uncorrelated mean values of i_{th} genotype for 't' character,

Y_{jt} = Uncorrelated mean values of j_{th} genotype for 't' character,

D^2_{ij} = D^2 between i_{th} and j_{th} genotype,

3.2.3.1.4.4 Testing of significance of D^2 values

The D^2 values obtained for a pair of population is taken as the calculated values of X^2 and is tested against the tabulated value of X^2 for P degrees of freedom, where P is the number of characters considered.

3.2.3.1.4.5 Contribution of individual characters towards divergence

In all the combinations each character was ranked on the basis of their contribution towards divergence between two entries ($d_i = r_t^i - r_t^j$) Rank 1 is given to the highest mean difference and rank P to the lowest difference, where P is the total number of characters. Percentage contribution of each character towards genetic divergence was calculated using the formula:

$$\text{Percentage contribution of characters} = \frac{N \times 100}{M}$$

Where,

N = Number of genotype contribution where the character was ranked first

M = All possible combinations of number of genotypes considered

3.2.3.1.4.6 Grouping of genotypes into various clusters

Grouping of populations into different clusters was done using Tocher's method as described by Rao (1952). The criterion used in clustering by this method is that any two varieties belonging to same cluster should at least on an average show a similar D^2 value than those belonging to different clusters. For this purpose, D^2 values of all combinations of each genotype were arranged in an increasing order of magnitude in a tabular form as described by Singh and Chaudhary (1996). To start with two populations having the smallest distance from each other were considered to which a third population having the smallest D^2 value from the first two populations was added. Similarly, next nearest fourth population was considered and this procedure is continued. At certain stage where it was felt that after adding a particular population there was abrupt increase in the average D^2 value, that population was not considered for including in that cluster. The group of the first cluster was then omitted and the rest was treated in a similar way. This process was continued till all the genotypes were included into one or other cluster.

3.2.3.1.4.7 Average intra cluster distance

For the measurement of intra cluster distance, the formula used was $\Sigma D^2_i/n$

Where,

ΣD^2_i = Sum of distances between all possible combinations (n) of the populations included in a cluster

n = Number of clusters

3.2.3.1.4.8 Average inter-cluster distance

Clusters were taken one by one and their distances from other clusters were calculated. The distance between two clusters was the sum of D^2 values between the numbers of one cluster to each of the number of other cluster divided by the product of number of genotypes in both the clusters under consideration.

The square root of the average D^2 value gave the genetic distance between the clusters. Based on D^2 values (inter cluster distance) the scale given by Rao (1952) for rating of the distance was adopted and the cluster diagram was prepared.

$$\text{Average inter cluster distance} = \frac{D^2}{n_1 \times n_2}$$

n_1 and n_2 are number of genotypes of two clusters

Category	'D' values
Closely related	Below 22
Moderately divergent	Between 22 and 30
Highly divergent	Above 30

3.2.3.1.5 Estimation of correlation coefficients

Correlation coefficients were calculated at genotypic and phenotypic level using the formulae suggested by Falconer (1964).

$$\text{Genotypic coefficient of correlation } (r_g) = r(x_i, x_j)_g = \frac{\text{Cov. } (x_i, x_j)_g}{\sqrt{V(x_i)_g \cdot V(x_j)_g}}$$

Where,

$r(x_i, x_j)_g$ is genotypic correlation between i^{th} and j^{th} characters

$\text{Cov. } (x_i, x_j)_g$ is genotypic covariance between i^{th} and j^{th} characters

$V(x_i)_g$ is genotypic variance of i^{th} character

$V(x_j)_g$ is genotypic variance of j^{th} character

$$\text{Phenotypic coefficient of correlation } (r_p) = r(x_i, x_j)_p = \frac{\text{Cov. } (x_i, x_j)_p}{\sqrt{V(x_i)_p \cdot V(x_j)_p}}$$

Where,

$r(x_i, x_j)_p$ is phenotypic correlation between i^{th} and j^{th} characters

$\text{Cov. } (x_i, x_j)_p$ is phenotypic covariance between i^{th} and j^{th} characters

$V(x_i)_p$ is phenotypic variance of i^{th} character

$V(x_j)_p$ is phenotypic variance of j^{th} character

3.2.3.1.6 Path coefficient analysis

The direct and indirect effects both at genotypic and phenotypic levels were estimated by taking grain yield as dependant variable, using path coefficient analysis suggested by Wright (1921) and Dewey and Lu (1959). The following equations were formed and solved simultaneously for estimating the various direct and indirect effects.

$$r_{1y} = P_{1y} r_{11} + P_{2y} r_{12} + P_{3y} r_{13} \dots\dots\dots + P_{ny} r_{1n}$$

$$r_{2y} = P_{1y} r_{21} + P_{2y} r_{22} + P_{3y} r_{23} \dots\dots\dots + P_{ny} r_{2n}$$

$$\cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot$$

$$: \quad : \quad : \quad : \quad :$$

$$r_{ny} = P_{1y} r_{n1} + P_{2y} r_{n2} + P_{3y} r_{n3} \dots\dots\dots + P_{ny} r_{3n}$$

Where,

1, 2 n = Independent variables

y = Dependant variable

$r_{1y}, r_{2y} \dots\dots\dots r_{ny}$ = Coefficient of correlation between casual factors `1` to `n` on dependent character 1

$P_{1y}, P_{2y} \dots\dots P_{ny}$ = Direct effect of character 1 to n on character Y

The above question can be written in matrix form as:

$$\begin{array}{ccc}
 \text{A} & \text{C} & \text{B} \\
 \left(\begin{array}{c} r_{1y} \\ r_{2y} \\ \cdot \\ \cdot \\ \cdot \\ r_{ny} \end{array} \right) & \left(\begin{array}{cccc} 1 & r_{12} & r_{13} & \dots\dots\dots r_{1n} \\ r_{21} & 1 & r_{23} & \dots\dots\dots r_{2n} \\ \cdot & \cdot & \cdot & \\ \cdot & \cdot & \cdot & \\ \cdot & \cdot & \cdot & \\ r_{n1} & r_{n2} & r_{n3} & \dots\dots\dots 1 \end{array} \right) & \left(\begin{array}{c} p_{1y} \\ p_{2y} \\ \cdot \\ \cdot \\ \cdot \\ p_{ny} \end{array} \right)
 \end{array}$$

Then

$$B = (C)^{-1} A \text{ where } C^{-1} = \left(\begin{array}{cccc} c_{11} & c_{12} & c_{13} \dots\dots\dots & c_{1n} \\ c_{21} & c_{22} & c_{23} \dots\dots\dots & c_{2n} \\ \cdot & \cdot & \cdot & \cdot \\ \vdots & \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots & \vdots \\ c_{n1} & c_{n2} & c_{n3} \dots\dots\dots & c_{nn} \end{array} \right)$$

Direct effects were as follows:

$$p_{1y} = \sum_{i=1}^k c_{1i} r_{iy}$$

$$p_{2y} = \sum_{i=1}^k c_{2i} r_{iy}$$

$$p_{ny} = \sum_{i=1}^k c_{ni} r_{iy}$$

Residual effect, which measures the contribution of characters not considered, was obtained as:

$$P_{ry} = \sqrt{1 - (p_{1y} r_{iy} + p_{2y} r_{iy} + \dots + p_{ny} r_{ny})}$$

Where,

p_{ny} = direct effect of x_n on Y

r_{iy} = correlation coefficient of x_n on Y

3.2.3.1 Statistical analysis for molecular data

3.2.3.1.1 Scoring of SSR Bands

Amplified products from SSR markers analysis were scored qualitatively for the presence or absence of the corresponding band among the genotypes. Only the clear and unambiguous amplified bands were scored. The presence or absence of each band in all genotypes was scored manually by binary data matrix with '1' indicating the presence of the band and '0' indicating the absence of the band.

To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR marker was calculated according to the formula suggested by (Weir, 1996) and it is detailed below.

$$PIC = 1 - (\sum p_i^2)$$

where, 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the i^{th} plus allele in the set of seventy six genotypes investigated. PIC value estimate the discriminatory power of the SSR marker.

3.2.3.1.2 Cluster analysis

Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering was performed on Squared Euclidean distance matrix and similarity matrix using Jacquard's coefficient utilizing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. Data analysis was done using the software NTSYSpc version 2.02 (Rohlf, 1999). Details of the SSR markers used in the present study were tabulated in Table 3.2

Table3.1 List of rice genotypes studied for genetic diversity for cold tolerance

S.No	Genotypes	S.No	Genotypes
1	Himalaya 741	39	Lalankanda
2	CT-6	40	Tomozomachi
3	CT-327	41	CTH-1
4	CT-376	42	Rajendra
5	ECJ-21	43	Kakatiya
6	IR-64	44	Takari
7	Tellahamsa	45	Pusa superfast
8	BPT-5204	46	Kupparalavadlu
9	JyothiPrasad	47	Varalu
10	Kasturi	48	WGL-14377
11	IET-19600	49	JGL-18270
12	JGL-17970	50	Singadis
13	JGL-3828	51	Duppitokalu
14	Kahara	52	Gedanzipeton
15	CT-19	53	Erramallelu
16	BRS-7	54	VD-82
17	HPR-2177	55	AC-630
18	RP-2421	56	WGL-283
19	VD-62	57	AC-41036
20	JGL-18047	58	AC-41038
21	JGL-384	59	AC-41028
22	IR-83222-174	60	AC-41037
23	JGL-1798	61	AC-41039
24	Himalaya-2	62	AC-41015
25	JGL-11118	63	AC-40778
26	Shanta	64	AC-41016
27	JGL-17004	65	AC-41009
28	AC-3283	66	AC-41010
29	Emergency	67	AC-3569
30	JGL-11727	68	AC-38460
31	Bu-Pu-Mui	69	AC-38453
32	JGL-3855	70	AC-38459
33	JGL-11470	71	AC-34983
34	JGL-19621	72	AC-40776
35	PusaBasmathi	73	AC-41043
36	China 988	74	AC-40779
37	Padma	75	AC-41022
38	JGL-1853	76	AC-41015

Table 3.2. List of SSR markers employed in molecular diversity analysis for cold tolerance

S.No	PRIMER	MOTIF
1	RM 71	(ATT)10T(ATT)4
2	RM 243	(CT)18
3	RM129	(CGG)8
4	RM 302	(GT)30(AT)8
5	RM220	(CT)17
6	RM 206	(CT)21
7	RM428	(AG)15
8	RM 334	(CTT)20
9	RM 208	(CT)17
10	RM 307	(AT)14(GT)21
11	RM 292	(GT)10-6-(TGA)2TGT(TGA)4
12	RM 404	(GA)33

Chapter IV

RESULTS AND DISCUSSION

The present investigation was intended to address the variation present in seventy six genotypes of rice for cold tolerance, yield and yield related traits. Genetic diversity was assessed both at morphological and molecular level for cold tolerance, facilitating their effective utilization in future hybridization programmes.

4.1 DIVERSITY ANALYSIS OF RICE GENOTYPES USING MORPHOLOGICAL TRAITS

- 4.1.1 Mean performance of genotypes
- 4.1.2 Analysis of variance
- 4.1.3 Analysis of variability parameters
- 4.1.4 Genetic divergence
- 4.1.5 Character Association
- 4.1.6 Path Coefficient analysis
- 4.1.7 Screening of rice genotypes for cold tolerance using morphological traits

4.2 DIVERSITY ANALYSIS THROUGH MOLECULAR MARKERS

4.1.1 Mean performance of genotypes

The mean performance of genotypes were tabulated in Table 4.1a and 4.1b.

4.1.1.1 Number of days to germination

The genotypes exhibited a range of 2 (IR-64) to 10.33 days (Varalu) for germination with an average of 5.1 days.

4.1.1.2 Germination percentage

Germination percentage was found to vary from 33.99 per cent (Gedangipeton) to 99.65 per cent (Pusasuperfast) and the average value for the trait was 74.44 per cent. Kotaka and Abe observed a wide range of variation in low temperature germinability of rice in more than 700 varieties from Japan, Europe, China, Russia and other regions. A range of 64 - 93.6 per cent germination was reported by Nilanjaya *et al.* (2003) in the evaluation of boro rice germplasm for cold tolerance.

4.1.1.3 Seedling growth

Seedling growth rate in terms of dry matter accumulation ranged from 0.201 mg/day (JGL-11727) to 0.5577 mg/day (IR-64) with mean value of 0.3483 mg/day. Seedlings showing cold tolerance in terms of leaf yellowing score exhibited higher growth rate than the susceptible.

4.1.1.4 Seedling vigour index

The trait exhibited a range from 359.8 to 1804.3 with an average of 938.7. Highest vigour was observed with IR-83222-174 and the lowest by the genotype Kahara.

4.1.1.5 Leaf yellowing

Leaf yellowing is the earliest symptoms of cold temperature damage in rice (IRRI, 1982). In the present study IR-83222-174, Tellahamsa, IR-64 scored 1 for leaf yellowing in the scale of 1-9 and appeared to be tolerant to low temperature stress during seedling stage of growth. Leaf yellowing score at seedling stage in all genotypes ranged from 1 to 7. The leaf yellowing scores were tabulated in (Table 4.2). The genotypes based on leaf yellowing score were grouped into Tolerant, Moderately tolerant, Susceptible and highly susceptible. Seedlings of the genotypes with leaf yellowing score of 1 remained green but, with a score of 7 turned into brown indicating their cold susceptibility. Similar study was conducted by Nilanjaya *et al* (2003) to evaluate cold tolerance of boro rice germplasm.

4.1.1.6 Days to 50 per cent flowering

The genotypes exhibited a range of 86.00 to 119.00 days with a general mean of 103.6 days. Among all the genotypes CTH-1 was found to be early in flowering (86 days) and JGL-11727 (119.00 days) was found to be late.

4.1.1.7 Plant height (cm)

This trait exhibited a range from 1242.44 cm to 109.75 cm with an average of 75.91 cm. The genotype AC-40778 was found to be the shortest, while the tallest one was AC-38459 (109.75 cm).

4.1.1.8 Panicle length (cm)

This trait recorded a range of 14.06 cm to 27.31 cm with a general mean value of 19.90 cm for these rice genotypes. Among these 76 genotypes, the genotype Takari (14.06 cm) and JGL-11727 (27.31 cm) recorded the shortest and the longest panicle length, respectively.

4.1.1.9 Grain yield per plant (g)

The mean values for this trait ranged from 6.08 g to 31.95 g with a general mean of 19.10 g and the genotype AC-38453 (6.08 g) recorded the lowest grain yield, while, the genotype ECJ-21 (31.95 g) recorded the highest grain yield per plant.

4.1.1.10 1000 grain weight (g)

This trait recorded a general mean value 17.26 g and ranged from 10.62 g to 26.42g. The genotypes JGL-19621 (10.62 g) and AC41015 (26.42 g) were found to have the lowest and the highest 1000 grain weight, respectively.

4.1.1.11 Number of tillers per plant

The mean values for number of tillers per plant ranged from 4.8 tillers (Kahara) to 21.8 tillers (Padma) with a general mean of 13.7 tillers per plant.

4.1.1.12 Number of filled grains per panicle

The mean values for number of filled grains per panicle ranged from 26.16 (AC-38453) to 175.44 (JGL-11727) with general mean 95.04

4.1.1.13 Spikelet sterility (%).

Spikelet sterility ranged from 2.51 (CT-376) to 60.42 (AC-38453) with a general mean of 16.51. Satake and Shibata *et al.*(1992) reported induced spikelet sterility by low temperatures during the reproductive growth phase especially during the booting stage.

4.1.1.14 Number of Unfilled grains per panicle

The mean values for number of unfilled grains per panicle ranged from 2.44 (CT-6) to 91.71 (HPR-2177) with general mean of 19.13.

4.1.1.15 Total number of grains per panicle

The mean values for number of total grains per panicle ranged from 46.38 (AC-34983) to 215.93 (JGL-1798) with general mean 114.18.

Some of the characters showed a wide range of values viz. plant height (42.44-109.75 cm), number of total grains per plant (46.38-215.93), grain yield per plant (6.08-31.95 g), number of unfilled grains (2.44-91.71), number of filled grains (26.16-175.44). Moderate range was noticed for days to 50% flowering (86-119days), panicle length (14.06-27.31 cm), number of tillers per plant (4.8-21.8) and Spikelet sterility per centage (2.51-60.42). Very low range was noticed for the traits 1000 grain weight (10.62-26.42).

In the seedling stage germination percentage ranged from 33.9 to 99.65, days to germination 2 to 10.33 days, growth rate 0.2010 mg/day to 0.5577 mg/day on dry weight basis and seedling vigour index 359.85 to 1804.34.

4.1.2 Analysis of variance

Analysis of variance for the experiment involving a set of seventy six rice genotypes for ten characters, viz., days to 50 per cent flowering, plant height, panicle length, number of tillers per plant, 1000-grain weight, number of filled grains per panicle, number of unfilled grains per panicle, total grains per panicle, spikelet sterility percentage and grain yield per plant revealed that the mean sum of squares were highly significant for all the characters indicating the greater diversity among the 76 genotypes studied (Table 4.3a).

In the seedling stage germination percentage, days to germination, seedling growth rate and seedling vigour index showed significant difference at given level of significance. (Table 4.3b).

4.1.3 Analysis of variability parameters

The results pertaining to genetic variability parameters viz., mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (h^2) and genetic advance as per cent of mean for 10 characters are presented in Table 4.5 and the genetic parameter at seedling stage were tabulated in Table 4.4.

GENETIC VARIABILITY, HERITABILITY AND GENETIC ADVANCE

The genotypic and phenotypic coefficients of variation, heritability and genetic advance as per cent of mean were estimated for 76 genotypes. Results are furnished in Table 4.4, 4.5, Fig 4.3 and 4.4. The characters studied in the present investigation exhibited moderate to high PCV and GCV values.

4.1.3.1 Days to 50 per cent flowering

The genotypic and phenotypic coefficients of variation were low i.e., 7.47 and 7.52, respectively. The observed heritability estimate for this trait was high (98.7) while genetic advance as per cent of mean (15.3) were moderate. The result was in conformity with Manoj Kumar prajapate (2011) for low GCV and PCV. Chikkalingaiah *et al.* (1999) for high heritability and Mulugeta Seyoum, *et al.* (2012) for genetic advance as percent of mean. Akinwale *et al.* (2011) recorded broad sense heritability estimates of 98.9 per cent for days to 50 per cent flowering.

4.1.3.2 Plant height (cm)

The genotypic and phenotypic coefficients of variation estimates observed for this trait were moderate i.e., 17.2 and 18.07, respectively. The observed heritability estimates for this character was high (90.7) with high genetic advance as per cent of mean (33.76). The similar results were reported by Nayak *et al.* (2002) for moderate GCV and PCV, Umadevi *et al.* (2009) and Mohan lal and Devendra (2011) for high heritability coupled with high genetic advance as percent of mean for plant height.

4.1.3.3 Number of tillers per plant

The genotypic and phenotypic coefficients of variation for this trait were high i.e., 24.04 and 24.90 respectively. The observed heritability estimate was high (93.3%) and with high genetic advance as per cent of mean (47.83). The similar result reported by Akhtar *et al.* (2011) for high GCV and PCV and Immanuel Selvaraj *et al.* (2011) reported high heritability coupled with high genetic advance as per cent of mean for number of productive tillers per plant in rice.

4.1.3.4 Panicle length (cm)

The GCV and PCV for this trait were moderate i.e., 12.92 and 13.13, respectively. The heritability observed for this trait was high (96.8). This character recorded moderate genetic advance as per cent of mean (26.19). Sadeghi (2011) reported broad sense heritability estimates of 93.15% for panicle length.

4.1.3.5 Number of filled grains per panicle

A high GCV (32.44) and PCV (32.89) were observed for this trait. The heritability estimate for this trait was very high (97.3) with high genetic advance as per cent of mean (65.91). The similar results were observed by Pankaj Garg *et al.* (2010) and Mulugeta seyoun *et al.* (2012) for high GCV and PCV, Pankaj Garg *et al.* (2010) for high heritability and Umadevi *et al.* (2009) for high genetic advance as percent of mean.

4.1.3.6 1000-grain weight (g)

Moderate genotypic (17.81) and phenotypic (18.21) coefficients of variation were recorded with high heritability estimate of 95.6 per cent. The genetic advance as per cent of mean was high (35.87). The similar results was found by Mohan lal and Devendra (2011) for moderate GCV and PCV, Mohan lal and Devendra (2011) and Pankaj Garg *et al.* (2010) for high heritability coupled with high genetic advance as percent of mean. Sadeghi (2011) reported broad sense heritability estimates of 93.77% for 1000 grain weight.

4.1.3.7 Grain yield per plant (g)

A high genotypic coefficient of variation (32.23) and phenotypic coefficient of variation (32.99) was observed for this trait. High heritability estimates (95.5%) coupled with high genetic advance as per cent of mean (53.80) was recorded for this trait. The results were in conformity with Pankaj Garg *et al.* (2010) and Mulugeta seyoun *et al.* (2012) for high GCV and PCV.

4.1.3.8 Number of unfilled grains per panicle

The GCV and PCV for this trait were high i.e., 70.478 and 71.473, respectively. The heritability estimate for this trait was very high (97.2%) with high genetic advance as per cent of mean (27.398).

4.1.3.9 Total grains per panicle

The genotypic and phenotypic coefficients of variation estimates observed for this trait were high i.e., 31.385 and 31.728, respectively. The observed heritability estimates for this character was high (97.8 per cent) with high genetic advance as per cent of mean (63.95).

4.1.3.10 Spikelet sterility percentage

Spikelet sterility percentage ranged from 2.51 (CT-376) to 60.42 (AC-3845) with mean value of 16.51. The estimates of genotypic (60.86) and phenotypic (62.15) coefficients of variability were found to be moderate. The heritability estimate was very high (80.04%) with high genetic advance (20.27) and high genetic advance as percent of mean (122.78%).

4.1.3.11 Germination percentage

Germination percentage was ranged from 33.9 to 99.65 and the mean germination percentage was 74.44. The estimates of genotypic (18.57) and phenotypic (18.59) coefficients of variability were found to be moderate. The heritability estimates were high for the character.

4.1.3.12 Days to germination

Days to germination ranged from 2 to 10.33 days with an average 5.10. The genotypic and phenotypic coefficients of variation estimates observed for the traits were high i. e. 32.56 and 34.62, respectively.

4.1.3.13 Growth rate

Seedling growth rate on dry weight basis was range from 0.2010 mg/day to 0.5577 mg/day with a mean of 0.3483 mg/day. The character showed higher heritability with high genetic advance as percent of mean.

4.1.3.14 Seedling Vigour Index (SVI)

The index value ranged from 359.85 to 1804.3 with an average value of 938.76. The heritability observed for the character was high. The PCV and GCV values were 24.61 and 24.92 respectively.

The results revealed that the estimates of phenotypic coefficient of variation (PCV) were slightly higher than those of genotypic coefficient of variation (GCV) for all the traits studied. The extent of the environmental influence on traits is explained by the magnitude of the difference between GCV and PCV. Large differences between GCV and PCV values reflect high environmental influence on the expression of traits. In this study, slight differences indicated minimum environmental influence and consequently greater role of genetic factors on the expression of traits. In contrary to the present study which showed close values between GCV and PCV Akinwala *et al.* (2011) and Sageghi (2011) recorded wide differences.

In the present study the estimates of broad sense heritability were high, indicating high component of heritable portion of variation that can be exploited by breeders in the selection of superior genotypes on the basis of phenotypic performance.

High to moderate heritability was reported for different quantitative traits studied in rice (Zahid *et al.*, 2006; Kole *et al.*, 2008; Khan *et al.*, 2009; Akinwale *et al.*, 2011; Sadeghi, 2011). Low heritability estimates indicates greater role of environment on the expression of the trait.

4.1.4 GENETIC DIVERGENCE

4.1.4.1 Mahalanobis's generalized distance D^2 values

In order to assess the genetic diversity among 76 genotypes, D^2 statistic was used by following the procedure given by Rao (1952). Since the entire 9 yield component characters were correlated, they were transformed into uncorrelated linear combination through pivotal condensation method.

4.1.4.2 Grouping of genotypes into various clusters

Seventy six genotypes were grouped into nine clusters based on D^2 values using Tocher's method (Rao 1952) such that the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. The distribution of genotypes into various clusters is shown in Table 4.6 and Fig 4.5. Out of nine clusters, cluster II was the largest comprising of 22 genotypes followed by clusters V with 17 genotypes, cluster I with 12 genotypes, cluster III with eight genotypes, cluster VIII with seven genotypes, cluster VII with six genotypes and cluster IV with two genotypes. The clusters VI and IX were represented by single genotype indicating high degree of heterogeneity among the genotypes. The formation of solitary clusters theoretically may be due to total isolation preventing the gene flow or intensive natural/human selection for diverse adaptive complexes. These genotypes may be very unique and useful in breeding point of view.

4.1.4.3 Average inter and intra cluster distances

The average D^2 values of intra and inter cluster distances are presented in Table 4.7. Maximum differences among the genotypes within the same cluster were shown by cluster VII (369.139) followed by cluster I (357.677), cluster IV (355.485), cluster V (235.461), cluster III (191.498), cluster VIII (175.616) and cluster II (171.915). Solitary clusters (VI, and IX) showed zero intra cluster distances. Diversity among the cluster varied from 302.174 to 5805.13. The maximum inter-cluster distance (5805.13) was noticed between the clusters VI and IX. The lowest inter-cluster distance was noticed between clusters II and III (302.174).

The intra-cluster distance varies from 171.915 (cluster II) to maximum distance of 369.139 (cluster VI). This reveals the presence of less divergent genotypes within the clusters. The inter-cluster D^2 values were ranged widely with minimum value of 302.174

between clusters II and III and maximum value of 5805.13 between clusters VI and IX indicating high diversity among the genotypes of these clusters. The maximum amount of heterosis is expected from the crosses with parents belonging to the most divergent clusters as has been reported by Katiyar and Singh (1990), Arora *et al.* (1991) and Umakanth *et al.* (2003). Hence, it is desirable to select the genotypes from the cluster showing high inter-cluster distance in breeding programme for obtaining the desirable segregants.

The inter-cluster distances are higher than the intra-cluster distances indicating the presence of wider genetic diversity between the clusters rather than within the clusters.

The average intra and inter cluster D^2 values are presented in Table 4.5 and Intra cluster D^2 values ranged from zero (cluster VI and IX) to 369.139 (cluster VII). Maximum intra cluster distance was observed in cluster VII (369.139), followed by cluster I (357.677), cluster IV (355.485), cluster V (235.461), cluster III (191.498), cluster VIII (175.616) and cluster II (171.915) indicating that some genetic divergence still existed among the genotypes. This could be made use of in the yield improvement through recombination breeding.

From the inter cluster D^2 values of the nine clusters, it can be seen that the highest divergence occurred between cluster VI and IX (5805.13) followed by cluster IV and IX (3193.751), cluster V and IX (3090.438), cluster I and IX (2974.966) and cluster III and IX (2901.064) suggesting that the crosses involving genotypes from these clusters would give wider and desirable recombination. While the lowest was noticed between cluster II and III (302.174), followed by cluster III and V (358.166) cluster I and II (374.057) and cluster II and V (395.85).

It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters. But for a plant breeder, the objective is not only high heterosis but other quality characters also. The greater the distance between two clusters, the wider the genetic diversity between the genotypes. Keeping this in view, it is indicated that hybridization between the genotypes of cluster IX and cluster VI, cluster IX and IV, cluster IX and V would produce encouraging results. The genotypes of these clusters may be used as parents in the crossing programme to generate breeding material with high diversity.

4.1.4.4 Cluster means of the characters

The cluster means for each of ten characters were presented in Table 4.8. From the data it can be seen that considerable differences existed for all the characters under study. The data indicated that the cluster mean for days to 50 per cent flowering was highest in cluster VIII (114.42) and the lowest in cluster I (92.0). Plant height was highest in cluster IV (105.475 cm) and lowest in cluster III (67.702 cm). Cluster IX recorded the highest number of tillers per plant (19.13) and the lowest number of productive tillers per plant was in cluster III (10.57). Cluster IV recorded the highest panicle length (25.09 cm) and the lowest was recorded in cluster I (17.70 cm). The number of filled grains per panicle was highest in cluster VIII (150.88) and the lowest in cluster VI (26.16). Highest 1000 grain weight was recorded in cluster IV (26.04 g) and the lowest in cluster VIII (12.87 g). Cluster VIII recorded the highest grain yield per plant (23.77 g) while in cluster VI it was low (6.08 g). Cluster IV recorded low spikelet percentage (9.75%) whereas it was high in cluster VI (60.42%). Higher number of unfilled grains was recorded in cluster IX (91.71) while it was low in cluster IV (11.65). Total number of grains per panicle was highest in cluster VIII (178.91 cm) and lowest in cluster VI (66.1 cm) and these results indicate that selection of genotypes having high values for particular trait could be made and used in the hybridization programme for improvement of that character.

It is observed that no cluster contained at least one genotype with all the desirable traits, which ruled out the possibility of selecting directly one genotype for immediate use. Similar results were reported by Bose and Pradhan (2005). Therefore, hybridization between the selected genotypes from divergent clusters is essential to judiciously combine all the targeted traits.

The cluster VIII is having highest mean value for number of filled grains per panicle and grain yield per plant. Cluster IX for number of tillers per plant and cluster IV for panicle length. The cluster IV is having least mean value for number of unfilled grains and spikelet sterility. The genotypes JGL11118, JGL1853, JGL18270, AC41015, JGL384, JGL3828, JGL11727, HPR2177, AC38459, AC41015 from these clusters having high mean values for grain yield per plant, number of filled grains, longer panicles, less number of unfilled grains and low percent of spikelet sterility may be directly used for adaptation or may be used as parents in future hybridization programme.

4.1.4.5 Relative contribution of characters towards genetic divergence

The number of times that each of the ten characters appeared in first rank and its respective per cent contribution towards genetic divergence is presented in Table 4.9. The results showed that the contribution of Days to fifty percent flowering was highest towards genetic divergence (41.58%) by taking 1185 times ranking first, followed by Number of unfilled grains per panicle (12.49%) by 356 times, Number of filled grains per panicle (11.16%) by 318 times, panicle length (10.42%) by 297 times, spikelet sterility (7.72%) by 220 times, Grain yield per plant (6.67%) by 190 times, Number of tillers per plant (4.14%) by 118 times, 1000 grain weight (3.82%) by 109 times, plant height (2%) by 57 times, total grains per panicle by zero times, respectively to the genetic divergence in decreasing order. Similar results were reported by Subudhi *et al.* (2008) and Deepak *et al.* (2006). No contribution towards genetic divergence was from the trait total number of grains per panicle. In order to select genetically diverse genotypes the material should be screened for the important traits viz., days to 50% flowering, unfilled grains per panicle, filled grains per panicle, panicle length and spikelet sterility.

The genotypes showing cold tolerance in terms of leaf yellowing score, germination percentage, number of days to germination and growth rate at seedling stage were grouped in cluster I, II, III and V indicating their divergence to cold tolerance from other clusters.

4.1.5 CHARACTER ASSOCIATION

Crop yield is the end product of the interaction of a number of other, often interrelated attributes. A thorough understanding of the interaction of characters among themselves had been of great use in plant breeding. The efficiency of selection for yield mainly depends on the direction and magnitude of association between yield and its component characters and also among themselves. Character association provides information on the nature and extent of association between pairs of metric traits and helps in selection for the improvement of the character. Phenotypic and genotypic correlations were worked out on yield and yield contributing characters in 76 genotypes. In general, genotypic correlations were found to be higher than phenotypic correlations, which indicate that though there is strong inherent association between character studies, its expression is lessened due to

influence of environment and considering the importance of phenotypic correlation it was discussed in the results which were presented in table 4.10.

4.1.5.1 Plant height (cm)

The plant height registered positive significant correlation with panicle length (0.3767), 1000 grain weight (0.2234), number of unfilled grains per panicle (0.1556) and spikelet sterility percentage (0.1551) where as it showed non significant positive phenotypic correlation with grain yield per plant (0.0985), number of tillers per plant (0.0754), Days to 50% flowering (0.0470), number of filled grains per panicle (0.0460) and total number of grains per panicle (0.0992).

In contrary, Ravindra babu *et al.* (2012) and Satish chandra *et al.* (2009) reported a significant negative association between plant height and grain yield per plant.

4.1.5.2 Number of Tillers per Plant

Number of tillers per plant exhibited significant positive phenotypic correlation with grain yield per plant (0.4306). It exhibited non significant positive correlation with panicle length (0.0918), plant height (0.0754), days to 50 per cent flowering and total number of grains per panicle but negative correlation with 1000 grain weight (-0.1189) and number of filled grains per panicle (-0.0238). Similar association between number of tillers per plant and grain yield were recorded by Kayvan Agahi *et al.* (2007).

4.1.5.3 Panicle Length (cm)

Panicle length registered significant positive phenotypic correlation with plant height (0.3767), days to 50% flowering (0.4132), number of filled grains per panicle (0.3622), number of unfilled grains per panicle (0.3579), total number of grains per panicle (0.4482), spikelet sterility percentage (0.1655) and grain yield per plant (0.2960). It showed negative significant association with 1000 grain weight and non significant positive correlation with number of tillers per plant. Similar association between panicle length and grain yield were reported by Satish Chandra *et al.* (2009) and Basavaraja *et al.* (2011).

4.1.5.4 1000 Grain Weight (g)

The character showed significant negative association with grain yield per plant (-0.4004), panicle length (-0.1353), days to 50 per cent flowering (-0.2364), number of filled grains per panicle (-0.5508), number of unfilled grains per panicle (-0.2939) and total number of grains per panicle (-0.5865). It showed a significant positive phenotypic correlation with plant height (0.2234) and non significant association with spikelet sterility percentage.

In contrary, Zulqarnain *et al.* (2012) and Ravindra babu *et al.* (2012) reported a significant positive association between 1000 grain weight and grain yield per plant.

4.1.5.5 Days to 50 per cent flowering

Days to 50 per cent flowering recorded a significant positive phenotypic correlation with grain yield per plant (0.1324). It also recorded a positive association with panicle length (0.4132), number of filled grains per panicle (0.2661), number of unfilled grains per panicle (0.1753) and total number of grains per panicle. The characters, plant height (0.0470), number of tillers per plant (0.1065) and spikelet sterility percentage (0.0778) showed positive and non-significant association. Similar results were reported by Laxuman *et al.*, (2011). Whereas, significant negative association was recorded between days to 50 percent flowering and grain yield by Pankaj *et al.*, (2010).

4.1.5.6 Number of Filled Grains per Panicle

Number of filled grains per panicle exhibited a significant positive phenotypic correlation with grain yield per plant (0.4796), total number of grains per panicle (0.9248), unfilled grains per panicle (0.1719), days to 50% flowering (0.2661) and panicle length (0.3622) whereas, negative and significant correlation with spikelet sterility percentage (-0.2789). It showed non significant positive association with plant height and negative non significant association with number of tillers per plant. Similar results were recorded for the association between number of filled grains per panicle and grain yield per plant by mulugeta *et al.* (2012). Whereas, significant negative correlation was reported between number of filled grains per panicle and grain yield by Pankaj *et al.*, (2010).

4.1.5.7 Unfilled Grains/panicle

Number of unfilled grains per panicle exhibited a non significant negative phenotypic correlation with grain yield per plant (-0.0223) and significant positive correlation with plant height (0.1556), panicle length (0.3579), days to 50% flowering (0.1753), number of filled grains per panicle (0.1719), total grains per panicle (0.5261) and spikelet sterility percentage (0.8384). The trait showed negative and significant correlation with 1000 grain weight (-0.2939) and positive non significant association with number of tillers per plant.

The contrasting results were reported by Ravindra babu *et al* (2012) and Sarawgi *et al*, (1997) for the association of number of unfilled grains per panicle and grain yield. They recorded a negative significant association between number of unfilled grains per panicle and grain yield per plant.

4.1.5.8 Total Grains/panicle

This trait had a significant positive correlation with grain yield per plant (0.4040), panicle length (0.4482), days to 50 per cent flowering (0.2956), number of filled grains per panicle (0.9248) and number of unfilled grains per panicle. It showed negative significant correlation with 1000 grain weight (-0.5865) and non significant positive correlation with spikelet sterility percentage (0.0776).

4.1.5.9 Spikelet sterility percentage

Spikelet sterility percentage exhibited significant negative phenotypic correlation with grain yield per plant (-0.2410) and number of filled grains per panicle (-0.2789). The trait showed positive and significant correlation with plant height (0.1551), panicle length (0.1655) and number of unfilled grains per panicle. Positive non significant association was recorded with number of tillers per plant (0.0328), 1000 grain weight (0.0242), days to 50% flowering (0.0778) and total number of grains per panicle (0.0776). Similarly Sarawgi *et al*. (1997) observed a high significant negative correlation between spikelet sterility percentage and grain yield per plant.

4.1.5.10 Grain Yield per Plant

Phenotypic correlations revealed that grain yield per plant had significant positive association with tillers per plant (0.4306), panicle length (0.2960), days to 50 per cent flowering (0.1324), number of filled grains per panicle (0.4796) and total number of grains per panicle (0.4040). Non significant positive association was observed with plant height (0.0985). The character showed negative significant association with 1000 grain weight (-0.4004), spikelet sterility percentage (-0.2410) and negative non significant with number of unfilled grains per panicle (-0.0223). Similar results were reported earlier in rice for the association of grain yield with number of filled grains per panicle by Mulugeta *et al*, (2012)

Grain yield per plant showed positive significant association with number of tillers per plant, panicle length, days to 50% flowering, number of filled grains per panicle and total number of grains per panicle. This indicated that all these characters were important for yield improvement. The character plant height also positively associated with grain yield per plant, indicates it's consideration in selection for yield improvement.

The study of phenotypic correlation studies showed that selection of plants with more number of filled grains per panicle, more number of tillers per plant, more days to 50% flowering, tall plants, longer panicles and more number of total grains per panicle would result in improvement of yield.

Character association at seedling stage

4.1.5.11 Germination percentage:

Germination percentage recorded a negative significant phenotypic correlation correlation (-0.4479) with days to germination. It showed positive significant phenotypic correlation with growth rate (0.2792) and seedling vigour index (0.7827).

4.1.5.12 Days to germination:

Days to germination recorded a negative significant phenotypic correlation with seedling growth rate (-0.1812) and seedling vigour index (-0.4247).

4.1.5.13 Growth rate:

Seedling growth rate on the dry weight basis recorded positive significant phenotypic correlation with seedling vigour index (0.4059).

4.1.6 PATH COEFFICIENT ANALYSIS

Correlation gives only the relation between two variables whereas path coefficient analysis allows separation of the direct effect and their indirect effects through other attributes by partitioning the correlations (wright, 1921). Hence, this objective was undertaken in the present investigation.

Based on the data recorded on the genotypes in the present investigation, the genotypic and phenotypic correlations were estimated to determine direct and indirect effects of yield and yield contributing characters.

As discussed in character association based on the importance of phenotypic effects the present results of phenotypic path coefficient of yield and yield contributing characters discussed here under, which were presented in table 4.12 and Fig 4.8 - 4.9.

4.1.6.1 Plant height

Plant height had positive phenotypic direct effect on grain yield per plant (0.0684). It had shown positive indirect effects on grain yield per plant via number of tillers per plant (0.0314), panicle length (0.0795), number of filled grains per panicle (0.0206) and spikelet sterility percentage (0.0255). While the rest of the characters showed negative indirect effects. Similar results were reported by Mulugeta *et al.* (2012) and Pankaj *et al.* (2010).

4.1.6.2 Number of tillers per plant

Number of Tillers per plant exhibited a phenotypic positive direct effect on grain yield per plant (0.4166) while the correlation with grain yield per plant was also positive and significant. The correlation was positive and significant mainly due to positive indirect effect contribution through 1000 grain weight (0.0308), plant height (0.0052) and panicle length (0.0194). Similar results were observed by Laxuman *et al.* (2011) and Sathish Chandra *et al.* (2009).

4.1.6.3 Panicle length (cm)

Panicle length expressed positive direct effect of 0.2112 on grain yield per plant. It had shown positive indirect effects on grain yield per plant via plant height (0.0258), number of tillers per plant (0.0382), 1000 grain weight (0.0351), number of filled grains

per panicle (0.1622) while the rest of the characters showed negative indirect effects. Similar results were reported by Ravindra babu *et al.* (2012).

4.1.6.4 1000-grain weight (g)

1000 grain weight expressed a negative direct phenotypic effect on grain yield per plant (-0.2591) while its correlation with grain yield was negative. The correlation between 1000 grain weight and grain yield per plant was negative mainly due to negative indirect effect influence through number of tillers per plant (-0.0495), panicle length (-0.0286) and number of filled grains per panicle (-0.2467). Similar results reported by Basavaraja *et al* (2011), Mulugeta *et al.* (2012) and Ravindra babu *et al.* (2012).

4.1.6.5 Days to 50 per cent flowering

The days to 50 per cent flowering had direct phenotypic negative effect (-0.1163) on grain yield and the correlation between days to 50 per cent flowering and grain yield was positive and significant (0.1324) mainly due to positive indirect effect through plant height (0.0032), number of tillers per plant (0.0444), panicle length (0.0873), 1000 grain weight (0.0612) and spikelet sterility percentage. Similar results were reported by Ravindrababu *et al.* (2012). But Mulugeta *et al.* (2012) observed a positive direct effect of days to 50 per cent flowering on grain yield.

4.1.6.6 Number of filled grains per panicle

Number of filled grains per panicle had direct phenotypic positive effect (0.4479) on grain yield per plant. Its correlation with grain yield per plant was also positive and significant. The correlation between number of filled grains per panicle and grain yield per plant was positive and significant mainly due to positive indirect effect contribution through plant height (0.0031), 1000 grain weight (0.1427) and panicle length (0.0765). Filled grains per panicle had the maximum positive direct effect on grain yield per plant. The present results were in conformity with Halil and Necmi (2003).

4.1.6.7 Number of Unfilled grains per panicle

Number of unfilled grains per panicle had direct phenotypic negative effect (-0.3833) on grain yield per plant. Its correlation with grain yield per plant was also

negative. The correlation between number of unfilled grains per panicle and grain yield per plant was negative and non significant mainly due to negative indirect effect contribution through days to 50% flowering (-0.0204) and total number of grains per panicle (-0.0216). Similar results were reported by Ravindra babu *et al.* (2012)

4.1.6.8 Total number of grains per panicle

Total number of grains per panicle had direct phenotypic negative effect (-0.0411) on grain yield per plant. Its correlation with grain yield per plant was also negative. The correlation between number of unfilled grains per panicle and grain yield per plant was positive and significant mainly due to positive indirect effect contribution through plant height (0.0068), number of tillers per plant (0.0008), panicle length (0.0946), 1000 grain weight (0.1520), filled grains per panicle (0.4142) and spikelet sterility percentage (0.0128).

4.1.6.9 Spikelet sterility percentage

Spikelet sterility percentage showed a positive direct effect on grain yield per plant. The correlation between spikelet sterility percentage and grain yield per plant was negative and significant mainly due to negative indirect effect contribution through 1000 grain weight (-0.0063), days to 50% flowering (-0.0090), number of filled grains per panicle (-0.1249), number of unfilled grains per panicle (-0.03213) and total number of grains per panicle (-0.0032). These results were in contrary with the findings of Sarawgi *et al.* (1997).

The association of different component characters among themselves and with yield is quite important for devising an efficient selection criterion for yield. The total correlation between yield and component characters may be some times misleading, as it might be an over-estimate or under-estimate because of its association with other characters. Hence, indirect selection by correlated response may not be some times fruitful. When many characters are affecting a given character, splitting the total correlation into direct and indirect effects of cause as devised by Wright (1921) would give more meaningful interpretation to the cause of association between the dependent variable like yield and independent variables like yield components. This kind of information will be helpful in formulating the selection criteria. Indicating the selection for these characters is likely to bring about on overall improvement in single plant yield directly.

Path coefficient analysis revealed that number of filled grains per panicle exerted the highest positive direct effect on grain yield followed by number of tillers per plant, panicle length and plant height. The negative direct effect on grain yield by unfilled grains per panicle, 1000 grain weight and days to 50 per cent flowering.

Path analysis revealed that numbers of filled grains per panicle, number of tillers per plant, panicle length and plant height are the most important characters which could be used as selection criteria for effective improvement on grain yield. Therefore, it is suggested that preference should be given to these characters in the selection program to isolate superior lines with genetic potentiality for higher yield in rice genotypes.

4.1.7. Screening of rice genotypes for cold tolerance using morphological traits

In the present study visible symptoms such as yellowing of leaves, germination percentage, days to germination, growth rate and seedling vigour index were used to assess the cold damage.

Yellowing of leaves score varied from 1-Tolerant, 3-Moderately tolerant, 5-Susceptible and 7-highly susceptible. Genotypes Tellahamsa, IR-83222-174, Emergency and Pusa superfast showed the signs of cold tolerance (score of leaf yellowing 1) and had germination more than 90 per cent (Table 4.13). Delayed and lower percentage of germination was reported by da Cruz and Milach (2000) for cold susceptible genotypes. Seedlings of the genotypes with leaf yellowing score of 1 remained green but, with a score of 7 turned into brown indicating their cold susceptibility. Similar study was conducted by Nilanjaya *et al* (2003) to evaluate cold tolerance of boro rice germplasm.

Germination percentage was found to vary from 33.99 per cent (Gedangipeton) to 99.65 per cent (Pusasuperfast) and the average value for the trait was 74.44 per cent. Kotaka and Abe observed a wide range of variation in low temperature germinability of rice in more than 700 varieties from Japan, Europe, China, Russia and other regions. A range of 64 - 93.6 per cent germination was reported by Nilanjaya *et al*. (2003) in the evaluation of boro rice germplasm for cold tolerance. Genotypes Tellahamsa, Emergency, IR-83222-174 and Pusa superfast showed higher germination percentage indicating their tolerance to cold. Similarly survival percentage was used to evaluate cold tolerance at

seedling stage by Morsy *et al.* (2007). Genotypes having higher germination percentage and tolerance score of leaf yellowing showed higher growth rate and seedling vigour index. Based on this study the genotypes can be classified as follows

Tolerant(1):	Tellahamsa, IR-83222-174, Emergency, Pusa superfast
Moderately Tolerant(3):	Himalaya 741, CT-327, IR-64, Jyothiprasad, JGL-384, Himalaya-2, JGL-11470, Takari, Kupparalavadlu, Duppitokalu, Erramallelu, AC-41036, AC-41038, AC-41037, AC-41010, AC-40776.
Susceptible(5):	CT-6, CT-376, ECJ-21, BPT-5204, Kasturi, CT-19, BRS-7, HPR-2177, RP-2421, JGL-1798, JGL-11118, Shanta, AC-3283, JGL-11727, Bu-Pu-Mui, JGL-3855, JGL-19621, PusaBasmathi, Padma, JGL-1853, Tomozomachi, CTH-1, Rajendra, Kakatiya, Varalu, JGL-18270, Singadis, VD-82, AC-630, WGL-283, AC-41039, AC-41015, AC-40778, AC-41016, AC-3569, AC-41043, AC-40779, AC-41015.
Highly susceptible(7):	IET-19600, JGL-17970, JGL-3828, Kahara, VD-62, JGL-18047, JGL-17004, China 988, Lalankanda, WGL-14377, Gedanzipeton, AC-41028, AC-41009, AC-38460, AC-38453, AC-38459, AC-34983, AC-41022.

4.2 GENETIC DIVERSITY ANALYSIS USING MOLECULAR MARKERS

4.2.1 Polymorphism of SSR markers

The first published study on microsatellite in plants was made by Candit and Hubbell, 1991. Among the classes of repetitive DNA sequences that have showed acquiescent for PCR amplification, SSRs remain the unsurpassed choice of markers (Jacob *et al.*, 1991). SSR markers are valuable genetic markers because they detect high levels of allelic diversity, co-dominant, easy and economically assayed by PCR (Weber and May, 1989), easily automated (Reed *et al.*, 1994; Westman and Kresovich, 1997 and Smith 1998), abundance and even genomic distribution (Weber and May, 1989), high level of

polymorphism (Saghai – Maroof *et al.*, 1994). It have an average polymorphism atleast 1.5 times higher than AFLP and RAPD markers (Mackill *et al.*,1996). It is found that genetically mapped microsatellite markers cover the whole rice genome with at least one microsatellite for every 16 to 20 cM (Chen *et al.*,1997). SSR's are highly polymorphic even between closely related lines (Gupta *et al.*, 1999).

The polymorphism in SSR could be due to change in SSR region itself caused by the expansion or contraction of SSR or interruption (Li *et al.*, 2007).

In the present study, a total of 12 SSR markers which were dispersed throughout the 12 chromosomes were used to assess the extent of genetic diversity across 76 rice genotypes collected from different parts. Out of 12 SSR markers tested, all markers were found to be polymorphic.

4.2.2 Number of Alleles

The SSR markers have significantly superior allelic diversity of microsatellites over RFLPs (McCouch *et al.*,1997) and high numbers of alleles for rice microsatellite markers. In the present study, a total of 49 alleles were detected across 76 rice genotypes by 12 polymorphic SSR markers. The number of alleles generated per locus by each marker ranged from 2 (RM 129) to 5 (RM 220 and RM 302). This was quite comparable to 2.0-5.5 alleles per SSR locus for various classes of microsatellites reported by Cho *et al.*, (2000) using a different set of rice germplasm.

4.2.3 Polymorphism Information Content (PIC) Values

Polymorphism information content (PIC) value is the reflection of allele diversity and frequency among the genotypes. To measure the informativeness of each SSR marker, PIC value was calculated. In the present study, the PIC values varied widely among SSR loci tested and ranged from 0.688 (RM129) to 0.907 (RM302), with an average of 0.855 per locus. The average PIC value in the study was higher than the average PIC value of 0.54 obtained by Joshi and Behera (2006) who studied indigenous non-Basmati aromatic rice genotypes.

4.2.4 Genetic Diversity Pattern by Cluster Analysis

The cluster analysis performed by using UPGMA based on similarity co-efficient values. It resolved 76 genotypes into two major clusters with 36% dissimilarity (Fig 4.14).

Cluster I consisted of single genotypes which showed 35% dissimilarity. Cluster II consisted of 75 genotypes which revealed 35% dissimilarity which was again subgrouped into two sub-clusters namely IIA and IIB and each subgroup comprised of 1 and 74, genotypes respectively.

Cluster IIA comprised of single genotype with 34% dissimilarity with other subclusters and Cluster IIB comprised of 74 genotypes with 32% dissimilarity with other subclusters. IIB again subgrouped into two sub-clusters namely IIB1 and IIB2 and each subgroup comprised of 1 and 73 genotypes, respectively.

IIB2 was a major cluster comprised of 73 genotypes and further grouped into IIB2a and IIB2b with 31 and 42 genotypes respectively. In a study conducted by Victoria et al., (2007) the cultivars were grouped into 3 major clusters with additional sub-clustering within each cluster.

Genotypes Tellahamsa, IR-83222-174, Himalaya 741, Emergency were grouped in IIB2b which were showed cold tolerance at seedling stage in terms of leaf yellowing score.

Many discrepancies were observed between the clustering patterns using morphological and SSR markers data, which might be due to presence of wider diversity among the genotypes and different heritability levels of the morphological traits used for evaluation. Better correspondence among these two diversity estimates could be achieved by increasing the number of SSR markers used for analysis and also multilocal evaluation of morphological traits.

4.2.5 Similarity matrix

A similarity matrix based on the proportion of shared SSR fragments was used to establish the level of relatedness between the genotypes for cold tolerance. Pair-wise estimates of similarity ranged from 0.51 to 0.96 (Table 4.15). VD62-JGL18047, CT19-BRS7, JGL1797-JGL3828, JGL19621-China988, JGL19621-Emergency, JGL19621-JGL11727, China 988-IR83222174, Padma-Himalaya2, Padma- JGL19621, China988-Padma, AC630-Erramallelu and AC38453-AC38459 were the closest genotypes with the highest similarity index of 96%. This was followed by 94% similarity between two pairs of genotypes. The lowest level of similarity (51%) was obtained between 'Jyothiprasad' and 'JGL18047'. Although comparison of genotypes for cold tolerance indicated that these were closely related, no genotype was exactly identical to any other cultivar.

Table 4.9 Relative contribution of characters towards genetic divergence

S. No	Source	Times Ranked 1 st	Contribution %
1	Days to 50% Flowering	1185	41.58
2	Unfilled Grains/ Panicle	356	12.49
3	Filled Grains/ Panicle	318	11.16
4	Panicle Length cm	297	10.42
5	Spikelet Sterility %	220	7.72
6	Grain Yield/ Plant gm	190	6.67
7	Tillers/ Plant	118	4.14
8	1000 Grain Wt gm	109	3.82
9	Plant Height cm	57	2.00
10	Total Grains/ Panicle	0	0.00

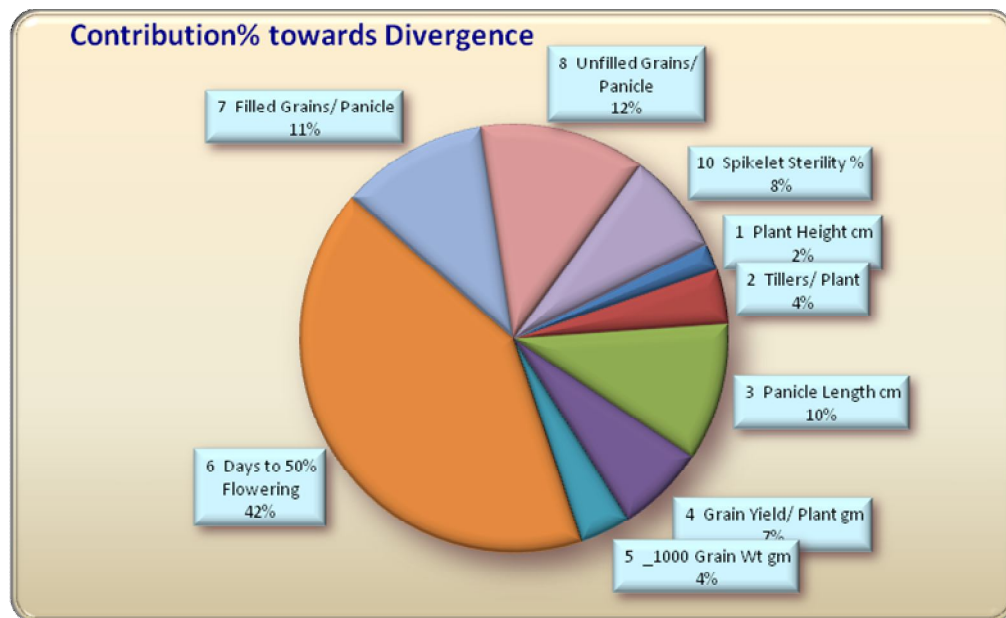


Fig.4.7 Relative contribution of characters towards genetic divergence

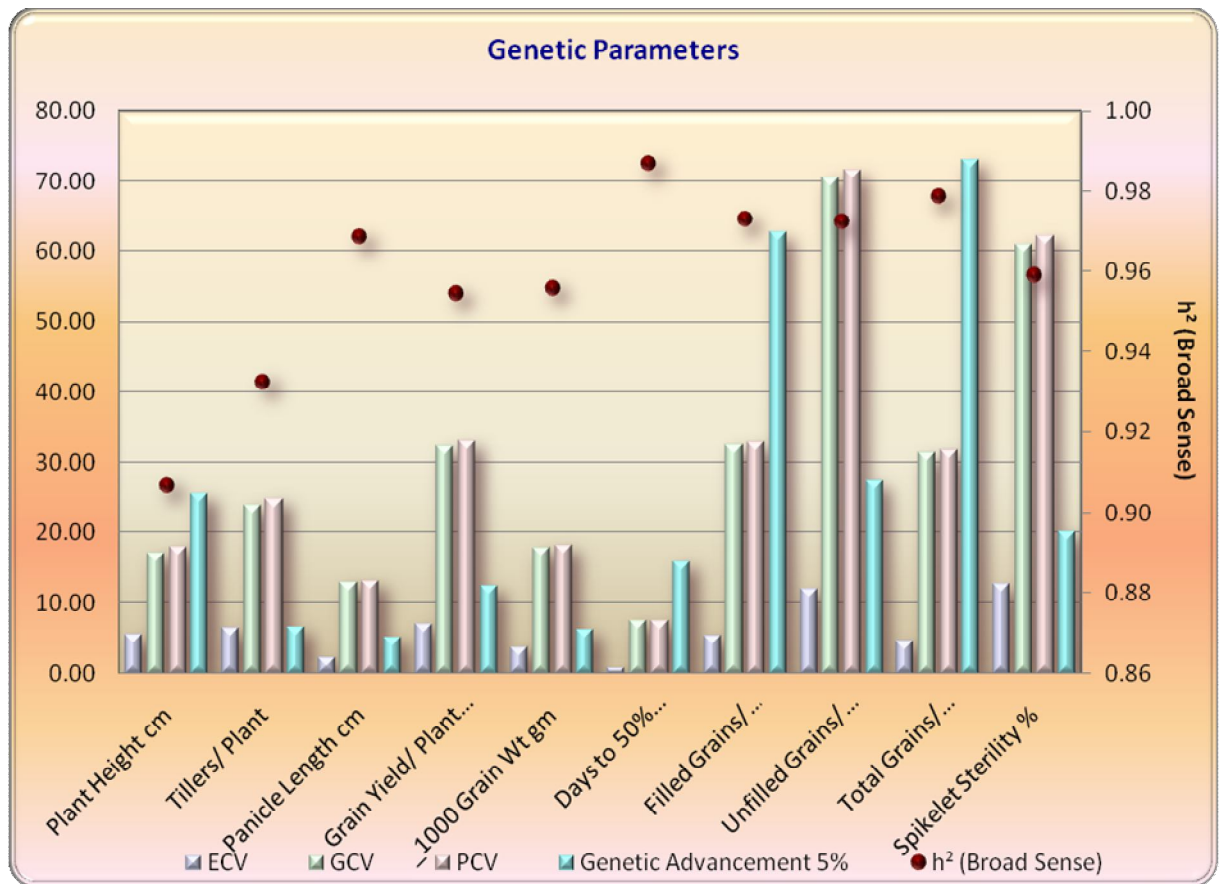


Fig.4.4 Genetic Parameters of rice genotypes for various characters

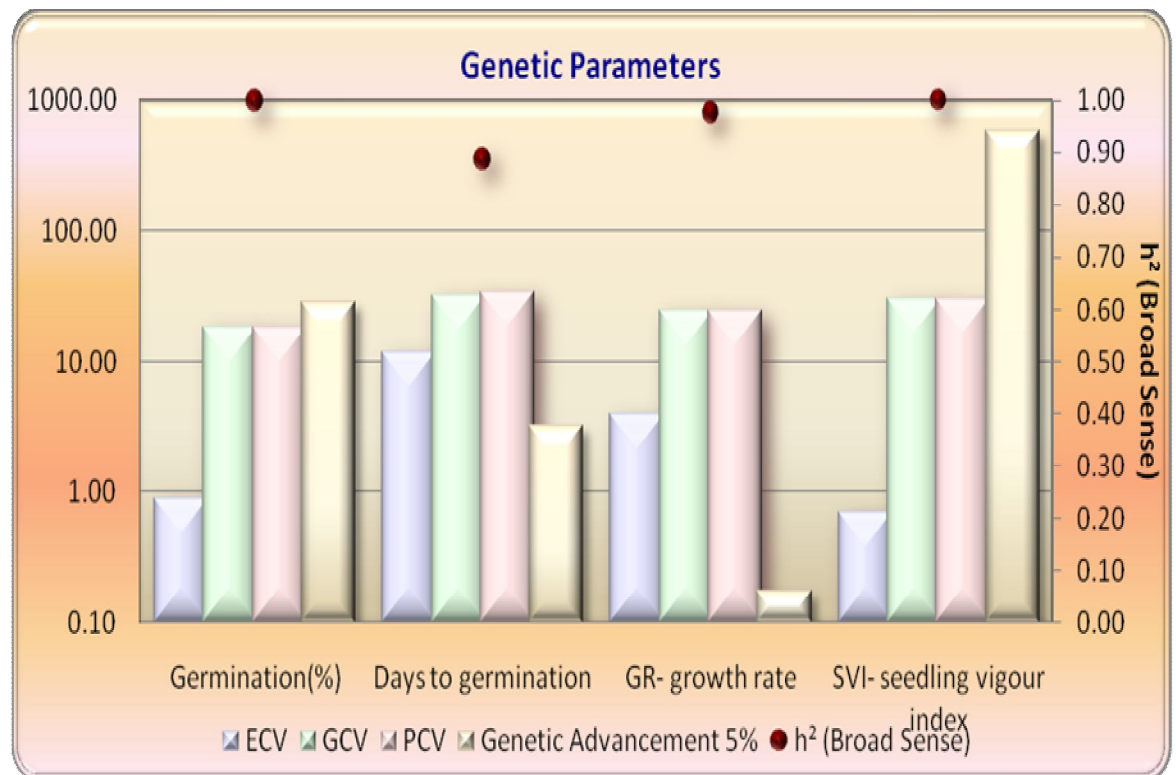
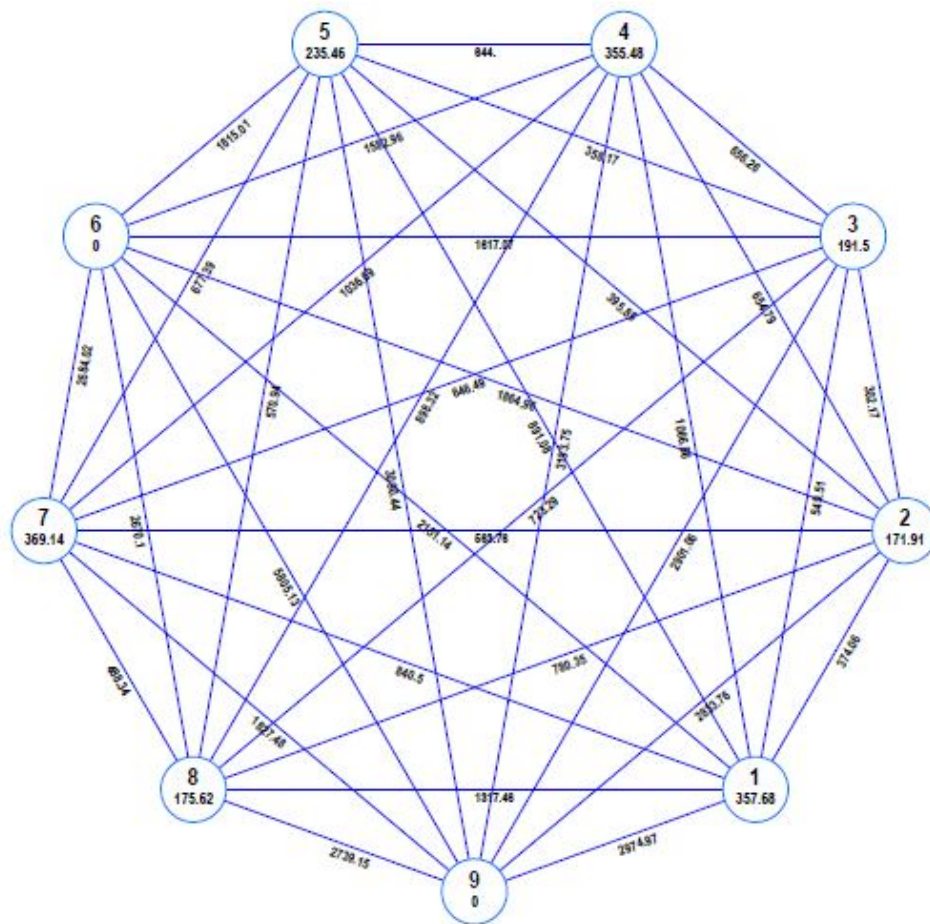


Fig.4.3 Genetic Parameters for cold tolerance at seedling stage



Euclidean² Distance (Not to the Scale)

Fig 4.5 Cluster Diagram for rice genotypes

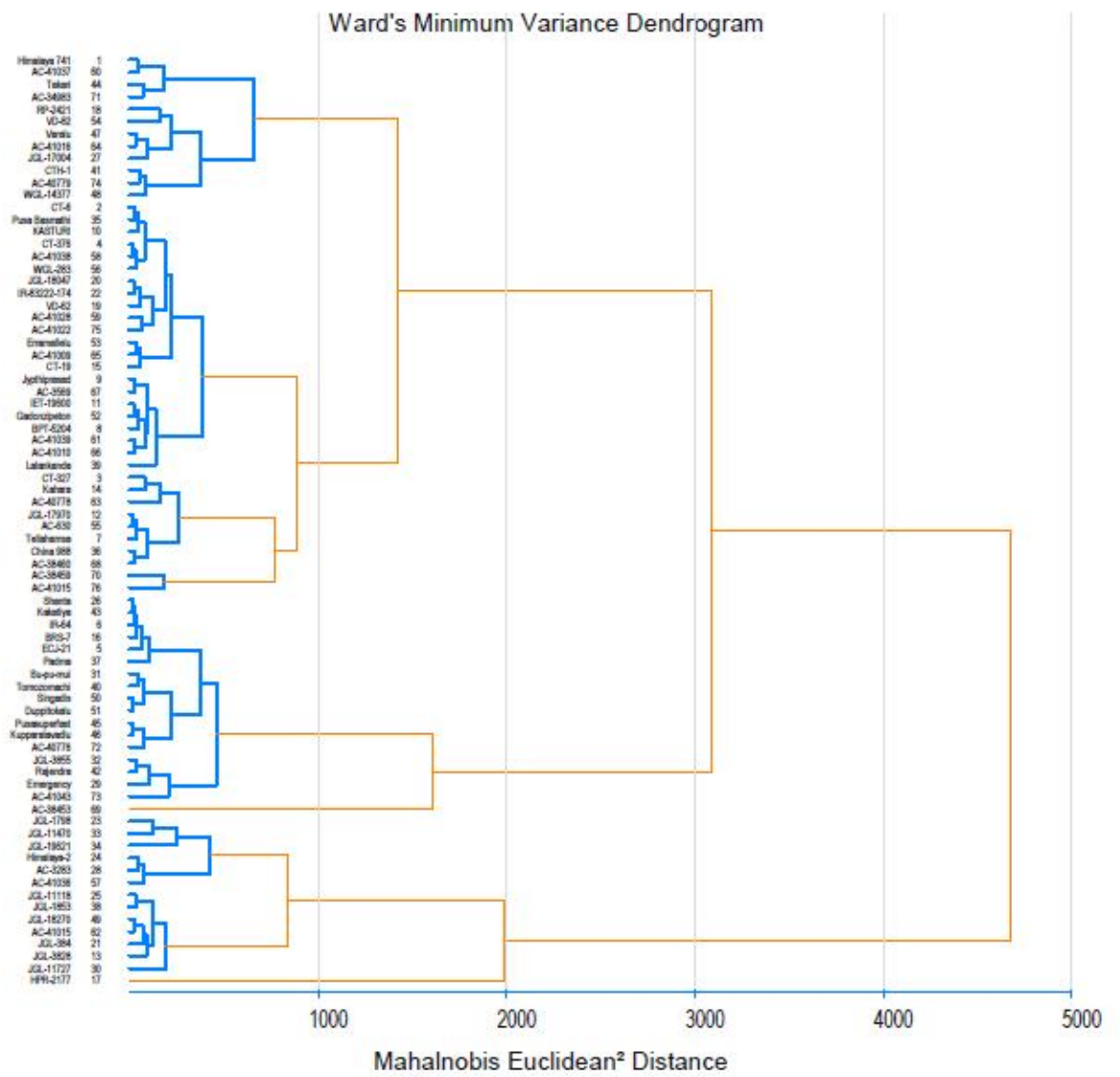


Fig 4.6 Ward's Minimum Variance Dendrogram

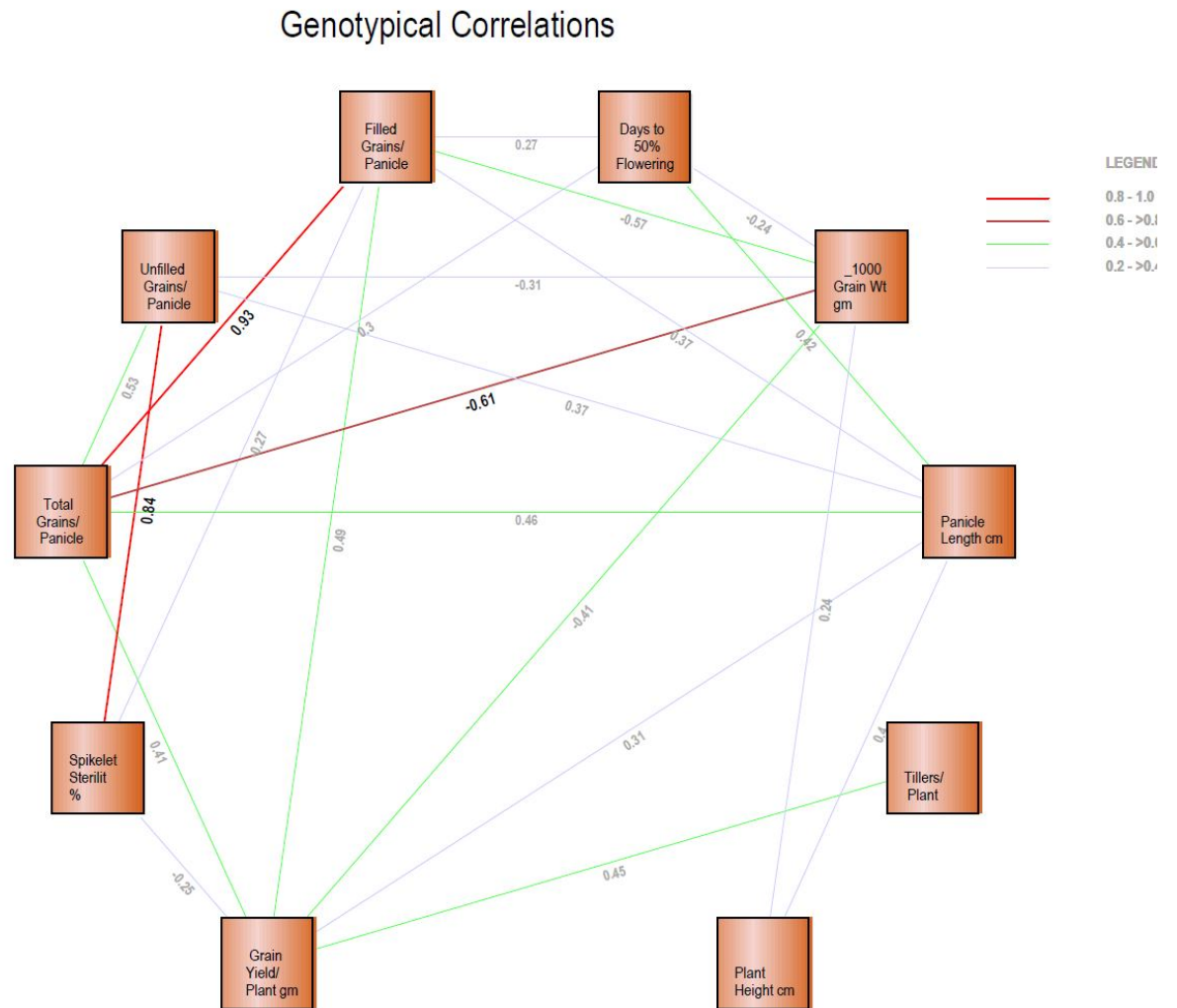


Fig 4.11 Genotypic Correlation of Rice genotypes

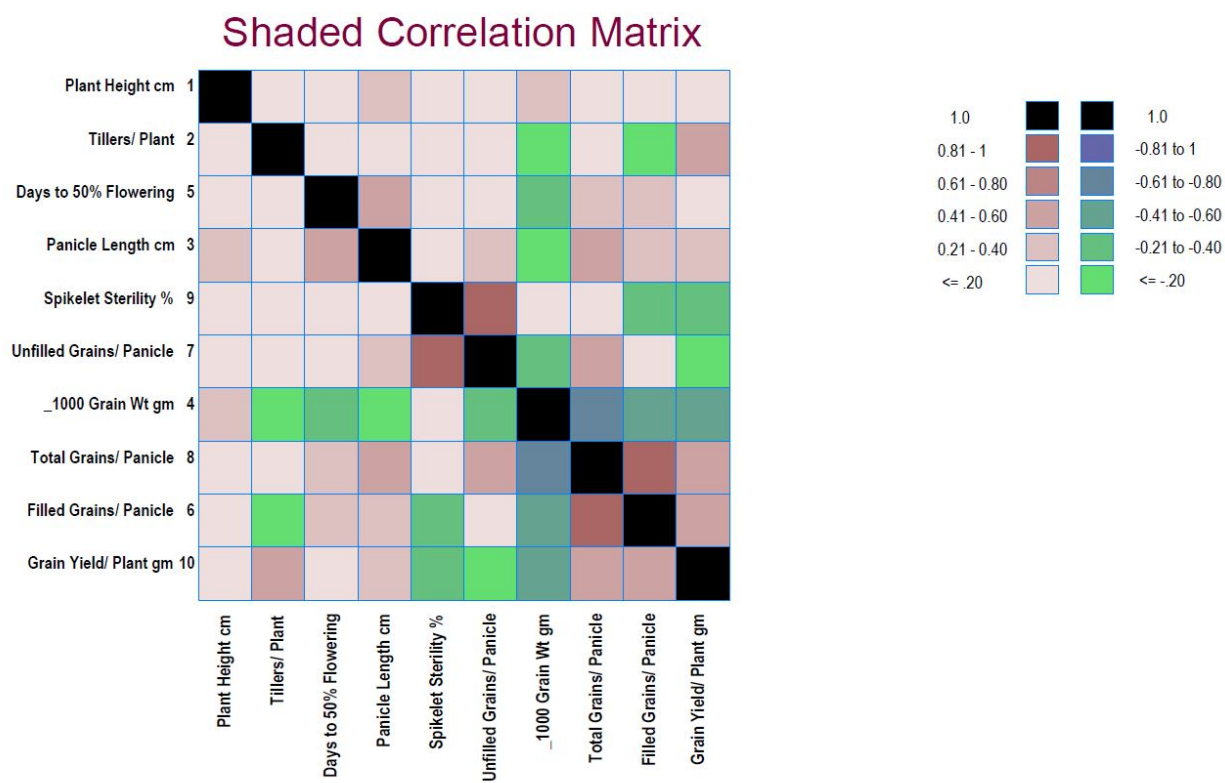


Fig 4.13 Shaded Genotypic Correlation Matrix of Rice genotypes

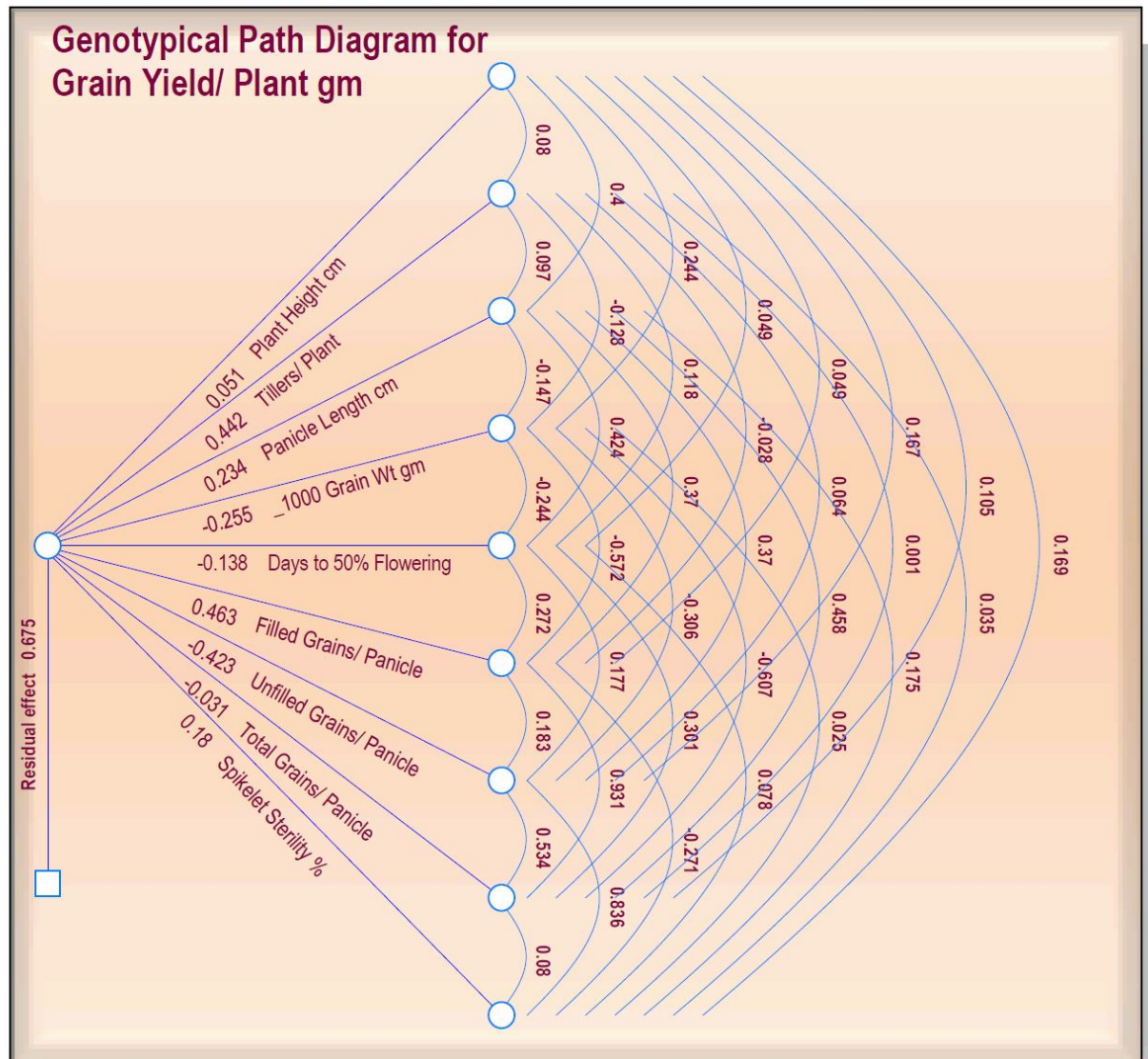


Fig 4.9 Genotypic path diagram for grain yield per plant

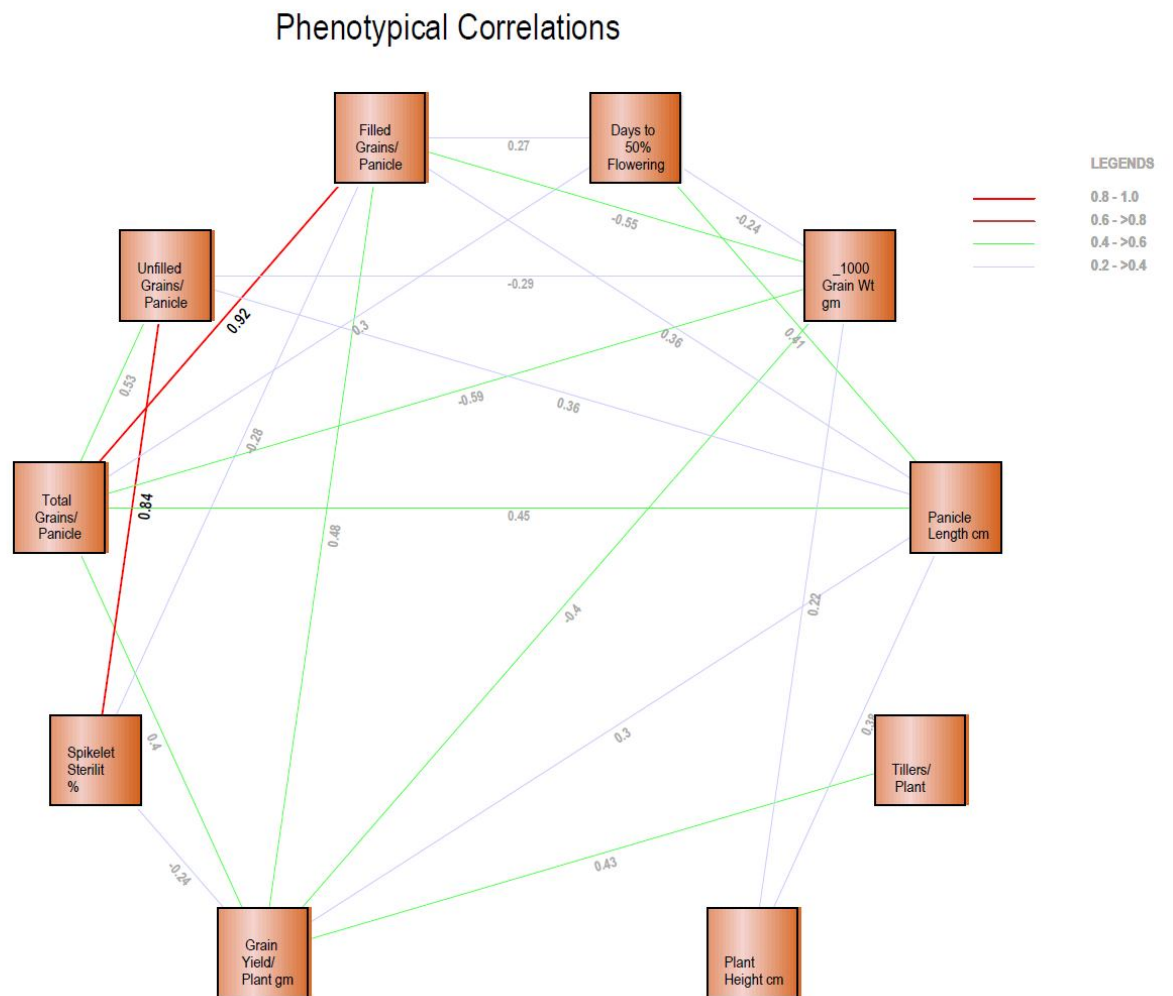


Fig 4.10 Phenotypic Correlations

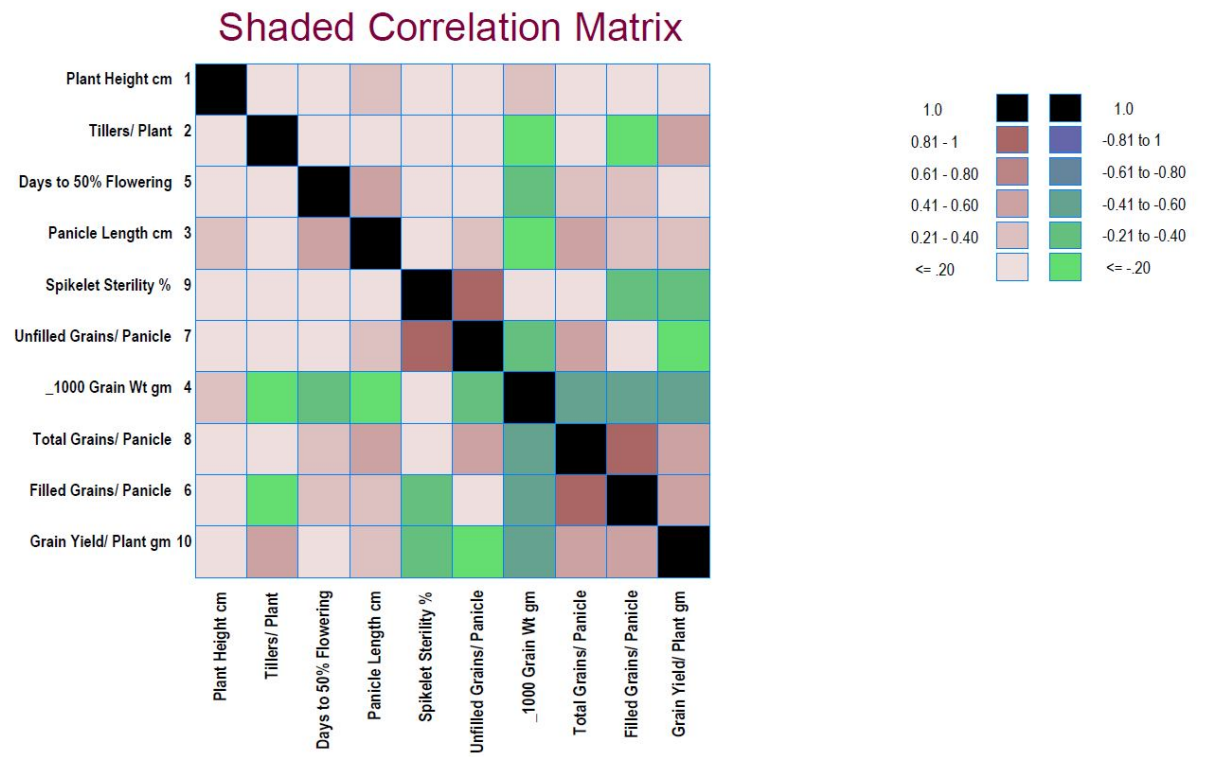


Fig 4.12 Shaded Phenotypic Correlation Matrix

Table 4.14 Allelic frequency and polymorphism information content values of twelve SSR markers

S.No	Primer	Motif	No. of Alleles	PIC Value
1	RM 71	(ATT)10T(ATT)4	3	0.870
2	RM 243	(CT)18	4	0.905
3	RM129	(CGG)8	2	0.688
4	RM 302	(GT)30(AT)8	5	0.907
5	RM220	(CT)17	5	0.932
6	RM 206	(CT)21	8	0.943
7	RM428	(AG)15	3	0.806
8	RM 334	(CTT)20	3	0.848
9	RM 208	(CT)17	5	0.915
10	RM 307	(AT)14(GT)21	4	0.830
11	RM 292	(GT)10-6-(TGA)2TGT(TGA)4	3	0.745
12	RM 404	(GA)33	4	0.882

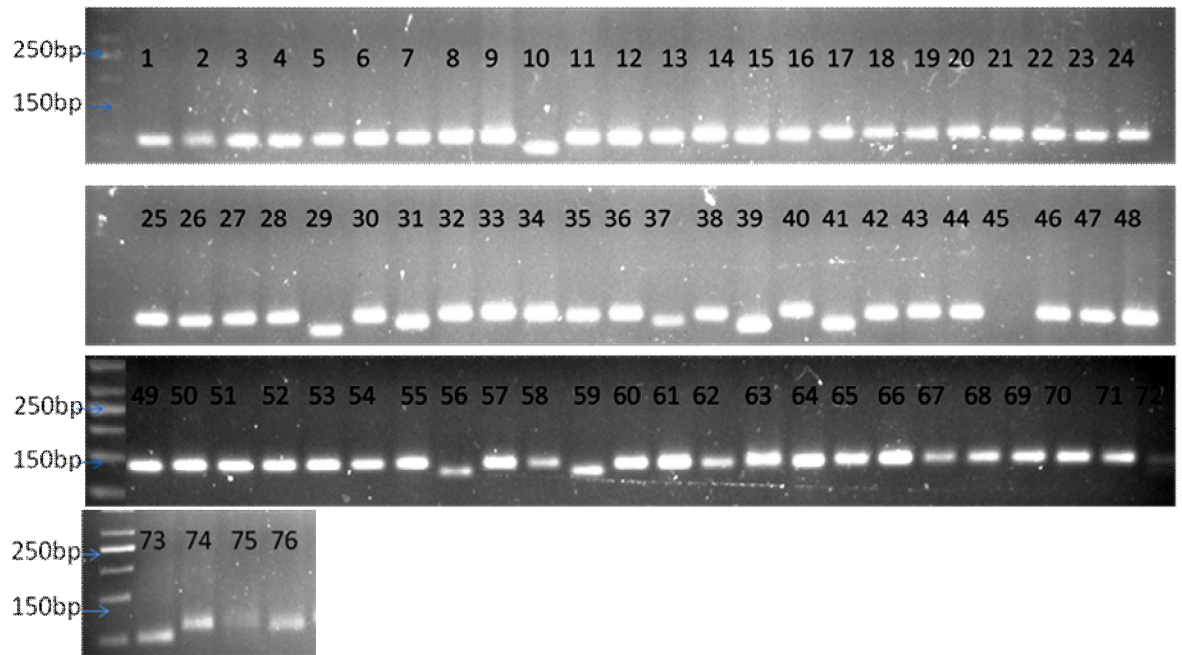


Fig. 4.15. SSR banding pattern of the rice genotypes with RM 220.

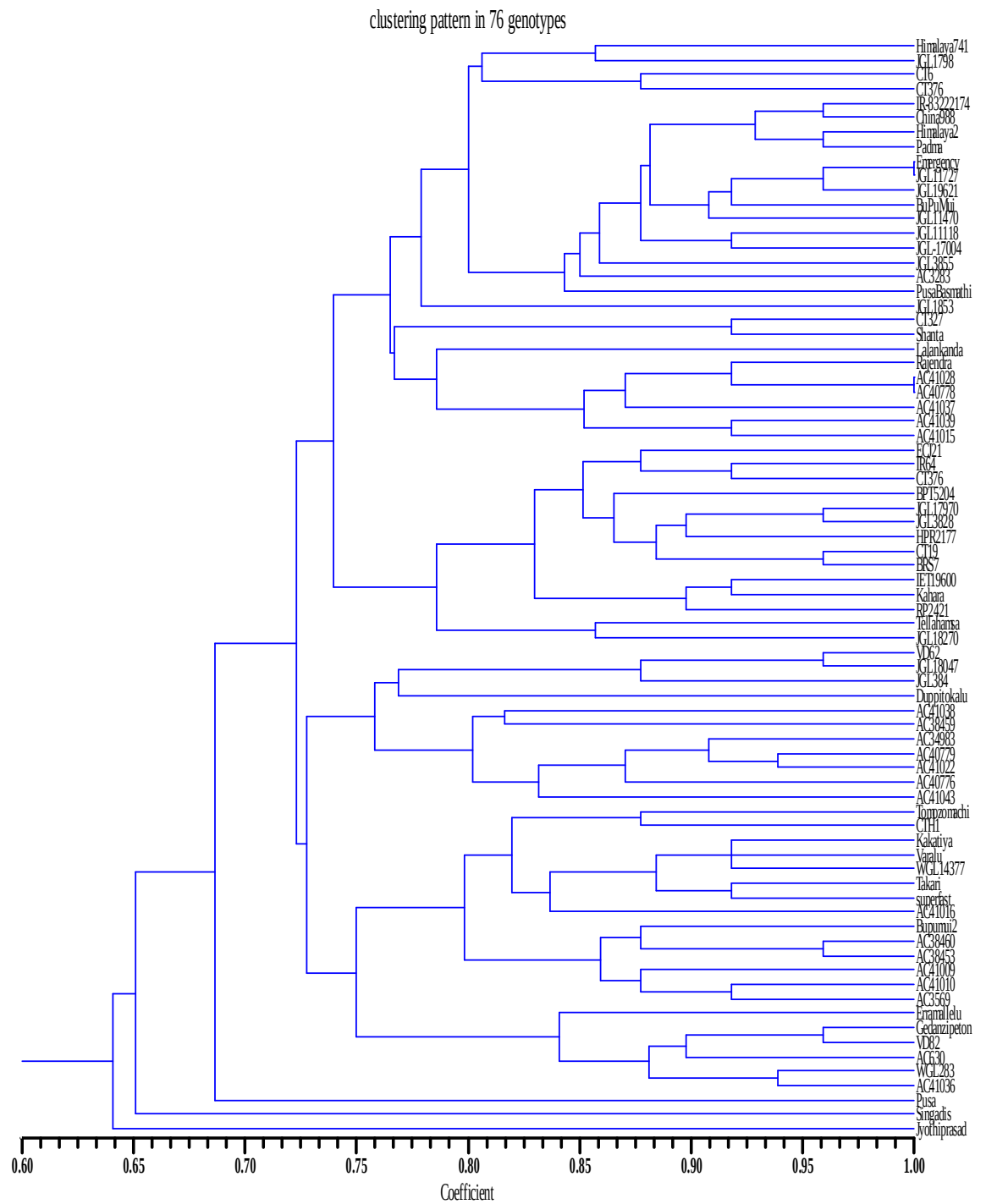


Fig. 4.14 Dendrogram resulting from UPGMA cluster analysis of 76 rice genotypes based on data derived from 12 polymorphic SSR markers

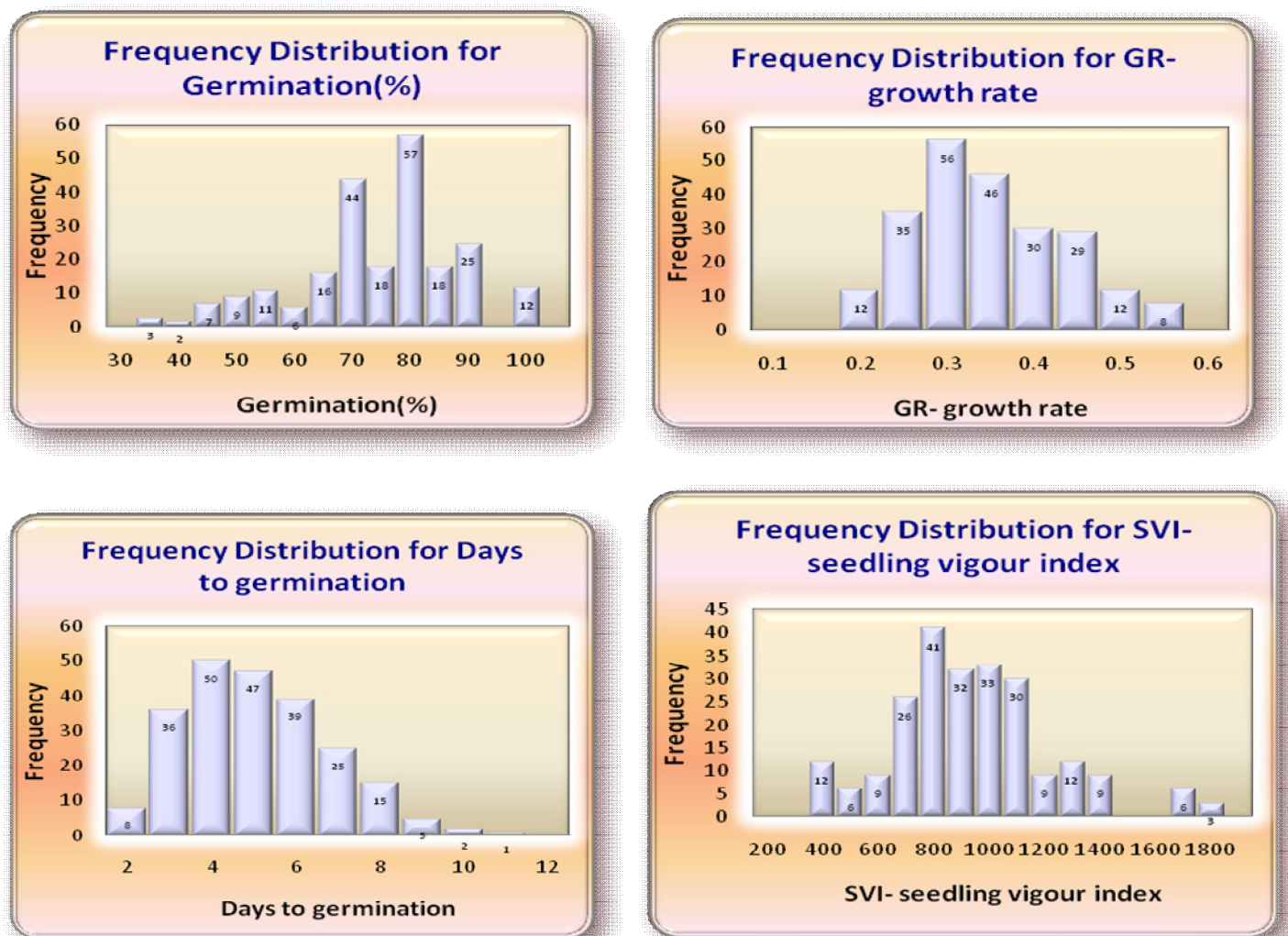


Fig.4.1. Frequency Distribution of various morphological characters for cold tolerance at seedling stage.

Character		Plant height(cm)	Tillers/plant	Panicle length (cm)	1000 grain weight (g)	Days to 50% flowering	Filled Grains/panicle	Un Filled Grains/panicle	Total Grains/panicle	Spikelet sterility%	Grain yield/ plant(g)
Plant height(cm)	P	1.0000	0.0754	0.3767**	0.2234**	0.0470	0.0460	0.1556*	0.0992	0.1551*	0.0985
	G	1.0000	0.0803	0.3996**	0.2443**	0.0494	0.0490	0.1674*	0.1048	0.1690*	0.0903
Tillers/plant	P		1.0000	0.0918	-0.1189	0.1065	-0.0238	0.0616	0.0019	0.0328	0.4306**
	G		1.0000	0.0969	-0.1284	0.1178	-0.0276	0.0644	0.0009	0.0348	0.4514**
Panicle length(cm)	P			1.0000	-0.1353*	0.4132**	0.3622**	0.3579**	0.4482**	0.1655*	0.2960**
	G			1.0000	-0.1471*	0.4236**	0.3704**	0.3698**	0.4576**	0.1749**	0.3086**
1000 grain weight (g)	P				1.0000	-0.2364**	-0.5508***	-0.2939**	-0.5865**	0.0242	-0.4004**
	G				1.0000	-0.2438**	-0.5720**	-0.3058**	-0.6071**	0.0246	-0.4117**
Days to 50% flowering	P					1.0000	0.2661**	0.1753**	0.2956**	0.0778	0.1324*
	G					1.0000	0.2720**	0.1770**	0.3007**	0.0785	0.1339*
Filled Grains/panicle	P						1.0000	0.1719**	0.9248**	-0.2789**	0.4796**
	G						1.0000	0.1829**	0.9308**	-0.2715*	0.4929**
Un Filled Grains/panicle	P							1.0000	0.5261**	0.8384**	-0.0223
	G							1.0000	0.5337**	0.8364**	-0.0279
Total Grains/panicle	P								1.0000	0.0776	0.4040**
	G								1.0000	0.0804	0.4143**
Spikelet sterility%	P									1.0000	-0.2410**
	G									1.0000	-0.2547**

Table 4.10 Phenotypic (P) and Genotypic (G) correlation coefficients

* Significant at 5 per cent level; ** Significant at 1 per cent level

Table 4.11 Phenotypic (r_p) and Genotypic (r_g) correlation coefficients for four characters at seedling stage

No	Character		Germination(%)	Days to germination	GR- growth rate	SVI- seedling vigour index
1	Germination(%)	P	1	-0.4479**	0.2792**	0.7827**
		G	1	-0.4794**	0.2833**	0.7838**
2	Days to germination	P		1	-0.1812**	-0.4247**
		G		1	-0.1917**	-0.4517**
3	GR- growth rate	P			1	0.4059**
		G			1	0.4109**
4	SVI-seedling vigour index	P				1
		G				1

*** Significant at 5 per cent level; ** Significant at 1 per cent level**

Table 4.12. Phenotypic (P) and Genotypic (G) Path coefficient analysis of yield and yield contributing characters in rice

[illegible]

Table 4.8 Character means in different clusters of rice genotypes

Cluster	1	2	3	4	5	6	7	8	9	10
I	71.733	11.632	17.706	16.385	17.722	92.000	91.372	14.061	105.433	13.208
II	76.418	13.837	19.736	21.220	17.889	99.758	90.103	13.893	103.996	13.264
III	67.702	10.578	18.496	10.626	17.110	105.708	75.793	15.683	91.476	16.975
IV	105.475	12.717	25.093	11.217	26.043	106.500	118.512	11.657	130.168	9.755
V	76.808	16.561	20.251	21.170	17.593	110.882	83.552	15.404	98.955	15.648
VI	95.200	12.800	17.800	6.089	25.967	104.000	26.167	39.933	66.100	60.420
VII	74.250	14.806	20.461	23.077	14.219	104.500	121.746	40.275	162.021	26.043
VIII	74.980	12.654	22.542	23.744	12.877	114.429	150.887	28.026	178.913	15.629
IX	103.533	19.133	25.053	10.283	16.587	99.667	67.933	91.713	159.647	57.447

1. Plant Height in cm
2. Number of Tillers per Plant
3. Panicle Length in cm
4. Grain Yield per plant in grams

5. 1000 Grain Weight in grams
6. Days to 50 per cent Flowering
7. No. of Filled Grains/ Panicle
8. No. of Unfilled Grains/ Panicle

9. Total Grains/ Panicle
10. Spikelet Sterility (%)

Table 4.7 Intra cluster (in bold) and inter cluster distance for 10 characters in rice

[illegible]

Table 4.6 Distribution of rice genotypes in different clusters

Cluster	No. of genotypes	Genotypes
I	12	Himalaya741, AC41037, Takari, AC34983, RP2421, VD82, Varalu, AC41016, JGL17004, CTH1, AC40779, WGL14377
II	22	CT6, Pusabasmathi, Kasturi, CT376, AC41038, WGL283, JGL18047, IR83222-174, VD62, AC41028, AC41022, Erramallelu, AC41009, CT19, Jyothiprasad, AC3569, IET19600, Gadonzipeton, BPT5204, AC41039, AC41010, Lalankanda
III	8	CT327, Kahara, AC40778, JGL17970, AC630, Tellahamsa, China988, AC38460
IV	2	AC38459, AC41015
V	17	Shanta, Kakatiya, IR64, BRS7, ECJ21, Padma, Bu-pu-mui, Tomazomachi, Singadis, Duppitokalu, pusasuperfast, Kupparalavadlu, AC40776, JGL3855, Rajendra, Emergency, AC41043
VI	1	AC38453,
VII	6	JGL1798, JGL11470, JGL19621, Himalaya2, AC3283, AC41036
VIII	7	JGL11118, JGL1853, JGL18270, AC41015, JGL384, JGL3828, JGL11727
IX	1	HPR2177

Table 4.5 Genetic Parameters for cold tolerance at seedling stage (Summary)

	Germination percentage	Days to germination	GR-growth rate	SVI-Seedling vigour Index
Var Environmental	0.45	0.36	0	43.02
ECV	0.9	11.75	3.88	0.7
Var Genotypical	191.07	2.76	0.01	82986.33
GCV	18.57	32.56	24.61	30.69
Var Phenotypical	191.52	3.12	0.01	83029.34
PCV	18.59	34.62	24.92	30.69
h ² (Broad Sense)	1	0.88	0.98	1
Genetic Advancement 5%	28.44	3.22	0.17	593.28
Gen.Adv as % of Mean 5%	38.2	63.1	50.09	63.2
General Mean	74.44	5.11	0.35	938.76

Table 4.4 Genetic Parameters (Summary)				
	Germination percentage	Days to germination	GR-growth rate	SVI-Seedling vigour Index
V _p	191.52	3.12	0.01	83029.34
V _g	191.07	2.76	0.01	82986.33
V _e	0.45	0.36	0	43.02
PCV	18.59	34.62	24.92	30.69
GCV	18.57	32.56	24.61	30.69
ECV	0.9	11.75	3.88	0.7
h ² (Broad Sense)	1	0.88	0.98	1
GA at 5%	28.44	3.22	0.17	593.28
GAPM at 5%	38.2	63.1	50.09	63.2
General Mean	74.44	5.11	0.35	938.76
V _p = Phenotypic variance		PCV= Phenotypic coefficient of variation		
V _g = Genotypic variance		GCV= Genotypic coefficient of variation		
V _e = Environmental variance		ECV= Environmental coefficient of variation		
h ² (bs)=Heritability broad sense		GA at 5%= Genetic advance at 5% level		
		GAPM at 5%= Genetic advance as percent of mean at 5% level		

Table 4.4 Genetic Parameters (Summary)

	Plant height(cm)	Tillers/plant	Panicle length (cm)	Grain yield/plant	1000 grain weight	Days to 50 per cent flowering	No. of filled grains	No. of unfilled grains	Total grains/panicle	Spikelet sterility(%)
Var Environmental	17.57	0.79	0.22	1.81	0.44	0.81	26.62	5.18	28.27	4.33
ECV	5.52	6.47	2.34	7.04	3.83	0.87	5.43	11.89	4.66	12.59
Var Genotypical	170.71	10.93	6.62	37.93	9.46	60.02	950.63	181.93	1284.16	101.06
GCV	17.21	24.05	12.92	32.24	17.81	7.48	32.44	70.48	31.38	60.87
Var Phenotypical	188.29	11.72	6.83	39.73	9.90	60.83	977.24	187.10	1312.42	105.38
PCV	18.08	24.90	13.14	33.00	18.22	7.53	32.89	71.47	31.73	62.16
h ² (Broad Sense)	0.91	0.93	0.97	0.95	0.96	0.99	0.97	0.97	0.98	0.96
Genetic Advancement 5%	25.63	6.58	5.21	12.39	6.19	15.85	62.64	27.40	73.02	20.28
Gen. Adv as per cent of Mean 5%	33.76	47.84	26.20	64.88	35.87	15.30	65.91	143.16	63.95	122.79
General Mean	75.91	13.75	19.90	19.10	17.27	103.61	95.04	19.14	114.18	16.52

Table 4.5 Genetic Parameters (Summary)

	Plant height(cm)	Tillers/plant	Panicle length (cm)	Grain yield/plant	1000 grain weight	Days to 50 per cent flowering	No. of filled grains	No. of unfilled grains	Total grains/panicle	Spikelet sterility (%)
V _p	188.29	11.72	6.83	39.73	9.90	60.83	977.24	187.10	1312.42	105.38
V _g	170.71	10.93	6.62	37.93	9.46	60.02	950.63	181.93	1284.16	101.06
V _e	17.57	0.79	0.22	1.81	0.44	0.81	26.62	5.18	28.27	4.33
PCV	18.08	24.90	13.14	33.00	18.22	7.53	32.89	71.47	31.73	62.16
GCV	17.21	24.05	12.92	32.24	17.81	7.48	32.44	70.48	31.38	60.87
ECV	5.52	6.47	2.34	7.04	3.83	0.87	5.43	11.89	4.66	12.59
h ² (bs)	0.91	0.93	0.97	0.95	0.96	0.99	0.97	0.97	0.98	0.96
GA at 5%	25.63	6.58	5.21	12.39	6.19	15.85	62.64	27.40	73.02	20.28
GAPM at 5%	33.76	47.84	26.20	64.88	35.87	15.30	65.91	143.16	63.95	122.79
General Mean	75.91	13.75	19.90	19.10	17.27	103.61	95.04	19.14	114.18	16.52
V _p = Phenotypic variance			PCV= Phenotypic coefficient of variation				h ² (bs)=Heritability broad sense			
V _g = Genotypic variance			GCV= Genotypic coefficient of variation				GA at 5%= Genetic advance at 5% level			
V _e = Environmental variance			ECV= Environmental coefficient of variation				GAPM at 5%= Genetic advance as percent of mean at 5% level			

Table 4. 3(a) ANOVA (RBD) for ten quantitative characters

S.No.	Character	Mean sum of squares		
		Replications	Treatments	Error
		(d.f.=2)	(d.f.=75)	(d.f.=150)
1	Days to 50 percent flowering	0.157895	180.859649 **	0.811228
2	Plant height (cm)	6.643408	529.716593 **	17.572391
3	Number of tillers per plant	2.21075	33.585637	0.791176
4	Panicle length (cm)	0.162107	20.065898 **	0.216956
5	1000 grain weight (g)	0.858984	28.815697 **	0.436931
6	Grain yield per plant (g)	5.422686	115.589872 **	1.807266
7	Number of filled grains per panicle	49.98495	2878.492334 **	26.617048
8	Number of unfilled grains per panicle	17.69608	550.954328 **	5.175688
9	Total grains per panicle	5.555058	3880.739748 **	28.267048
10	Spikelet sterility percentage	3.575422	307.494031 **	4.325527
** Significant at 1% level * Significant at 5% level				

S.No.	Character	Mean sum of squares	
		Treatments	Error
		(d.f =75)	(d.f =152)
1	Days to germination	8.6508 **	0.3596
2	Germination percentage	573.6535 **	0.4537
3	Growth rate	0.0222 **	0.0002
4	Seedling Vigour Index	249002 **	43.0199
** Significant at 1% level * Significant at 5% level			

Table 4.3 (b) ANOVA (CRD) for four characters at seedling stage

Table 4.1a. Mean Performance of genotypes with respect of four characters at seedling stage						
S. No	Genotype	Germination %	Days to germination	GR	SVI	Leaf yellowing
	MEAN PERFORMANCE					
1	Himalaya 741	76.27	8.67	0.3423	830.42	3
2	CT-6	71.00	7.33	0.2430	797.83	5
3	CT-327	80.50	3.67	0.2490	830.50	3
4	CT-376	75.83	4.33	0.3467	794.03	5
5	ECJ-21	78.65	3.67	0.2907	967.52	5
6	IR-64	87.54	2.00	0.5577	1392.79	3
7	Tellahamsa	91.05	5.33	0.5153	1659.61	1
8	BPT-5204	69.40	5.33	0.3203	922.81	5
9	Jyothiprasad	82.15	5.67	0.2627	804.54	3
10	Kasturi	70.36	6.33	0.2270	579.88	5
11	IET-19600	88.20	6.00	0.4097	1343.46	7
12	JGL-17970	92.27	4.67	0.3380	1033.39	7
13	JGL-3828	90.27	4.33	0.3250	731.16	7
14	Kahara	45.08	8.33	0.3667	359.85	7
15	CT-19	42.32	7.33	0.3077	472.92	5
16	BRS-7	71.22	7.33	0.3733	533.45	5
17	HPR-2177	81.21	7.00	0.2810	1075.58	5
18	RP-2421	81.34	7.33	0.3123	1152.55	5
19	VD-62	75.74	3.67	0.3390	927.32	7
20	JGL-18047	83.33	4.67	0.3300	941.52	7
21	JGL-384	82.37	3.33	0.3800	726.88	3
22	IR-83222-174	98.43	3.33	0.5347	1804.34	1

23	JGL-1798	84.21	5.67	0.4267	1015.80	5
S. No	Genotype	Germination %	Days to germination	GR	SVI	Leaf yellowing
24	Himalaya-2	81.77	6.00	0.3540	938.01	3
25	JGL-11118	78.48	4.33	0.4477	753.43	5
26	Shanta	56.97	5.33	0.4323	444.77	5
27	JGL-17004	91.30	3.67	0.3830	922.31	7
28	AC-3283	70.10	3.67	0.2300	720.38	5
29	Emergency	98.35	3.33	0.4733	1390.61	1
30	JGL-11727	81.55	5.33	0.2010	1027.04	5
31	Bu-Pu-Mui	50.13	5.33	0.3127	396.63	5
32	JGL-3855	84.35	5.00	0.3123	850.38	5
33	JGL-11470	84.50	4.33	0.2290	945.80	3
34	JGL-19621	65.01	6.33	0.3010	735.11	5
35	PusaBasmathi	72.53	7.00	0.3843	1025.63	5
36	China 988	80.63	6.00	0.5277	1288.58	7
37	Padma	55.18	8.33	0.4100	782.94	5
38	JGL-1853	80.30	6.00	0.4377	906.72	5
39	Lalankanda	75.03	4.67	0.3823	1094.49	7
40	Tomozomachi	82.42	5.67	0.4247	1075.33	5
41	CTH-1	48.52	7.00	0.2873	685.60	5
42	Rajendra	50.24	6.67	0.3357	611.26	5
43	Kakatiya	54.23	6.33	0.3277	667.07	5
44	Takari	68.78	5.33	0.2207	703.21	3
45	Pusa superfast	99.65	4.00	0.5100	1702.87	1

46	Kupparalavadlu	71.03	5.33	0.2157	1079.37	3
S. No	Genotype	Germination %	Days to germination	GR	SVI	Leaf yellowing
47	Varalu	65.30	10.33	0.2523	660.52	5
48	WGL-14377	66.98	8.33	0.2877	816.76	7
49	JGL-18270	61.80	4.67	0.2613	886.38	5
50	Singadis	74.76	5.33	0.3233	835.48	5
51	Duppitokalu	46.13	4.33	0.2190	564.11	3
52	Gedanzipeton	33.90	7.33	0.3110	371.95	7
53	Erramallelu	68.77	4.67	0.2547	794.03	3
54	VD-82	71.36	5.67	0.3500	1055.13	5
55	AC-630	82.23	4.67	0.2750	1110.13	5
56	WGL-283	98.27	5.33	0.3177	1236.49	5
57	AC-41036	90.12	2.67	0.2923	1342.06	3
58	AC-41038	89.21	3.33	0.3533	1266.25	3
59	AC-41028	80.14	3.67	0.2390	1120.29	7
60	AC-41037	78.25	3.00	0.3323	985.95	3
61	AC-41039	69.76	3.00	0.3177	955.39	5
62	AC-41015	82.14	2.33	0.2733	1246.98	5
63	AC-40778	78.14	3.33	0.3077	1116.41	5
64	AC-41016	59.58	4.00	0.3133	738.85	5
65	AC-41009	66.23	6.33	0.4523	941.80	7
66	AC-41010	71.20	2.67	0.4523	1073.98	3
67	AC-3569	72.18	3.67	0.4210	951.12	5
68	AC-38460	82.18	3.67	0.4477	992.41	7

69	AC-38453	82.14	4.67	0.4410	1012.02	7
S. No	Genotype	Germination %	Days to germination	GR	SVI	Leaf yellowing
70	AC-38459	70.17	4.33	0.5100	776.52	7
71	AC-34983	87.83	3.67	0.2723	1387.07	7
72	AC-40776	66.13	7.33	0.3450	819.35	3
73	AC-41043	55.14	5.67	0.3413	813.11	5
74	AC-40779	72.24	3.00	0.4177	912.50	5
75	AC-41022	76.24	3.67	0.4457	1082.41	7
76	AC-41015	79.45	3.00	0.4257	1034.75	5
	Mean	74.44	5.11	0.3483	938.76	
	C.V.	0.90	11.75	3.8835	0.70	
	F ratio	1264.34	24.05	121.5173	5788.07	
	S.E.	0.39	0.35	0.0078	3.79	
	C.D. 5%	1.09	0.97	0.0218	10.58	
	C.D. 1%	1.43	1.28	0.0288	13.97	
	Range Lowest	33.90	2.00	0.2010	359.85	
	Range Highest	99.65	10.33	0.5577	1804.34	

SVI-Seedling Vigour Index

GR-Seedling growth rate in terms of Dry weight basis

Table 4.1b. Mean performance of 76 rice genotypes with respect of ten characters

	PH	T/P	PL	GY/P	1000Gwt	FF	FG	UG	TG	SS%
Himalaya 741	55.93	10.10	14.98	7.87	18.90	92.00	79.03	18.13	97.17	18.65
CT-6	70.87	16.30	19.57	21.40	18.07	97.33	93.09	2.45	95.54	2.55
CT-327	77.27	13.47	16.27	10.26	15.54	105.00	65.41	4.83	70.24	6.88
CT-376	68.47	12.37	19.93	20.05	17.81	101.33	102.00	2.63	104.63	2.51
ECJ-21	68.67	18.03	18.83	31.96	17.17	110.00	110.00	8.00	118.00	6.77
IR-64	73.43	15.13	20.78	21.21	18.50	110.33	101.93	15.20	117.13	12.87
Tellahamsa	67.60	13.23	20.75	15.42	16.38	105.33	90.23	19.28	109.52	17.60
BPT-5204	51.20	13.20	17.09	19.75	19.07	101.67	78.09	14.60	92.69	15.74
Jypthiprasad	64.33	14.27	18.29	17.26	18.04	98.00	64.42	23.14	87.56	26.42
KASTURI	79.00	14.10	21.31	29.77	18.84	99.33	82.08	9.57	91.64	10.43
IET-19600	69.27	13.13	18.20	23.44	17.13	102.33	97.99	22.57	120.56	18.73
JGL-17970	78.70	10.03	18.73	11.45	17.32	103.00	92.86	14.93	107.79	13.87
JGL-3828	70.42	10.80	22.60	18.35	12.37	117.67	138.50	19.47	157.97	12.36
Kahara	73.67	4.80	15.49	6.33	19.10	106.33	71.60	9.57	81.17	11.76
CT-19	84.17	12.73	24.07	29.11	17.17	102.67	84.57	17.80	102.37	17.39
BRS-7	66.00	19.53	19.95	26.13	14.94	112.33	101.60	22.30	123.90	18.00
HPR-2177	103.53	19.13	25.05	10.28	16.59	99.67	67.93	91.71	159.65	57.45
RP-2421	86.77	5.87	19.45	15.17	15.07	99.33	132.99	29.49	162.49	18.19
VD-62	101.73	9.00	18.25	21.60	15.64	99.67	95.58	19.00	114.58	16.52
JGL-18047	73.80	11.40	21.00	19.58	19.11	101.67	98.32	22.13	120.45	18.39
JGL-384	67.40	10.33	21.29	18.10	12.50	116.67	130.82	38.19	169.02	22.59
IR-83222-174	82.66	8.87	19.25	18.63	20.91	101.00	98.86	18.81	117.67	16.25
JGL-1798	69.20	16.00	21.41	22.64	11.10	108.67	169.00	46.93	215.93	21.75
Himalaya-2	76.33	11.33	19.12	19.39	17.31	106.67	100.67	45.02	145.69	30.91
JGL-11118	70.93	16.00	20.60	27.29	13.49	108.67	154.67	26.07	180.73	14.41
Shanta	71.17	19.13	20.51	26.44	18.55	113.67	112.17	14.83	127.00	11.77
JGL-17004	59.20	11.20	17.83	13.77	11.42	89.00	133.62	12.81	146.43	8.75
AC-3283	83.00	12.83	21.07	17.75	16.50	102.00	77.73	48.43	126.17	38.38
Emergency	63.07	16.87	19.37	15.45	18.09	116.33	82.47	32.80	115.27	28.72
JGL-11727	81.20	11.27	27.31	27.30	14.15	119.00	175.45	30.45	205.89	14.76
Bu-pu-mui	75.87	14.33	20.44	20.42	19.11	104.67	64.51	20.07	84.57	23.72

JGL-3855	72.04	16.31	24.89	16.87	15.97	116.67	76.67	20.00	96.67	20.87
JGL-11470	76.17	13.30	23.85	24.61	11.20	105.00	117.20	39.68	156.88	25.29
JGL-19621	65.93	19.83	17.13	29.92	10.63	100.33	170.24	29.31	199.55	14.67
Pusa Basmathi	84.87	17.77	21.10	22.32	19.24	95.33	87.23	9.67	96.90	9.97
China 988	64.33	10.67	19.45	10.22	18.67	101.33	55.83	19.90	75.73	26.26
Padma	81.67	21.80	21.47	30.83	13.27	106.00	98.15	19.51	117.66	16.61
JGL-1853	80.20	14.87	21.31	26.32	12.65	112.00	134.33	20.17	154.50	13.05
Lalankanda	76.17	19.17	15.87	22.88	15.32	100.33	56.40	8.00	64.40	12.39
Tomozomachi	82.13	10.33	21.83	21.53	17.61	109.33	76.07	11.27	87.33	12.90
CTH-1	79.20	14.27	20.09	22.66	21.20	86.00	79.13	8.00	87.13	9.18
Rajendra	74.47	13.00	22.19	14.98	16.45	114.00	78.67	22.67	101.33	22.35
Kakatiya	76.53	17.57	19.81	23.04	16.42	113.33	100.87	9.93	110.80	8.96
Takari	49.57	14.64	14.07	11.89	20.91	87.00	63.47	2.73	66.20	4.14
Pusasuperfast	95.23	20.27	19.54	19.09	19.00	105.33	67.17	7.07	74.24	9.50
Kupparalavadlu	93.18	16.73	17.93	16.81	19.96	107.67	64.33	7.97	72.30	10.62
Varalu	77.44	13.47	18.01	20.84	15.59	92.33	104.56	14.78	119.34	12.41
WGL-14377	75.73	9.83	19.53	13.78	18.77	90.00	64.48	20.55	85.03	24.17
JGL-18270	82.11	11.28	23.06	25.42	12.90	115.33	158.76	29.56	188.31	15.75
Singadis	68.16	14.52	20.21	22.81	19.00	109.67	86.53	8.00	94.53	8.47
Duppitokalu	53.40	13.00	19.61	17.73	19.49	110.67	73.45	8.78	82.23	10.67
Gadonzipeton	76.67	13.00	17.02	21.78	18.50	101.67	113.41	13.07	126.48	10.32
Erramallelu	74.43	18.63	22.91	26.69	15.28	98.67	108.40	14.67	123.07	11.91
VD-82	106.80	11.17	18.83	27.32	16.43	95.00	142.74	9.31	152.05	6.11
AC-630	69.63	10.40	19.51	11.58	17.81	105.33	83.60	23.85	107.45	22.18
WGL-283	73.00	9.73	20.23	17.63	17.61	100.00	77.81	8.10	85.91	9.44
AC-41036	74.87	15.53	20.19	24.15	18.58	104.33	95.63	32.27	127.90	25.26
AC-41038	64.53	15.33	19.96	19.63	18.71	102.00	90.87	12.40	103.27	12.00
AC-41028	92.40	15.87	21.05	18.14	15.59	101.33	73.92	9.55	83.47	11.40
AC-41037	60.47	8.67	16.27	11.66	18.91	98.33	81.20	10.49	91.69	11.44
AC-41039	75.92	13.37	17.34	21.21	20.15	93.00	89.73	11.56	101.29	11.40
AC-41015	72.60	14.03	21.61	23.42	12.08	111.67	163.68	32.28	195.97	16.47
AC-40778	42.44	9.19	18.79	6.47	15.23	113.67	85.27	6.93	92.20	7.52

AC-41016	68.80	15.47	18.69	14.99	15.88	87.33	94.75	18.80	113.55	16.55
AC-41009	81.47	12.30	22.67	26.44	15.77	101.00	118.07	20.53	138.60	14.81
AC-41010	72.17	15.67	16.87	18.08	19.31	97.67	86.24	13.34	99.58	13.40
AC-3569	61.58	15.88	18.95	15.01	16.31	97.33	84.33	17.83	102.17	17.45
AC-38460	67.97	12.83	18.97	13.27	16.83	105.67	61.55	26.17	87.71	29.72
AC-38453	95.20	12.80	17.80	6.09	25.97	104.00	26.17	39.93	66.10	60.42
AC-38459	109.75	13.43	23.03	9.85	25.67	108.67	149.60	9.87	159.47	6.19
AC-34983	64.53	11.67	14.97	10.93	21.01	95.33	42.22	4.17	46.39	8.98
AC-40776	100.07	16.77	17.93	17.53	18.75	115.33	77.43	5.27	82.70	6.38
AC-41043	90.67	18.20	18.96	17.07	16.80	109.67	48.37	28.20	76.57	36.85
AC-40779	76.36	13.24	19.75	25.74	18.59	92.33	78.27	19.47	97.73	19.92
AC-41022	102.50	12.33	23.23	16.45	20.00	101.33	100.87	14.23	115.10	12.37
AC-41015	101.20	12.00	27.16	12.58	26.42	104.33	87.42	13.45	100.87	13.32
Mean	75.91	13.75	19.90	19.10	17.27	103.61	95.04	19.14	114.18	16.52
C.V.	5.52	6.47	2.34	7.04	3.83	0.87	5.43	11.89	4.66	12.59
F ratio	30.14	42.45	92.49	63.96	65.95	222.95	108.14	106.45	137.29	71.09
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E.	2.42	0.51	0.27	0.78	0.38	0.52	2.98	1.31	3.07	1.20
C.D. 5%	6.76	1.44	0.75	2.17	1.07	1.45	8.32	3.67	8.58	3.36
C.D. 1%	8.93	1.89	0.99	2.86	1.41	1.92	10.99	4.85	11.33	4.43
Range Lowest	42.44	4.80	14.07	6.09	10.63	86.00	26.17	2.45	46.39	2.51
Range Highest	109.75	21.80	27.31	31.96	26.42	119.00	175.45	91.71	215.93	60.42

PH-Plant height in cm; T/P-Tillers per plant; PL-Panicle length in cm ; GY/P-Grain yield per plant; 1000 GW-1000 grain weight; FF-50 per cent flowering; FG-number of filled grains per panicle; UG-number of unfilled grains per panicle; TG-Total grains per panicle; SS%-Spikelet sterility percentage.

Table 4.2 Response of genotypes to cold at seedling stage			
	Response to cold	Genotype	Leaf yellowing score
1	Tolerant	Tellahamsa	1
2	Tolerant	IR-83222-174	1
3	Tolerant	Emergency	1
4	Tolerant	Pusa superfast	1
1	Moderately Tolerant	Himalaya 741	3
2	Moderately Tolerant	CT-327	3
3	Moderately Tolerant	IR-64	3
4	Moderately Tolerant	Jyothiprasad	3
5	Moderately Tolerant	JGL-384	3
6	Moderately Tolerant	Himalaya-2	3
7	Moderately Tolerant	JGL-11470	3
8	Moderately Tolerant	Takari	3
9	Moderately Tolerant	Kupparalavadlu	3
10	Moderately Tolerant	Duppitokalu	3
11	Moderately Tolerant	Erramallelu	3
12	Moderately Tolerant	AC-41036	3
13	Moderately Tolerant	AC-41038	3
14	Moderately Tolerant	AC-41037	3
15	Moderately Tolerant	AC-41010	3
16	Moderately Tolerant	AC-40776	3
1	Susceptible	CT-6	5
2	Susceptible	CT-376	5
3	Susceptible	ECJ-21	5
4	Susceptible	BPT-5204	5
5	Susceptible	Kasturi	5
6	Susceptible	CT-19	5
7	Susceptible	BRS-7	5
8	Susceptible	HPR-2177	5
9	Susceptible	RP-2421	5
10	Susceptible	JGL-1798	5
11	Susceptible	JGL-11118	5
12	Susceptible	Shanta	5
13	Susceptible	AC-3283	5
14	Susceptible	JGL-11727	5
15	Susceptible	Bu-Pu-Mui	5
16	Susceptible	JGL-3855	5
17	Susceptible	JGL-19621	5

18	Susceptible	PusaBasmathi	5
19	Susceptible	Padma	5
20	Susceptible	JGL-1853	5
21	Susceptible	Tomozomachi	5
22	Susceptible	CTH-1	5
23	Susceptible	Rajendra	5
24	Susceptible	Kakatiya	5
25	Susceptible	Varalu	5
26	Susceptible	JGL-18270	5
27	Susceptible	Singadis	5
28	Susceptible	VD-82	5
29	Susceptible	AC-630	5
30	Susceptible	WGL-283	5
31	Susceptible	AC-41039	5
32	Susceptible	AC-41015	5
33	Susceptible	AC-40778	5
34	Susceptible	AC-41016	5
35	Susceptible	AC-3569	5
36	Susceptible	AC-41043	5
37	Susceptible	AC-40779	5
38	Susceptible	AC-41015	5
1	Highly susceptible	IET-19600	7
2	Highly susceptible	JGL-17970	7
3	Highly susceptible	JGL-3828	7
4	Highly susceptible	Kahara	7
5	Highly susceptible	VD-62	7
6	Highly susceptible	JGL-18047	7
7	Highly susceptible	JGL-17004	7
8	Highly susceptible	China 988	7
9	Highly susceptible	Lalankanda	7
10	Highly susceptible	WGL-14377	7
11	Highly susceptible	Gedanzipeton	7
12	Highly susceptible	AC-41028	7
13	Highly susceptible	AC-41009	7
14	Highly susceptible	AC-38460	7
15	Highly susceptible	AC-38453	7
16	Highly susceptible	AC-38459	7
17	Highly susceptible	AC-34983	7
18	Highly susceptible	AC-41022	7



Figure 4.2.a. Experimental Plot at College Farm



Figure 4.2.b. Screening for cold tolerance at seedling stage



Figure 4.2.c. Normal and Dead Seedlings



Figure 4.2.e. Cold tolerant genotypes of Rice at seedling stage (IR-83222-174, Emergency and CT-376).

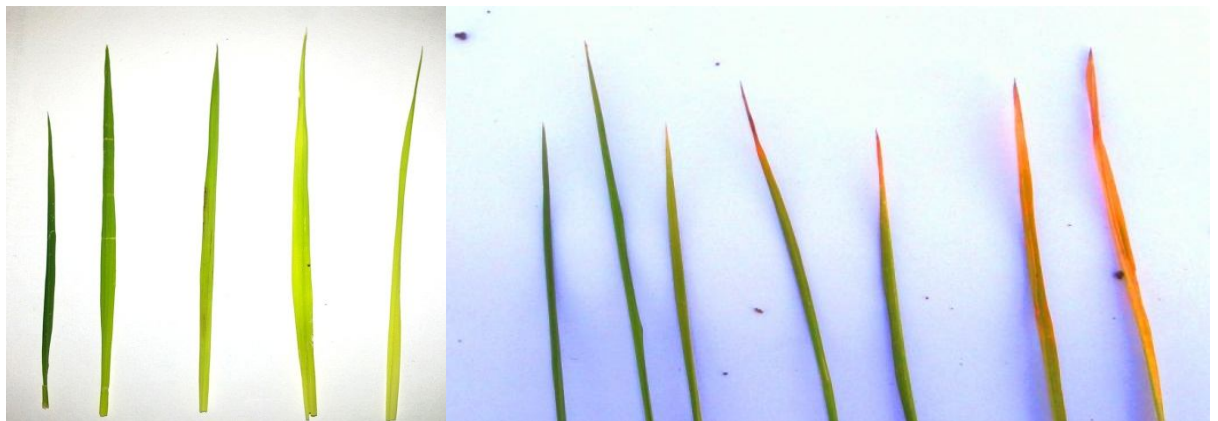


Figure 4.2.d. Leaf yellowing symptoms due to cold at seedling stage



Figure 4.2.d(i) Symptoms of leaf yellowing due to cold



Figure. 4.2.d(ii).Symptoms of leaf yellowing due to cold at seedling stage

Chapter V

SUMMARY AND CONCLUSIONS

Low temperature is a common production constraint in rice cultivation in temperate zones and high-elevation environments, with the potential to affect growth and development from germination to grain filling. There is a wide range of genotype-based differences in cold tolerance among rice varieties, these differences often reflecting growth conditions in the place of origin, as well as breeding history. However, improving low temperature tolerance of varieties has been difficult, due to a lack of clarity of the genetic basis to low temperature tolerance for different growth stages of the rice plant.

In the present study Seeds and plants of 76 rice genotypes were exposed to low temperature during germination, seedling and vegetative stages to assess their cold tolerance. Seventy six rice genotypes were studied with regard to genetic diversity for cold tolerance, employing SSR markers and morphological traits. The investigation was performed in RBD with three replications at college farm, as well as in CRD at the laboratory, Institute of Biotechnology, College of Agriculture, Rajendranagar, Hyderabad during *rabi* 2011.

A genotype with higher germination percentage, higher growth rate, lower leaf yellowing score and higher seedling vigour index at the seedling stage indicated its tolerance to cold at the seedling stage. Genotypes with low germination percentage, more number of days to germination, slow growth rate and higher leaf yellowing score indicated cold susceptibility.

Analysis of variance revealed significant differences for all the characters studied indicating the presence of considerable amount of variability among the genotypes.

The PCV and GCV were high for number of tillers per plant, grain yield per plant, number of filled grains per plant, total grains per plant, spikelet sterility percentage, days to germination, growth rate and seedling vigour index. Heritability estimates were high for all the traits under study. Genetic advance as percent of mean was high for the traits viz., plant height, number of tillers per plant, panicle length, grain yield per plant, 1000 grain weight, number of filled grains per panicle, number of unfilled grains per panicle, total grains per panicle, spikelet sterility percentage, germination percentage, days to germination, growth rate and seedling vigour index.

From the studies on morphological variability it would be concluded that high PCV, GCV, heritability and genetic advance as percent of mean were observed for the traits viz., number of tillers per plant, grain yield per plant, number of filled grains, total grains per plant, days to germination, growth rate and seedling vigour index indicating predominance of additive gene action in the inheritance of these characters.

At the seedling stage yellowing of leaves, germination percentage, days to germination, growth rate and seedling vigour index were used to assess the cold damage. Yellowing of leaves score varied from 1-Tolerant, 3-Moderately tolerant, 5-Susceptible and 7-highly susceptible. Genotypes Tellahamsa, IR-83222-174, Emergency and Pusa superfast showed the signs of cold tolerance (score of leaf yellowing 1) and had germination more than 90 per cent. Seedlings of the genotypes with leaf yellowing score of 1 remained green but, with a score of 7 turned into brown indicating their cold susceptibility.

Morphological divergence by Euclidian² method indicated the existence of significant diversity among genotypes which were grouped into nine clusters. Days to 50 percent flowering, unfilled grains per panicle, filled grains per panicle and panicle length contributed maximum towards genetic diversity. Maximum difference among the genotypes within the same cluster were shown by cluster VII. The maximum inter cluster distance was noticed between the cluster VI and IX. From the inter cluster D² values of nine clusters the highest divergence occurred between cluster VI and IX indicating their importance to contributing genetic diversity. Cluster VI and IX were represented by single genotype indicating high degree of heterogeneity among the genotypes.

The genotypes showing cold tolerance in terms of leaf yellowing score, germination percentage, days to germination and growth rate at seedling stage were grouped in cluster I, II, III and V indicating their divergence to cold tolerance from other clusters.

Character association studies revealed that the character grain yield per plant showed significant positive association with filled grains per panicle, number of tillers per plant, total grains per panicle and panicle length. This indicated that simultaneous selection of all these characters was important for yield improvement.

Analysis using cold QTL linked markers revealed considerable amount of diversity with coefficient of similarity value ranging from 0.51 to 0.96. The genotypes were grouped into two main clusters. cluster I consist of only one genotype while the remaining 75 genotypes were grouped in the cluster II, which was a major cluster. Cluster II was classified into two sub-clusters IIA and IIB . cluster IIA consisted of single genotype while IIB had 74. Cluster IIB sub grouped into IIB1 and IIB2. Cluster IIB1 consisted of single genotype while IIB2 had 73. IIB2 was a major cluster sub grouped into IIB2a and IIB2b. Cluster IIB2a contains 31 genotypes while cluster IIB2b had 42.

The SSR markers used in this study were polymorphic, and can be used to distinguish rice genotypes for cold tolerance. In addition, this study indicated that although morphological characterization was influenced by the environment and is time consuming in general, it might still be an important and practical means of making progress in rice genotypes evaluation for cold tolerance.

The study also indicate the low correspondence between the diversity estimate of morphological and molecular marker data. Hence, a judicious use of diversity estimate from morphological and molecular data may be required for the identification and selection of diverse genotypes for cold tolerance.

From the results we can conclude the following

- Direct selection for yield improvement through traits such as number of filled grains per panicle, number of tillers per plant, days to 50% flowering, plant height, panicle length and number of grains per panicle would be rewarding.
- Cold tolerant genotypes Tellahamsa, Pusa superfast, Emergency, Himalaya 741, JGL-384, JGL-11470 can be used to breed for tolerance to cold.
- Both morphological traits and SSR markers were highly effective in estimating genetic diversity for cold tolerance and distinguishing genotypes in rice.

Future line of work

- The genotypes identified in this study can be used as parents in breeding programme to develop cold tolerant cultivars.
- The genotypes screened in the study can be used in the development of mapping population and identification of QTLs for cold tolerance.

- The outcome of this study can be utilized for development of cold tolerant varieties for the benefit of farmers.

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* Original not seen.

Weather data during crop growth			
Date	Max. Temp. (°C)	Min. Temp. (°C)	Mean Temp(°C)
12-Dec-11	31.5	16.1	23.8
13-Dec-11	30.0	18.1	24.05
14-Dec-11	28.5	12.1	20.3
15-Dec-11	29.5	11.1	20.3
16-Dec-11	29.5	11.6	20.55
17-Dec-11	29.0	10.6	19.8
18-Dec-11	29.0	12.1	20.55
19-Dec-11	29.0	12.1	20.55
20-Dec-11	29.0	12.1	20.55
21-Dec-11	29.0	11.1	20.05
22-Dec-11	29.0	10.6	19.8
23-Dec-11	29.0	14.1	21.55
24-Dec-11	29.0	11.1	20.05
25-Dec-11	30.0	8.1	19.05
26-Dec-11	30.0	8.1	19.05
27-Dec-11	30.0	9.1	19.55
28-Dec-11	29.5	11.1	20.3
29-Dec-11	29.5	11.1	20.3
30-Dec-11	29.5	16.1	22.8
31-Dec-11	30.5	18.1	24.3
1-Jan-12	26.0	21.1	23.55
2-Jan-12	29.0	21.1	25.05
3-Jan-12	31.0	19.1	25.05
4-Jan-12	31.0	17.1	24.05
5-Jan-12	33.5	17.1	25.3
6-Jan-12	33.0	16.1	24.55
7-Jan-12	33.0	15.1	24.05
8-Jan-12	34.0	16.1	25.05
9-Jan-12	32.0	15.6	23.8
10-Jan-12	32.0	19.1	25.55
11-Jan-12	26.0	17.1	21.55
12-Jan-12	26.5	15.6	21.05
13-Jan-12	28.5	9.6	19.05
14-Jan-12	26.0	8.1	17.05
15-Jan-12	26.5	4.6	15.55
16-Jan-12	28.0	6.1	17.05
17-Jan-12	30.5	7.1	18.8
18-Jan-12	32.0	10.1	21.05
19-Jan-12	31.5	10.1	20.8
20-Jan-12	32.0	13.1	22.55

21-Jan-12	30.0	16.5	23.25
22-Jan-12	29.5	14.1	21.8
23-Jan-12	29.5	14.1	21.8
24-Jan-12	30.0	15.1	22.55
25-Jan-12	31.0	13.1	22.05
26-Jan-12	30.5	12.1	21.3
27-Jan-12	32.0	12.0	22
28-Jan-12	31.5	14.1	22.8
29-Jan-12	30.5	16.1	23.3
30-Jan-12	29.0	16.1	22.55
31-Jan-12	28.5	14.1	21.3
1-Feb-12	29.50	14.10	21.8
2-Feb-12	29.50	16.10	22.8
3-Feb-12	29.50	15.10	22.3
4-Feb-12	29.00	15.10	22.05
5-Feb-12	31.00	14.10	22.55
6-Feb-12	30.50	14.10	22.3
7-Feb-12	33.00	13.10	23.05
8-Feb-12	33.00	13.10	23.05
9-Feb-12	34.00	17.10	25.55
10-Feb-12	31.00	13.10	22.05
11-Feb-12	31.00	18.60	24.8
12-Feb-12	30.00	19.10	24.55
13-Feb-12	31.00	18.10	24.55
14-Feb-12	33.50	17.10	25.3
15-Feb-12	34.00	15.10	24.55
16-Feb-12	34.50	16.10	25.3
17-Feb-12	34.50	19.60	27.05
18-Feb-12	32.00	17.10	24.55
19-Feb-12	32.50	14.10	23.3
20-Feb-12	34.50	14.10	24.3
21-Feb-12	35.00	13.60	24.3
22-Feb-12	35.50	13.60	24.55
23-Feb-12	35.00	15.10	25.05
24-Feb-12	35.00	15.10	25.05
25-Feb-12	35.50	14.10	24.8
26-Feb-12	35.50	14.10	24.8
27-Feb-12	35.50	14.10	24.8
28-Feb-12	36.00	15.60	25.8
29-Feb-12	36.50	16.10	26.3
1-Mar-12	35.50	14.10	24.8
2-Mar-12	37.00	12.10	24.55

3-Mar-12	37.00	14.10	25.55
4-Mar-12	35.00	14.10	24.55
5-Mar-12	36.00	13.10	24.55
6-Mar-12	36.00	16.10	26.05
7-Mar-12	37.00	16.10	26.55
8-Mar-12	36.50	19.10	27.8
9-Mar-12	35.50	15.10	25.3
10-Mar-12	34.50	14.10	24.3
11-Mar-12	35.00	17.60	26.3
12-Mar-12	35.00	20.10	27.55
13-Mar-12	35.00	21.60	28.3
14-Mar-12	35.00	20.60	27.8
15-Mar-12	36.00	21.10	28.55
16-Mar-12	36.50	17.10	26.8
17-Mar-12	36.50	16.10	26.3
18-Mar-12	37.00	20.50	28.75
19-Mar-12	38.00	21.10	29.55
20-Mar-12	39.00	17.10	28.05
21-Mar-12	39.00	16.10	27.55
22-Mar-12	39.00	19.10	29.05
23-Mar-12	38.00	17.10	27.55
24-Mar-12	38.50	15.10	26.8
25-Mar-12	38.00	16.10	27.05
26-Mar-12	37.50	16.10	26.8
27-Mar-12	39.00	17.10	28.05
28-Mar-12	38.00	18.10	28.05
29-Mar-12	37.50	18.10	27.8
30-Mar-12	37.50	21.60	29.55
31-Mar-12	39.00	22.10	30.55
1-Apr-12	37.0	21.1	29.05
2-Apr-12	36.5	20.1	28.3
3-Apr-12	38.5	22.1	30.3
4-Apr-12	38.0	20.1	29.05
5-Apr-12	39.5	20.1	29.8
6-Apr-12	39.0	21.1	30.05
7-Apr-12	37.5	21.6	29.55
8-Apr-12	37.0	20.1	28.55
9-Apr-12	37.5	21.1	29.3
10-Apr-12	38.0	23.1	30.55
11-Apr-12	38.5	22.1	30.3
12-Apr-12	37.0	23.1	30.05
13-Apr-12	36.5	17.1	26.8

14-Apr-12	36.0	22.1	29.05
15-Apr-12	37.0	21.6	29.3
16-Apr-12	36.5	21.6	29.05
17-Apr-12	38.5	22.1	30.3
18-Apr-12	37.5	24.1	30.8
19-Apr-12	38.5	24.1	31.3
20-Apr-12	38.0	25.1	31.55
21-Apr-12	39.5	25.1	32.3
22-Apr-12	39.0	23.1	31.05
23-Apr-12	39.0	22.1	30.55
24-Apr-12	33.0	21.1	27.05
25-Apr-12	37.0	24.6	30.8
26-Apr-12	37.5	24.1	30.8
27-Apr-12	35.0	25.1	30.05
28-Apr-12	37.5	26.1	31.8
29-Apr-12	36.0	27.1	31.55
30-Apr-12	39.0	25.1	32.05

