

Study of morphological and molecular diversity in drought tolerant rice (*Oryza sativa* L.)

काशी हिन्दू
विश्वविद्यालय



BANARAS HINDU
UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

Master of Science (Agriculture) IN Genetics and Plant breeding

Supervisor

Dr. S. K. Singh

Submitted by

Monika Singh

DEPARTMENT OF GENETICS AND PLANT BREEDING
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI-221005
INDIA

ID No. G-15113

2017

Enrolment No.-377981

DR. S. K. SINGH
Professor

Department of Genetics and Plant breeding
Institute of Agricultural Sciences
Varanasi – 221005

CERTIFICATE

Ref. No

Date:

To,
The Registrar (Academic)
Banaras Hindu University,
Varanasi-221005 (India).

Through: The Head, Department of Genetics and Plant breeding,
Institute of Agricultural Sciences, B.H.U, Varanasi.

Dear Sir,

I have great pleasure in forwarding the thesis entitled **“Study of morphological and molecular diversity in drought tolerant rice (*Oryza sativa* L.)”** submitted by **Miss. Monika Singh I.D. No. G-15113**, in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in **Genetics and Plant breeding**, from Department of Genetics and Plant breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire scheme of investigation reported herein, was planned and carried out by the candidate under my guidance to the best of my knowledge and belief, the data presented in the thesis are genuine and original.

Thanking you,

Forwarded by

Yours faithfully

Head

Prof. S. K. Singh
(Supervisor)

“Study of morphological and molecular diversity in drought tolerant rice (*Oryza sativa* L.)”

By

Monika Singh

Thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science (Agriculture)

in

Genetics and Plant breeding

**DEPARTMENT OF GENETICS AND PLANT BREEDING
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI - 221 005**

I.D. No. G-15113

2017

Enrolment No: 377981

APPROVED BY ADVISORY COMMITTEE

Advisor : **Dr. S. K. Singh**
Professor,
Department of Genetics and Plant breeding,
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi, India

Member : **Dr. J.P. Shahi**
Professor,
Department of Genetics and Plant breeding,
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi, India

Member : **Dr. A. K. Singh**
Professor,
Department of Horticulture,
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi, India

EXTERNAL EXAMINER:

Acknowledgement

With limitless humility, I bow my head towards almighty, merciful, compassionate and supreme power "Lord Kashi Vishwanath Ji" who showed his mercy on me and blessed me to complete my M.Sc successfully hitherto and present the piece of work for which I am eternally indebted.

Indeed, with knee down, I bow my head and offer flowers of reverence to Mahamana Pandit Madan Mohan Malaviyaji, the founder of the Banaras Hindu University, for his lifetime sacrifice and efforts in establishing such a great temple of learning for the cause of millions of students like me.

With immense pleasure and profound sense of gratitude, indeed, I take this opportunity to express my heartfelt and sincere thanks to my esteemed supervisor, Prof. S.K. Singh, Department of Genetics & Plant Breeding, Institute of Agricultural Sciences, B.H.U., for his meticulous guidance, indelible inspiration, persistent encouragement, ingenious suggestions, mellifluous nature and indefatigable attitude. I will ever cherish the fatherly affection that he bestowed upon me throughout my tenure as a student under him which helped me to come up with many difficult situations.

I wish to express my heartfelt and advent thanks to the members of my Advisory Committee, Internal Subject Expert – I Dr. J.P. SHAHI, Professor, in the Department of Genetics & Plant Breeding and External Subject Expert – II Prof. A.K. Singh, in the Department of Horticulture and for their valuable suggestions and liberal help rendered during the course of study and research work.

I am extremely grateful to the Director Sir Prof. A. Vaishampayan, of the Institute for rendering such a beautiful and enthusiastic environment which appealed me to perform in a better way.

I am also grateful to all the teachers of the Department of Genetics and Plant Breeding for providing me necessary help and guidance during the period of my investigation. I would like to express my heartiest thanks towards the non-teaching staff of the department for their help during my stay.

I acknowledge my heartfelt appreciation towards my friends and seniors Dinesh, Rathan, Amrita, Sonali, Ravi, Jagriti, Darpan manmode for being helpful throughout and their wonderful company which made my study period in the University cheerful.

I am extremely thankful to my seniors Prudvi Raj (research scholar), Dr. D.K. Singh (research scholar), Shri Niwas Reddy (research scholar), Dr. Shubhira (SRF) for their care,

support, love, affection, and friendly nature which helped me to survive and work hard even in difficult and tiresome situations.

Words fail to express my deep gratitude from the depths of my heart for my loving father, **Shri Anil Kumar Singh**, and my mother **Smt. Sunita Singh**, and my grand parents **Shri Sharda Saran Singh**, and **Smt. Lilawati Singh** whose selfless sacrifice, blessings and exhilaration have always animated me to rise against problems and face them smilingly, how so much formidable they may be. They kept enthusiastic throughout my educational career, which enabled me to acquire the present gratification. My mother **Smt. Sunita Singh** has always been a source of motivation, strength and patience, hope during moments of discouragement. I would also like to express my thanks to my dearest brother **Siddharth Singh**, and my dearest sisters **Nivedita Singh**, **Ankita Singh** whose displayed profound sense of fraternity and valuable suggestions and love to complete this gigantic task.

I am also extremely thankful to my uncle **Dr. K.N. Singh** for giving me constant inspiration, guidance and assistance throughout my educational carrier.

I wish to thank to all non-teaching staff of the department, **Shri Rakesh Giri**, **Shri Sant Lal** and **Chhote Lal** for their kind assistance during my entire P.G. studies.

A plethora of thanks also goes to **Shri Lal Bahadur** and the farm labours for their whole hearted help during field experimentation.

I have no words to express my gratitude towards my best friend **Dinesh** who always believed me and was with me at good and bad times. Thank you for tolerating me all these years. So, thanks a lot for your belief, encouragement, support.

I am indebted to the great institution Banaras Hindu University to complete the research work. Finally, I would also like to thank all those who could not find a separate name but have loved me and always wished for my welfare.

My thanks are also to those who knowingly or unknowingly helped me through their criticism & suggestions from time to time which inspired me to do my best all the time.

Date : June, 2015

Place : BHU Varanasi

(**Monika Singh**)



Contents

1.	<i>Introduction</i>	1-6
2.	<i>Review of Literature</i>	7-34
3.	<i>Material and Methods</i>	35-62
4.	<i>Experimental Findings</i>	63-79
5.	<i>Discussion</i>	80-88
6.	<i>Summary and Conclusion</i>	89-91
	• <i>References</i>	<i>i-xiv</i>
	• <i>Appendices</i>	

LIST OF TABLE

Table No.		Page No.
3.1	List of 20 genotypes and their sources.	
3.2	Classification of rice genotypes on the basis of amylose content	
3.3	Details of the SSR primers used in present study.	
3.4	List of various components of PCR master mixture (15 μ l).	
3.5	The schedule of temperature and duration programmed for PCR amplification using SSR primers.	
3.6	Analysis of variance (ANOVA)	
4.1	ANOVA of 20 rice genotypes for twenty seven yield and yield attributing trait	
4.2	Grouping of 20 rice germplasm into five clusters (by Tocher method)	
4.3	Inter and intra cluster distance of D^2 value of 20 rice genotype	
4.4	Inter and intra cluster distance of D value of 20 rice genotype	
4.5	Cluster mean of 20 rice genotypes	
4.6	Relative contribution of 27 characters to genetic diversity in 20 genotypes of rice	
4.7	Pic value of 10 polymorphic markers	
4.8	Jackard dissimilarity Matrix of 20 rice genotypes	

LIST OF FIGURE

Figure No.		Page No.
1	Clustering of 20 rice genotype	
2	Cluster by tocher method	
3	Dendogram	
4	Character contribution of 27 traits of 20 genotypes of rice	

INTRODUCTION

Rice is a cereal crop, belongs to genus *Oryza* of Poaceae family. The genus *Oryza* has twenty two wild and two cultivated species namely; *Oryza sativa* and *Oryza glaberrima*, all the germplasm found in Asia, America and Europe belongs to *Oryza sativa*, and while in West Africa belong to *Oryza glaberrima*. *Oryza sativa* is a cultivated diploid species having 24 chromosomes. The *sativa* rice is commonly divided into three sub-species i.e. *Indica*, *Japonica* and *Javanica*. Rice is a short day self-pollinated crop, needs a hot humid climate with average temperature 21 to 37°C throughout the life period of the crop. It is an extensively consumed cereal crop, serves as a major source of carbohydrate in human diet. Rice is placed on second position in cereal cultivation around the globe and occupies an important position in the economy of India as an export item as well as staple food.

It is cultivated in 114 countries across the globe, but 90 percent of world's rice is grown in Asia (FAO 2013). Rice production, consumption and trade are mostly concentrated in Asia. One third of Asia's rice production is consumed in China and one fifth in India. Among the rice growing countries in the world, India has the largest area under rice crop (about 45 million ha.) and ranks second in production next to China. (IRRI 2016, standard evaluation system for rice.) Rice is grown in almost all the states of India, but major rice producing states fall in the regions of middle and lower gangetic plains, as well as the coastal lowlands of peninsular India, Uttar Pradesh has total rice production of around 12.5 mt (DAE 3rd adv. estimate) which is second largest rice producer in India following West Bengal (14.6 mt).

In April 2017, United State department of Agriculture (USDA) estimated that the world rice production 2016/2017 will be 481.14 million tons, around 0.8 million tons more than previous year's projection. Similarly, the Indian rice production is

expected to be around 109 mt during the year 2016-17 which is the highest ever production of rice till date (AICRIP annual meeting report 2017).

Diversity in rice has been well utilized in the efforts to solve the present day food problem. Land races of rice were collected over past several decades to use them as parents for producing the high yielding varieties, disease and pest resistance. Well adapted varieties resulted in unprecedented rise in rice yield often referred to as ‘Green revolution’ (Jackson and Haggan, 1993). In India, the revolution in rice production was realized after the introduction of lodging resistant, fertilizer responsive and photo-insensitive semi-dwarf high yielding varieties of rice in mid sixties.

Large areas of rice are grown under lowland and upland rainfed conditions. These areas respectively occupy 31% and 11% of the global rice-growing area (IRRI, 2001). Drought is a major constraint for rice production in rainfed ecosystems. Evenson et al. (1996) projected global rice yield lost to drought to be 18 million tonnes annually or 4% of total rice production, which was valued conservatively at US\$ 3.6 billion at that time. On average, the estimated yield lost to drought is 144 kg/ha annually in eastern India (Dey and Upadhyaya, 1996). Imitation modeling indicates that reduction in yield of rainfed lowland rice outstanding to drought averages from 13% to 35% for different locations in northeast Thailand (Jongdee et al., 1997b).

Water is the critical and most important factor in rice production. The 70 percent of the world’s food-growing areas turn increasingly parched (IRRI, 2009), so that main challenge for rice producers is to develop a strategy to raise yields of the water intensive crop. Drought is a major abiotic stress that limits rice productivity in rainfed and upland ecosystems (Bimpong *et al.* 2011) and worldwide, drought affects approximately 27 million ha of rainfed (IRRI, 2011). In India, area under rice cultivation remained stagnant and even declined in the recent years due to water non-availability. Drought reduces yield by 15–50 per cent depending on the stress intensity and crop growth period at which the stress occurs in rice (Srividhya *et al.* 2011). Eastern India, comprising Jharkhand, Orissa, and Chhattisgarh

alone accounts loss of about 40 per cent of the total rice production due to severe drought (Pandey and Bhandari, 2009). Developing high yielding and drought resistant varieties for rainfed area is priority for improving rainfed rice production.

Plant response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular, and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants (Zhang *et al.* 2012). The effect of drought on rice plants considerably varies with genotypes, developmental stages, and degree and duration of drought stress (Wang *et al.* 2011). A better understanding of the complex physiological mechanisms underlying drought response is important to improve rice yields under water-limited environments. Roots, the hidden half of a plant, are important for numerous functions including water and nutrient uptake that make it difficult to overlook their importance to plant productivity (MacMillan *et al.* 2006). Root traits are key component in rice plant adaptation to drought stress (Courtois *et al.* 2009).

The semi-aquatic nature of rice and high water requirements for its cultivation make it much more prone to losses from drought than other cereals such as wheat and maize, which are better adapted to be grown with less water. As a result of the reduction in water availability and recent climate change scenarios, rice production is likely to be more severely affected by drought in Asia. Dry spells can occur at almost any time during the rice growth period in rain-fed areas, leading to drought stress of varying intensity. However, rice is highly sensitive to water stress at the reproductive stage (O'Toole 1982) as floral fertility in rice is extremely sensitive to water stress. Drought is the largest constraint to rice production in these systems, affecting 19 million ha of upland rice and over 14 million ha of rainfed lowland rice (Evenson *et al.*, 1996, Pandey *et al.*, 2000).

Genetic variability for agronomic traits is the key component of breeding programs for broadening the gene pool of rice and would require reliable estimates of heritability in order to plan an efficient breeding program. (Akinwale *et al.*, 2011). Yield component breeding to increase grain yield would be most effective, if the

components involved are highly heritable and genetically independent or positively correlated with grain yield. However, it is very difficult to judge whether observed variability is highly heritable or not. Moreover, knowledge of heritability is essential for selection based improvement as it indicates the extent of transmissibility of a character into future generations (Sabesan et al., 2009). The advantage of path analysis is that it permits the partitioning of the correlation coefficient into its components, one component being the path coefficient that measures the direct effect of a predictor variable upon its response variable; the second component being the indirect effect(s) of a predictor variable on the response variable through another predictor variable.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains. Diversity based on phenological and morphological characters usually vary with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. Protein or isozyme marker studies are also influenced by environment and reveal low polymorphism. Now, the rapid development of biotechnology allows easy analysis of a large number of loci distributed throughout the genome of plant. Microsatellites or simple sequence repeats (SSRs) are tandem arrays of short nucleotide repeats with 1–5 bp (Wu and Tanksley 1993) and are widely dispersed in all eukaryotic genomes. Dinucleotide repeats (AT/TA) and (GA/CT) are commonly found in vascular plants. SSRs are characterized as co-dominant, highly polymorphic, abundant and randomly distributed markers in genomes. SSR markers can be easily amplified by PCR and are probably selectively neutral (Akagi et al. 1997). Genetic mapping and breeding (McCouch et al. 1997), gene flow (Chase et al. 1996; Innan et al. 1997), genetic diversity and population differentiation (Byrne 1996; Cho et al. 2000). To date, more than two thousand SSR markers of cultivated rice are available, which provides a powerful tool for To date, more than two thousand SSR markers of cultivated rice are

available, which provides a powerful tool for studying *O. sativa*, as SSR markers have good cross-species amplification to the close relatives (Roa et al. 2000). Hybridization programme involving genetically diverse parents belonging to different clusters would provide an opportunity for bringing together gene constellations of diverse nature, promising hybrid derivatives resulted probably due to complementary interaction of divergent genes in parents. Several workers have emphasized the importance of genetic divergence for the selection of desirable parents (Murthy and Arunachalam, 1996 and Rahman, 1997). The use of Mahalanobis D2 statistics for estimating genetic divergence has been emphasized by many workers (Roy and Panwar, 1993; Ramya and Senthilkumar, 2008).

The success of any plant breeding programme largely depends on the existence of diversity among the genotypes (Allard, 1960). This helps in the choice of parents for hybridization in yield improvement programmes. Hence, estimation of genetic diversity for yields and its components among genotypes is important for planning the future crossing programme. In the present study an attempt was made to assess the genetic divergence using Mahalanobis D2 statistics and different clustering procedures, based on yield and its component characters and assessing the relative contribution of different components to total divergence (Rao, 1952).

Drought tolerance characters are quantitative traits, and so dissection of these complex traits into component genetic factors is a prerequisite to manipulate the traits. Genome mapping using molecular genetic markers offers an excellent opportunity to locate genes or QTLs controlling quantitative characters (Lander and Botstein 1989; Tanksley 1993). Markers linked with genes controlling a trait of interest could be used in the selection of genotypes in a breeding program. Hence the present study was aimed to know the drought tolerance in rice both under morphological and molecular level.

Considering the importance of genetic diversity based on quantitative traits and molecular markers, the present investigation entitled “**Study of morphological and molecular diversity in drought tolerant rice (*Oryza sativa* L.)**” has been formulated with the following objectives:

- I. To study genetic variability for yield & yield component in drought tolerant rice genotypes.
- II. To estimate genetic diversity based on polymorphic SSR Markers in Rice genotypes.
- III. To study Genetic diversity using D^2 statistics.

REVIEW OF LITERATURE

The present review of literature is an attempt to bring together findings on genetic diversity among rice genotypes. Rice is grown in diverse agro climatic conditions in various tropical and subtropical countries including India. To meet the future food demand, there is an urgent need to enhance the productivity of rice crop. Drought is the most important source of climate-related risk for rice production in rainfed areas (Pandey *et al.*, 2007).

The present investigation relates to the “STUDY OF MORPHOLOGICAL AND MOLECULAR DIVERSITY IN DROUGHT TOLERANT RICE (*Oryza sativa* L.)” in a set of twenty rice genotypes (drought donor including checks). Relevant literatures on these aspects have been reviewed here under the following heads:

2.1 Variability, heritability and genetic advance.

2.2 Markers based genetic diversity

2.3 Genetic divergence using D^2 statistics.

2.1 VARIABILITY, HERITABILITY AND GENETIC ADVANCE

The occurrence of differences among the individuals in a population of a plant species, either due to difference in their genetic constitution or the environment in which they are grown or the interaction between them, is called variability. The magnitude of variability present in a crop species is of almost importance as it provides the basis for effective selection and for all crop improvement programmes for evolving superior genotypes. This is the main concern of plant breeders.

Reddy (1992) studied highest genotypic and phenotypic coefficient of variability for number of grains per panicle. All the traits exhibited moderate to high estimates of heritability ranging from 67.9% for grain yield per hill to 99.5% for days

to 50% flowering. Plant height and number of grains per panicle showed high estimates of genetic advance along with high heritability.

Chaubey and Singh (1994) considered genetic variability in a set of twenty rice varieties and observed high heritability coupled with high genetic advance for effective tillers per plant, grain yield per plant and 1000-grain weight.

Reddy and De (1996) evaluated that there were significant differences among thirty six genotypes for the 12 characters. Grain yield per hill, grain per panicle and panicle weight had the highest estimates of genotypic and phenotypic variability. They observed high heritability for grain length, followed by 1000-grain weight, grain breadth, plant height, panicle weight, grain yield and grain per panicle.

Balan *et al.* (1999) performed study on twenty eight rice genotypes for genetic parameters and reported that high heritability estimates were combined with genetic advance for grain yield and moderate genetic advance for days to 50 per cent flowering and plant height.

Rather *et al.* (1998) considered fifty six rice cultivars belonging to three different eco-geographical races *viz.* *Indica*, *Japonica* and *Javanica* for genetic variability and genetic parameters with respect to fourteen traits. Spikelet sterility and grains per panicle exhibited high genotypic coefficient of variation along with moderate heritability and high genetic advance.

Kumar *et al.* (1999) studied significant variation for plant height, number of tillers per plant, flag leaf length, flag leaf width, number of panicle per plant, number of spikelets per plant, yield per plant and test weight.

Ali *et al.* (2000) studied ten rice varieties or purelines and reported significant genetic variability for all the character except for number of tillers per plant and panicle length. Heritability was observed maximum for plant height followed by 100-seed weight, number of tillers per plant and panicle length. However, genetic advance was observed highest for number of tillers per plant followed by plant height.

Sharma *et al.* (2000) studied 55 rice F₂ genotypes observed highest phenotypic as well as genotypic variability for grains per panicle followed by grain yield per plant and effective panicles per plant. Heritability in broad sense was highest for effective panicles per plant followed by grains per panicle and grain yield per plant. Grains per panicle also exhibited highest genetic advance as per cent of mean followed by effective panicles per plant and grain yield per plant.

Yadav (2000) estimated genetic variability in rice observed appreciable amount of genotypic coefficient of variation, heritability and genetic advance for total spikelets per panicle, fertile spikelets per panicle and grain yield per plant.

Shobha *et al.* (2001) studied genetic variability in four hundred eighty aromatic/quality rice germplasms. These germplasms showed a wide range of variation for all the agronomic and yield component traits. Coefficient of variation was highest for sterile spikelets per panicle followed by sterility per cent and fertile spikelets per panicle. Majority of the aromatic rice observed to be tall in nature having longer panicle.

Shanthi *et al.* (2001) studied 16 lines of M₆ generations of induced mutants along with parental line and observed that plant height, panicle length, 1000-grain weight and number of grains per panicle exhibited low difference between phenotypic and genotypic coefficients of variation. They suggested that these characters may be emphasized in selection programmes. Heritability in broad sense was high (more than 80%) for all characters except grain yield per plant (78.99%) and number of tillers per plant (63.04%). The highest genetic advance was recorded for number of grains per panicle (107.13). High heritability coupled with high genetic advance was observed for plant height, number of grains per panicle and 1000-grain weight. They suggested that these characters were governed largely by the additive effects of genes.

Pandey *et al.* (2002) worked with 21 aromatic varieties rice reported high genetic variability for most of the yield contributing traits.

Yadav (2002) estimated yield and its components like days to 50 per cent flowering, days to maturity, plant height, number of tillers per plant, panicle length,

spikelets per panicle, total grain per panicle, 1000-grain weight and grain yield per plant in 15 rice genotypes, and reported higher values for various variability parameters.

Mahto *et al.* (2003) evaluated twenty six early maturing upland rice genotypes for genetic variation. The genotypic coefficient of variation ranged from 5.36 for panicle length to 24.83 for grain yield. The difference between PCV and GCV was minimum for 1000-grain weight (0.12) and days to 50% flowering (0.13). Estimates of heritability in broad sense were highest for 1000-grain weight (98.30%) followed by days to 50% flowering (97.33). The number of grains per panicle and panicle length showed a wide difference between PCV and GCV.

Akter *et al.* (2004) studied the genetic variability in the irrigated nine hybrid rice varieties. All the characters studied by them showed significant variation. High heritability was observed for all the tested characters except grains per panicles. High heritability with high genetic advance as per cent of mean was found for 1000-grain weight.

Souroush *et al.* (2004) observed the significant differences among thirty six rice genotypes for all the quantitative and qualitative traits. The PCV was greater than GCV for all the traits although differences for most of them were small. The highest phenotypic and genotypic coefficients of variation were obtained for gel consistency, leaf area, leaf width and number of panicle per plant. The highest estimate of heritability was observed for plant height and lowest for panicle weight.

Vivek *et al.* (2005) evaluated 39 tropical *Japonica* lines during the wet season. The genotypes showed a wide range of variability for all characters. High genotypic and phenotypic coefficient of variation was observed for grain yield followed by harvest index and biological yield. They observed high heritability coupled with high genetic advance for grain yield followed by harvest index and biological yield.

Satyanarayana *et al.* (2005) worked on variability in sixty-six elite restorer lines of rice, recorded high variability, heritability and genetic advance for number of grains per panicle, spikelet fertility, days to 50 per cent flowering and plant height,

while test weight recorded high variability and heritability coupled with low genetic advance. The number of effective tillers per plant and panicle length recorded low estimates of heritability.

Madhavalatha *et al.* (2005) studied the genetic variability in fifty four rice genotypes. They observed high variability, heritability and genetic advance for number of grains per panicle, grain yield per plant, while days to maturity exhibited high heritability coupled with low genetic advance.

Patra *et al.* (2006) studied variability, heritability and genetic advance of 13 yield contributing characters in twenty long awned rice genotypes. The analysis of variation for all the traits indicated the existence of high genetic variability among the long awned rice genotypes. High heritability coupled with high genetic advance for characters like grain yield per plant per hill, leaf width, plant height, awn length, 1000-grain weight, number of effective tillers per plant and kernel (L/B) ratio showed contribution of high heritability and moderate genetic advance giving similar indication which also added more number of useful traits for widening the scope of meaningful selection.

Mustafa and Elsheikh (2007) assessed 14 rice genotypes for genetic variability and observed wider range of genetic variability among the genotypes for most of the characters studied. The highest GCV was recorded for grain yield, unfilled grains per panicle and number of grains panicle.

Singh *et al.* (2007) estimated genetic variability in 117 diverse rice lines (*indica* and *japonica*) for 11 different traits reported significant variation among the genotypes for all the traits. High GCV and PCV were noticed for grains per panicle. High heritability coupled with high genetic advance was observed for days to 50% flowering, grains per panicle and plant height, indicating effectiveness of selection for improvement of these traits. However, kernel length, kernel breadth and L/B ratio recorded high heritability accompanied with low genetic advance.

Jadhav and Dudhare (2008) studied genetic variability and genetic advance for 23 traits in 49 genotypes of indigenous and exotic rice. These genotypes were

studied for genetic seedling growth and yield attributing traits. The highest magnitude of genotypic coefficient variation was observed for plant height followed by seedling length. High estimates of heritability coupled with high genetic advance were recorded for plant height, 100 seed weight. They suggested that the selection for these characters likely to accumulate more additive gene effects leading to further improvement of their performance.

Kole *et al.* (2008) assessed variability, correlation and path coefficients for twelve morphological characters on 18 morphological distinct mutants in M₄ generations along with their two mother genotypes (IET 14142 and IET 14143), which were developed from Tulaipanja, an aromatic non-basmati rice cultivar of West Bengal.

Anbanandan *et al.* (2009) studied fifty four rice varieties for genetic variability. High values of heritability along with genetic advance were observed for grain yield per plant, 100 grain weight, productive tillers per plant, grains per panicle, grain length, kernel breadth, kernel length, panicle length and plant height.

Veerabadhiran *et al.* (2009) estimated extent of variability and genetic parameters in 15 rice hybrids and five checks for seventeen qualitative traits along with grain yield. The magnitude of difference between PCV and GCV was relatively low for all the traits, indicating less environmental influence. High GCV and PCV were recorded for gelatinization temperature and gel consistency. High heritability coupled with high genetic advance was observed for kernel breadth, kernel length/breadth ratio, kernel breadth after cooking, kernel length/breadth ratio after cooking, gel consistency, volume expansion ratio, gelatinization temperature, amylose content and grain yield per plant.

Krishna *et al.* (2010) performed studied 97 rice accessions with three check varieties and reported that highest magnitudes of Phenotypic and genotypic coefficient of variation were found for number of unfilled spikelets per panicle, number of total spikelets per panicle respectively. High estimates of heritability coupled with high genetic advance was found for number of total spikelets per panicle

and number of filled spikelets per plant, indicating that they can be effectively improved through selection.

Jayasudha and Sharma (2010) worked with 47 rice genotypes including thirty three hybrids and fourteen parents and reported considerable variability present among the genotypes for all the characters. Characters like days to 50 per cent flowering and grain yield per plant showed high value of heritability coupled with high genetic advance.

Gyanendrapal *et al.* (2011) assessed variability, heritability, genetic advance in per cent of mean and diversity for different characters in 60 genotypes under sodic situation. The maximum genotypic and phenotypic coefficients of variability were observed for spikelets per panicle followed by number of panicle bearing tillers per plant and grain yield per plant. The values of broad sense heritability were observed for all the characters except panicle length. High heritability, coupled with high genetic advance was recorded for grain yield/plant followed by spikelets per panicle, panicle bearing tillers per plant, flag leaf area and days to 50% flowering hence, these traits were predominantly under the control of additive gene action.

Prasad *et al.* (2011) investigated genetic variability for yield and its component characters in various rice germplasms lines and to know the inter-relation among different yield contributing characters and their association with grain yield. Eight superior genotypes, viz., Ujala depama, IR-64, Bhurma bhuqi, Malida, Tella hamsa, Kavya, Vldhan-221 and Krishna hamsa were found to be potential to be used as parents in heterosis breeding.

Rahaman (2012) assessed diversity of ten physiological characters along with yield. Analysis of variance revealed significant differences among the genotypes for all the traits. All the characters showed moderate to low phenotypic and genotypic coefficient of variation. Growth duration showed the highest heritability (98.97) followed by days to flowering (98.00) and flag leaf area (92.29). The highest genetic advance was found for NAR at flowering and the lowest for panicle exertion rate.

Gouda, P. K. (2012) studied heritability for grain yield and selection was done for grain yield per se using wild species *Oryza meridionalis* and identified physio-morphological traits. A population derived from *O. sativa* cv. IR58025B x *O. meridionalis* (BC2F3) was evaluated under control and stress conditions. Broad-sense heritability of grain yield under stress, although only 33.1%, was comparable to control 59.4%, indicating direct selection for grain yield per se can be practiced.

Gangashetty (2013) studied 42 genetically diverse local non-basmati aromatic genotypes of rice. He observed the moderate genotypic and phenotypic coefficients of variation were recorded for panicle length, and grain breadth however days to 50 per cent flowering had recorded lower values of genotypic and phenotypic variance. High heritability coupled with high genetic advance was observed for all traits except days to 50 per cent flowering. The traits were showed higher values of variance, and selection for such trait may be practiced based on phenotypic observation.

Chouhan *et al.* (2014) evaluate and characterize thirty five wild rice accessions for fourteen quantitative and twelve qualitative traits. Analysis of variance revealed sufficient variability for all the quantitative traits. The magnitude of phenotypic coefficient of variation was higher to corresponding genotypic coefficient of variation. High heritability coupled with high genetic advance as per cent of mean was found in the trait total number of grains per panicle and grain yield per plant.

Lingaiah,N (2015) conducted an experiment conducted to estimate the genetic variability parameters for the quantitative character rice cultivars. The magnitude of phenotypic co-efficient of variation was higher to genotypic co-efficient of variation for all the traits. The highest value of phenotypic and genotypic co-efficient of variation was observed for yield followed by number of grains per panicle and test weight. High heritability associated with high genetic advance as per cent of mean was found for the characters number of grains per panicle, yield, test weight and plant height indicating the role of additive gene action in controlling these characters.

Tiwari, G.C (2015) studied variability, heritability and genetic advance for 12 quantitative characters in ten diverse genotypes of rice were crossed in a diallel fashion. The estimates of phenotypic coefficient of variation were higher than those of

genotypic coefficient of variation for all the traits except plant height. High estimates of heritability and genetic advance were obtained for plant height, number of spikelets per panicle, days to 50 per cent flowering and days to maturity. These traits were mostly governed by additive gene action.

Muthuramu *et al.* (2016) worked with forty nine improved rice cultures obtained from All India Co-ordinated Rice Improvement Project. The analysis of variance revealed that all the treatments are highly significant for various characters studied *i.e.* days to 50% flowering, plant height, number of productive tillers per plant, number of productive tillers per square metre plot area, grain yield per plot, straw yield per plot and harvest index. The higher magnitude of PCV was recorded for grain yield per plot and straw yield per plot where as moderate magnitude of GCV was recorded for days to 50% flowering, plant height, grain yield per plot and harvest index. Days to 50% flowering and plant height showed high broad sense heritability and moderate for grain yield per plot and harvest index. High heritability coupled with high genetic advance was recorded for days to 50% flowering indicated the major role of additive gene action in the inheritance of this character. Thus, this character may serve as an effective selection parameter during breeding programme in the rainfed rice ecosystem.

Mishu *et al.* (2016) performed studied on 6 aromatic rice varieties. Variety Badshahbhog was the topmost yielder followed by Kataribhog yield and soon. Variability parameter revealed that the phenotypic coefficient of variation were higher than genotypic coefficient of variance for all the traits studied. The spikelet per plant and 1000- grain weight showed high heritability where as effective tillers per plant showed low heritability.

Karande *et al.* (2017) evaluated genetic variability and correlation between yield and other yield attributing characters of rice genotypes in *kharif* 2013. High genotypic coefficient of variation, high heritability with high genetic advance as per cent of mean was observed for grain yield per plant, total tillers per plant, productive tillers per plant and grains per panicle, whereas days to initiation of flowering, days to 50 % flowering and days to maturity had high heritability with low genetic advance as

per cent of mean. The genotypic correlation of grain yield was positive and significant with total tillers per plant, productive tillers per plant, spikelet fertility, 1000 grain weight and straw yield per plant where as phenotypic correlation of yield was positive and significant with productive tillers per plant, spikelet fertility and straw yield per plant.

Bhinda *et al.* (2017) investigated with objective to obtain information on genetic variability, heritability and genetic advance for Yield Contributing and Quality Traits in Advance Breeding Lines of Rice (*Oryza sativa* L.) The analysis of variance revealed significant differences among all the genotypes for all the 15 characters studied. High value of phenotypic coefficient of variability (PCV) was observed than genotypic coefficient of variability (GCV) for all the characters. Alkali spreading value exhibited highest GCV (19.44%) and PCV (21.07%) followed by filled grains per panicle (GCV 14.83% and PCV 19.90%). The highest broad sense heritability was estimated for gel-consistency (87.84%) followed by alkali spreading value (85.08%). Whereas the highest value of genetic advance was recorded for characters namely filled grains per panicle (41.01) and number of grains per panicle (38.26). High heritability coupled with high genetic advance was reported for gel-consistency, plant height, filled grains per panicle, number of grains per panicle and days to 50% flower.

2.2 MARKERS BASED GENETIC DIVERSITY

Knowledge about the extent of genetic diversity among the parental lines is imperative for their utilization in crop breeding programme. Earlier, morphological traits, isozymes and storage proteins were used to estimate genetic diversity; however, such information is not reliable due to influence of developmental stages and environment. Molecular markers being environmentally neutral, they are supposed to be most effective genetic apparatus for the estimate of genetic diversity. This section summarizes the result and conclusions of important genetic diversity assessment studies reported in rice employing molecular markers.

Mackill (1995) observed polymorphism on a sample of 14 rice genotypes including several closely related *japonica* cultivars and two *indica* cultivars of rice

using AFLP, RAPD and microsatellite markers. For AFLP, using 17 primers combination with seven primer and two enzymes (Eco RI, Mse I) 529 bands were generated out of which 147 were polymorphic accessions. With 21 RAPD primers, 43 bands showed polymorphism out of total 103 amplified. For microsatellite markers, the number of alleles per locus ranged from 1-6 with average of 3.3. All marker types gave same classification of rice accession into sub species. Within *japonica* cultivars the average per cent polymorphism between any two accessions was 29, 33 and 47 percent for AFLP, RAPD and microsatellite respectively. The average per cent polymorphism between *indica* and *japonica* accessions was 65 per cent for AFLP, 34 per cent for RAPD and 76 per cent for microsatellite respectively.

Akagi *et al.* (1997) studied on polymorphism of microsatellite loci using 59 *japonica* cultivars including both domestic and modern Japanese cultivars. Microsatellites consisting of AT repeat showed high gene diversity even within genetically closely related cultivars.

Sebastien *et al.* (1998) assessed the nuclear diversity of 78 rice cultivars developed by IRRI, the Philippines Rice Research Institute Bureau of Plant Industry and university of Philippines from 1968 to 1995 using 41 microsatellite markers representing 41 loci with a total of 174 alleles. Over the 30 years of improvement, the molecular diversity of material being produced had increased slightly and the number of frequently occurring alleles had decreased. The diversity was highest in irrigated lowland rice cultivars; intermediate in the upland material and lowest in the rainfed low land cultivars.

Kwon *et al.* (1999) estimated the genetic diversity within 25 *japonica* and six *indica* varieties using a combination of 19 microsatellite and 28 random decamer primers. Microsatellites showed polymorphism among all the varieties by revealing 91 alleles with an average of 48 bands per primer. The decamer primer generated 114 non-redundant bands with a mean of 4.07 bands per primer. They concluded that the microsatellite primers detected more alleles than random primers, although the mean difference was statistically non-significant.

Garland *et al.* (1999) performed study on 10 microsatellite markers for analyzing diversity in rice in a set of 43 cultivars from Australia and they concluded that the microsatellite markers are useful for cultivar identification and assessment of genetic relationships.

Sun *et al.* (2000) estimated genetic diversity of cultivated rice and common wild rice from 10 countries in Asia by RFLP analysis using 44 probes of single copy. They reported that the common wild rice from China has the largest genetic diversity in 10 countries and the secondary is the common wild rice from India.

Luce *et al.* (2001) assessed 419 cultivars of rice from gene banks in five European countries with reference to a collection of 57 Asian cultivars. The cultivars were examined using 16 microsatellite markers. The structure of rice germplasm from European collection is almost exclusively bipolar; 85 per cent of varieties are from the sub species *japonica* and 13 per cent from sub species *indica*. Average polymorphism depicted was 9.1 alleles per locus. All cultivars were identified and classified on the basis of their allelic patterns. A total of 338 accessions had different genotypes among 419. Groups with varietal affinities were identified using a method of hierarchial classification. The microsatellite polymorphism revealed that 82 cultivars displayed an intervarietal polymorphism with two alleles on one or more loci.

Qian *et al.* (2001) studied genetic variation within and between five population of *O.granulata* from two different regions of China using RAPD and ISSR markers. A total of 61 polymorphic loci were detected out of 199 amplified bands using 20 RAPD primers. RAPD marker revealed 73 percent of total genetic diversity resided between the two regions, whereas, only 19 and 6 per cent were present between populations within the region and within the population respectively. However, ISSR marker showed that a great amount of variation (49 per cent) was present between two regions, with 98 per cent between population within regions and 12 per cent within population. Result indicated that the per cent polymorphism detected by ISSR was higher than that detected by RAPD markers.

Sarla *et al.* (2003) worked with diverse *O. nivara* accessions using ISSR and SSR markers based on AG and GA repeats. Genetic diversity among 24 accessions of

O. nivara from 11 states of India and four *O. sativa* varieties, one each from Glaszmann's isozyme groups I, II, V and VI were analyzed using ISSR and SSR primers. The primers based on AG and GA repeats were informative, and their resolving power ranged from 4.2 to 10.8 and PIC value from 0.64-0.89. Ten alleles out of 40 amplified at six loci were unique to an accession. Two accessions each from Uttar Pradesh and Bihar and one from Madhya Pradesh were distinct from other accessions. *O.nivara* alleles in common with Jaya, Dular, Basmati 370 and Taipei 309 were identified.

Ravi *et al.* (2003) observed genetic diversity among the 40 cultivated varieties including hybrids and their A, B and R lines and five wild relatives of *O.sativa* L.(One of *O.nivara*, three of *O.rufipogon* and one of *Porteresia coractata*) using SSRs and RAPD markers. A total of 36 decamer RAPD primers and 38 SSR primers were used to evaluate for polymorphism. A total of 499 RAPD loci were produced with a polymorphism percentage of 9 and higher PIC value was 0.89. They concluded that the two-marker system contrasted most notably in pair by pair comparison of relationships and SSR analysis resulted in a more definitive separation of clusters of genotypes indicating the higher level of efficiency of SSR markers for the accurate determination of relationships between accessions that are too close to be accurately differentiated by RAPD markers.

Neeraja *et al.* (2005) estimated a set of tall races of rice (*Oryza sativa* L.) using gene derived simple sequence repeats and to compare the level of gene derived SSRs with other SSRs of rice. Experimental material comprises of a set of 21 tall rice landraces and a set of eight check genotypes, which included five semi dwarf or semi tall non lodging, high yielding varieties and three tall landraces which were not responsive to nitrogen under propped condition in terms of grain yield. These were characterized using 17 gene derived SSRs and eight other genomic SSRs. Gene derived SSRs had lower polymorphism information content (0.68) than the other SSRs (0.87). UPGMA analysis of gene derived SSR data separated tall landraces and high yielding varieties which were nitrogen responsive into major cluster and the other three landraces which were non-responsive to nitrogen into minor cluster at 0.37 similarity. The use of gene derived marker that target functional loci appears to be an

effective strategy in characterizing landraces for optimizing choice of parents for hybridization programmes.

Ram *et al.* (2005) analyzed genetic diversity among 35 rice accessions which included 19 landraces, 9 cultivars and 7 wild relatives using SSR markers. They reported that the genetic diversity was the highest among wild relatives (0.436) followed by land races (0.356) and the lowest for cultivars.

Vanaja *et al.* (2006) estimated genetic diversity analysis and pedigree evaluation were carried out using RAPD markers on 20 true breeding and high yielding rice genotypes of Kerala. Results showed that the genetic base of rice varieties bred in Kerala is narrowing down, indicating the need to use genetically diverse genotypes in breeding programmes.

Chakravarthi and Naravaneni (2006) observed genetic diversity and DNA fingerprinting of 15 elite rice genotypes belonging to indica and japonica types using 30 SSR primers present on chromosome numbers 7-12. All the 30 RM primers showed polymorphism between 15 rice cultivars. This dendogram revealed that the genotypes that are derivatives of genetically similar type clustered more together. The results revealed that all the primers showed distinct polymorphism among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism.

Lapitan *et al.* (2007) evaluated twenty-four rice cultivars for genetic diversity using 164 SSR markers. A total of 890 alleles were detected by 151 (92%) polymorphic markers with an average of 5.89 per locus. The PIC value was 0.68 per marker and it ranged from 0.18 (RM420) to 0.91 (RM473B). Using Shannon's diversity index, an overall genetic diversity of 0.71 was obtained indicating a high level of genetic variation among these cultivars. Cluster analysis of these cultivars enabled to identify 3 groups at 40% level of similarity with additional sub-clusters within each group. The results indicated that these quality rice cultivars exhibited a higher genetic diversity and therefore useful for rice breeding programs.

Alvarez *et al.* (2007) worked with microsatellite polymorphism in a sample of 39 traditional rice (*Oryza sativa* L.) varieties and 11 improved varieties which were planted in Cuba. They concluded that higher heterozygosity (H) was found higher in traditional varieties 0.72 than higher improved varieties 0.42, and 68% of the total microsatellite alleles were found exclusively in the traditional varieties.

Seetharam *et al.* (2008) Studied on 30 rice genotypes using SSR markers and morphological characters using 35 primer markers where 28 were found to be polymorphic. The number of SSR alleles ranged from two to four.

Rabbani *et al.* (2008) evaluated the genetic polymorphisms and identities of 10 traditional, 28 improved and 2 Japanese cultivars of rice using the RAPD technique. They reported that 40 cultivars were grouped into 3 main clusters corresponding to aromatic, non-aromatic and *japonica* groups.

Herrera *et al.* (2008) performed study on a set of 48 SSR markers to assess the genetic diversity of 11 Venezuelan rice cultivars. They concluded that these rice cultivars showed low diversity.

Tang *et al.* (2009) assessed genetic diversity in 370 aromatic rice accessions in china using 60 SSR markers. There was significant differentiation between accessions from Southern China and South western China, Central China and South western China, respectively. Cluster analysis showed that 370 aromatic rice varieties were mainly classified into *indica* and *japonica*, and aromatic rice varieties from the same areas or the neighbouring provinces were basically assigned to the same group.

Umadevi *et al.* (2010) studied on genetic diversity in 110 rice genotypes representing different origins on the basis of quantitative traits using Mahalanobis D2 statistics and SSR marker analysis. Categorized the genotypes into 29 clusters, cluster III was the largest with twenty five genotypes, cluster XXV exhibited the maximum mean values for number of productive tillers per plant and number of secondary branches per panicle two distinct groups resulted from SSR marker data analysis. Among the 50 primers, RM 401, RM 536, RM 5862, RM 7575 and RM 20A produced maximum number of alleles. All the IR lines were clustered in cluster II

(eleven) and all the RR lines (six) and ASD lines (six) were clustered in cluster I and also confirmed by molecular study. The highest contribution towards genetic divergence was recorded by water uptake followed by L/B ratio, volume expansion ratio, linear elongation ratio and gel consistency.

Singh *et al.* (2011) analyzed the genetic diversity of a set of 50 elite rice genotypes through SSR analysis. SSR marker analysis revealed distinct polymorphism among the cultivars with 28 primers and the polymorphic information content (PIC) value ranged from 0.139-0.99 with an average of 0.589 per primer. UPGMA Cluster analysis grouped the rice genotypes into eight clusters in which genetically similar type clustered together.

Priti Upadhyay *et al.* (2012) analyzed genetic diversity with respect to phylogenetic analysis, population substructure, and identification of molecular tags with 25 popular rice varieties and four landraces from different ecological belts of India employing a set of 52 simple sequence repeat (SSR) markers categorized the genotypes into two major clusters, distributed according to their pedigree. The molecular diversity and population structure analysis indicated that there is not much variation among the popular rice cultivars of India due to limited SSR markers considered.

Ravindra Kumar *et al.* (2012) assessed genetic diversity relationships among 64 rice genotypes of a germplasm with 20 SSR markers on chromosome number 7-12 were investigated resulting in eight polymorphic markers out of twenty. The cluster analysis showed higher level of genetic variation among the genotypes. Similarity coefficients ranged from 0.40 to 0.96. The dendrogram revealed 8 major distinct clusters. Higher range of similarity values for related genotypes using simple sequence repeats (SSR) provides greater confidence for the assessment of genetic diversity and relationships.

Haddad *et al.*, (2013) evaluated eight rice cultivars with 46 microsatellite and 245 AFLP markers where genetic diversity relationships are being accounted as about twelve SSR markers were polymorphic and 245 polymorphic AFLP markers were

generated using five AFLP primer combinations. Cluster analysis was achieved based on SSRs and AFLP data and the relationship between the rice cultivars was addressed.

Yadav *et al.* (2013) conducted an experiment to evaluate the 88 rice germplasm accessions for agronomic traits and molecular diversity. The molecular diversity based grouping indicated that varieties from a common center were genetically similar, with few exceptions. The trait-linked markers gave an average genetic dissimilarity of 0.45 as against that of 0.37 by random markers, along with an average polymorphic information constant value of 0.48 and 0.41 respectively. The correlation between the kinship matrix generated by trait-linked markers and the phenotype based distance matrix (0.29) was higher than that of random markers (0.19).

Umadevi *et al.* (2014) studied thirty-nine genotypes and found a total of 98 alleles with an average of 2.78 alleles per locus. The polymorphic information content (PIC) value of the markers ranged between 0.28 and 0.50 with a mean value of 0.45. The highest PIC value (0.50) was observed for ten primers that is, RM2, RM24, RM411, RM104, RM266, RM125, RM4674, RM247, RM3476 AND RM 3351 while the lowest was 0.28 for RM29. The genetic similarity coefficients for 39 genotypes obtained with SSR markers ranged from 23.8 to 78.4%.

Ramadan *et al.* (2015) evaluated seven rice genotypes for genetic diversity using forty-six SSR markers which indicated that the number of alleles generated by each marker varied from 2 to 6 alleles with an average of 2.76 alleles per locus. The overall size of amplified fragments ranged from 93 to 487 bp. The PIC values ranged from 0.21 to 0.79 with an average of 0.46. The Jaccard's similarity coefficients among the studied genotypes ranged from 0.07 to 0.804.

Singh *et al.* (2015) determined molecular screening and genetic diversity of major rice blast resistance genes in 192 rice germplasm accessions using simple sequence repeat (SSR) markers. The genetic frequencies of the 10 major rice blast resistance genes varied from 19.79% to 54.69%.

Singh *et al.* (2015) observed 76 rice accessions using simple sequence repeat (SSR) markers. The SSR markers were highly polymorphic across all accessions and altogether 79 alleles were detected. The overall PIC value ranged from 0.26 to 0.65 with an average of 0.47 indicating high level of genetic variation. The cluster analysis showed the rice germplasm accessions grouped in two major groups and 14 sub-groups.

Khatab *et al.* (2016) studied fourteen rice genotypes using 13 SSR primers found that the polymorphic information content (PIC) values varied from 0.47 to 0.78, the highest value belongs to RM2239 (PIC 0.78), while RM223 showed the lowest PIC value (PIC= 0.47). Moreover, SSR marker, RM420, was found to be related to generating four alleles, which could be distinguishable for early and late genotypes and would help to reduce the amount of water required for rice growth especially in areas with limited water availability.

Joshi *et al.* (2016) estimated 73 genotypes of rice using thirteen markers and observed that the markers produced a total of 34 alleles with an average of 2.62 alleles per locus. PIC value varied from 0.225 to 0.743. Amplification products ranged from 100 bp produced by marker K39512 to 220 bp produced by marker RM536. The UPGMA cluster diagram grouped the genotypes into eight major clusters. The Jaccard's similarity coefficient ranged from 0.16 to 1.00.

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

2.2.1 Genetic markers with special reference to SSRs

Simple sequence repeats (SSRs), commonly referred to as microsatellites are DNA sequences that are randomly repeated mono to hexa nucleotide units [(T), (AT), (CTT) (GATA) etc]. Their abundance and hyper variability make them valuable as genetic markers (Weber and May, 1989). They provide the basis of polymerase chain reaction (PCR) based multi-allelic and co-dominant marker system. Among various PCR based markers, SSR markers are more popular in rice because they are highly informative; mostly mono locus, co-dominant, easily analyzed and cost effective (Chambers and Avoy, 2000). PCR primers complementary to the flanking region are used to amplify SSR containing DNA fragment. Length polymorphism is created when PCR product from different individuals vary in length as a result of variation in the number of repeat units in the SSR. Of the several DNA markers available, microsatellites or SSRs are considered to be the ideal genetic markers.

The term microsatellite was coined by Litt and Luty (1989). The first report of microsatellites in plants was made by Candit and Hubbel, (1991) and suggested that they are abundant in plant system. They are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Giovannoni *et al.*, 1991) Among classes of repetitive DNA sequences that have showed acquiescent for amplification, SSRs remain the unsurpassed choice of markers (Jacob *et al.*, 1991). Rice microsatellites have been demonstrated to be polymorphic between (Wu and Tanksley, 1993; Panaud *et al.*, 1996) and within rice varieties (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 study was made between inter and intra cultivar polymorphism among the cultivated and wild rice and the extent of chloroplast genomic differentiation was quantified (Provan *et al.*, 1997). SSR's are superior molecular markers for hybrid 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) associated with submergence

tolerance (Xu *et al.*, 2004). 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. McCouch *et al.*, (1997) reported the significant superiority of microsatellites allelic diversity and higher number of alleles over RFLP markers. Liu and Wu (1998) stated that SSR markers were able to detect a high level of polymorphism among inbred lines. Thus, SSR markers are good molecular markers for hybrid rice research. The screening of microsatellites alleles in varieties would create a valuable database for variety identification and the development of molecular markers through marker assisted selected (Garland *et al.*, 1999). 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). Thousands of microsatellites have been identified in rice and over 2,500 have been developed as molecular markers (Temnkyh *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 semiautomated fluorescent detection. SSR's also applicable in estimation of genetic diversity among inbreds and parental lines of rice (Enoki *et al.*, 2002). Yashitola *et al.*, (2002) utilized SSR and STS (Sequence tagged Sites) markers in genetic purity assessment of rice varieties with objective of replacing GOT with DNA based assay. 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 specific locus (Akkaya and Bal, 2004). Microsatellite markers have been ideal for identification and purity checking of rice varieties (Singh *et al.*, 2004). Microsatellite markers were used in genetic diversity assessment of 18 African rice varieties (Semagan *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). SSR markers revealed varying degrees of genetic similarity among the accessions of cultivated and wild species of rice (Jayamani *et al.*, 2007). According to Li *et al.* (2007), polymorphism in SSR could be due to change on SSR region itself caused by the expansion or contraction of S SR 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 micro satellites 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; Chattopadhyaya *et al.*, 2008) and tagging of fragrance gene (Cordeiro *et al.*, 2002; Kibria *et al.*, 2008; Nguyen and Bui, 2008 and Madhav *et al.*, 2010). The markers which showed an average PIC value of more than 0.5 indicated that the SSR markers were highly informative and extremely useful for detection of more alleles in the germplasm accessions and distinguished the polymorphic rate of a marker at a specific locus (DeWoody *et al.* 1995).

2.3 GENETIC DIVERGENCE USING D² STATISTICS.

Crosses among diverse parents are expected to produce a broad spectrum of genetic variability, thereby providing a greater possibility of isolating high yielding segregants in advance generations. The more diverse the parent, more are the chances of increased spectrum of variability. Several plant breeders for assessing genetic divergence among the genotypes successfully used Mahalanobis' D²-statistics. Some of the important reports regarding this have been cited here under:

Hedge and Patil (2000) worked with genotypic divergence in 40 rain-fed rice varieties using D²-statistics. They obtained nine different clusters. Spikelet number per panicle contributed highest to the total divergence followed by 100-grain weight. Based on the genetic distance and per se performance of the genotypes, they suggested different genotypes for the purpose of hybridization to obtain desirable segregates.

Sarawgi and Rastogi (2001) studied genetic diversity among three hundred traditional accessions and could group them into 16 different clusters. They could select certain accessions on the basis of genetic diversity which could be utilized as donors in rice breeding programme for developing semi-dwarf high yielding early aromatic rice with good grain quality.

Sharma and Yadav *et al.* (2002) assessed the genetic diversity in 28 yield and morphological traits of 100 aromatic rice genotypes. Based on the D²-values these

genotypes were divided into 9 clusters. Based on mean performance, genetic distance and clustering pattern, inter-crossing of genotypes Gam poon, KhaoJaoHawan, Basmati sufaid 187, KCN 80152, Abor Bora and Hara, as they suggested, may be useful in creating wider variability for important agronomic and quality traits with high yielding segregants.

Ni. J *et al.*, (2002) worked with Thirty eight rice cultivars and two wild species accessions (*O. rufipogon* and *O. nivara*) were evaluated by means of 111 microsatellite markers distributed over the whole rice genome. Compared to *indica* cultivars, the *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.

Babu and Anbumalaramati *et al.* (2003) estimated genetic diversity in 33 rice varieties by using Mahalanobis' D^2 -statistics. The varieties were grouped into 10 clusters. The largest cluster contained seven genotypes followed by cluster II and III with six genotypes each. Three of the clusters were mono-genotypic. High order of divergence recorded between the clusters indicated possibilities of obtaining good segregants and high heterotic response by crossing parents from highly divergent clusters.

Purabi Das *et al.* (2004) determined the genetic divergence by employing Mahalanobis' D^2 -statistics in 50 land races collection of rice. The genotypes were grouped into 10 clusters. In their study they suggested a hybridization programme involving Taipei 309 and SIPI 681032 at cluster IV and Black Jeera, IR-50 and IR-54447-B-B-B-10-2 of cluster IX to obtain superior genotypes from the segregating generations.

Chaturvedi and Maurya (2005) observed the genetic divergence analysis in 26 genotypes of rice, belonging to seven different ecotypic situations, for 20 characters. The genotypes were grouped into 8 clusters. Comparison of cluster means revealed that cluster VIII gave exceptionally high values for 7 characters followed by cluster VII. The maximum inter-cluster D^2 -value was obtained between cluster III and VI and

III and VIII. It is suggested that for developing better cultivar, the genotypes of cluster III could be utilized in hybridization programme with the genotype of the cluster VI and VIII.

Suman *et al.*, (2005) evaluated 114 rice genotypes from Andhra Pradesh, India were evaluated for 16 metric characters to quantify the genetic diversity existing among them using Mahalanobis D^2 statistic. The genotypes fell into 10 clusters with maximum inter-cluster distance was observed between clusters V and X. Among the 16 quantitative characters studied, harvest index gave the highest contribution (26.42%) towards divergence followed by seed density (18.35%) and total number of tillers per plant (15.52%).

Madhavalatha *et al.*, (2005) analyzed 54 elite rice germplasm lines being maintained at the Agricultural Research Station, Nellore. For evaluating their genetic diversity with regards to grain yield, yield components (days to 50% flowering, days to maturity, harvest index, plant height, number of effective tillers per plant, panicle length, number of grains per panicle and fertility percentage. The genotypes were classified into nine clusters based on Mahalanobis D^2 statistic and also by presence of bands of seven peroxidase and five esterase bands obtained in the gel electrophoretic studies. Geographical and genetic diversity were observed to be unrelated as genotypes from diverse geographical regions were placed in the same cluster, while genotypes from the same center were grouped into different clusters. Results on inter-cluster distances revealed maximum diversity between genotypes of clusters IV and VIII. plant height and days to 50% flowering accounted for 82.04% of the total genetic divergence, indicating their importance.

Singh *et al.* (2006) studied genetic divergence of 52 traditional lowland rice genotypes from the five states of the North Eastern region of India, using Mahalanobis' D^2 -statistics. Based on the 15 agro-morphological characters, these genotypes were grouped into six clusters. Geographical origin was not found to be a good parameter of genetic divergence. Clusters II, IV and V had maximum inter-cluster distances and high values of plant height, days to 50 per cent flowering, panicle length, grain yield per plant and milling percentage, may be used for initiating hybridization programme.

Deepak Naik *et al.*, (2006) conducted genetic divergence to estimate the nature and magnitude of diversity in 50 aromatic rice accessions. The D^2 analysis indicated the presence of appreciable amount of genetic diversity in the material and these genotypes can be used as potential donors for future hybridization programme develop scented rice variety with good grain yield and quality traits.

Chandra *et al.* (2007) worked with 49 genotypes of non-scented rice including three checks to assess the nature and magnitude of genetic divergence by using Mahalanobis' D^2 -statistics for eight quality and seven quantitative characters. The genotypes were grouped into eight clusters based on the D^2 -values. Based on the mean performance genetic divergence and clustering pattern, they suggested that the genotypes MAUB-13, NLR-34450, RDR-916, MTU-1078 and NWGR-99014 from these clusters may be used as the potential donors for future hybridization programme in order to get the desirable recombinants in segregating generation with higher yield potential.

Parimalan *et al.* (2008) carried out an investigation among 51 genotypes of rice to study the nature and magnitude of genetic divergence using D^2 -statistics. They reported that all the genotypes were grouped into ten clusters and found that among the genotypes from the distant clusters viz. IV, VII and X; CO 31 and TPS 3 have superior performance, which can be utilized for future crop improvement programme.

Waghmare *et al.* (2008) studied genetic divergence in a set of 64 genotypes of rainfed rice using Mahalanobis' D^2 -statistics. The genotypes fell into 6 clusters. The genotypes R 1096-181-3-173-1 was found superior from cluster III for the character days to 50 per cent flowering and apparent translocation rate. In the same cluster R 1135-105-1-90-1 genotype was found superior for characters 100-seed weight, flag leaf area, photosynthetic-rate and grain yield per plant.

Kuchanur *et al.* (2009) studied genetic divergence based on Mahalanobis D^2 -statistic, grouped the 38 genotypes into seven clusters. Cluster I and III were the largest, while the clusters II, IV, V, VI and VII were mono genotypic. Genotypes in the cluster III had the highest intra-cluster distance, while maximum inter-cluster distance was observed between cluster IV and VII. Days to 50 per cent flowering had

the maximum contribution (74.96%) to the total divergence followed by yield (9.10%) while flag leaf breadth had no contribution. Cluster means for the twelve characters revealed that cluster VI showed highest means for yield (8829 kg/ha) followed by cluster V (6431.50 kg/ha), while cluster VII was early (90 days to 50 per cent flowering).

Rouf *et al.* (2009) studied genetic divergence on 75 rice genotypes based on Mahalanobis D^2 -statistic and grouped them into 11 clusters. Cluster VI, IX and X among themselves showed greater genetic separation. Though Cluster II and Cluster I had more strength which would help in achieving high genetic gain. As such the variability was evident for grain yield and major attributes.

Banumathy *et al.* (2010) studied fifty three rice genotypes to study genetic divergence based on Mahalanobis D^2 -statistic. The genotypes were grouped into 11 clusters, maximum number of 16 and 15 genotypes were grouped under cluster XI and I respectively, while clusters II, IV, V, VI, VIII, IX and X had only two genotypes each and clusters III and VII consisting of 3 and 5 genotypes respectively. Maximum inter cluster D^2 value was observed between cluster I and X (32.96) followed by cluster I and IV (32.90).

Singh *et al.* (2011) evaluated to thirty eight rice germplasm to study the genetic diversity based on Mahalanobis D^2 statistic and these germplasm grouped into eight clusters. Cluster III contained the highest thirteen germplasm. Maximum intra cluster distance was observed in cluster III (4.48) followed by cluster II (4.12), and the maximum inter cluster distance was observed between cluster I and III (14.40), and test weight had the maximum contribution (56.62%) to the total divergence.

Ovung *et al.* (2012) studied genetic divergence in 70 rice genotypes using Mahalanobis D^2 -statistics by considering 13 quantitative characters. The genotypes were grouped into nine clusters. Cluster I and cluster III constituted maximum number of genotypes i.e. 12 each. The inter cluster distance was maximum between cluster VI and VII (180.54) followed by cluster III and IX (125.20), suggesting that the genotypes constituted in these clusters may be used as parents for future hybridization programme.

Chouhan *et al.* (2014) worked with 35 wild germplasm accessions. The accessions were clustered into six groups, the cluster III contained the highest 12 accessions and cluster I and IV comprised 11 and 7 accessions respectively; while cluster II and VI have two accessions each and cluster V was monogenotypic. The maximum intra cluster distance was observed in cluster VI followed by clusters IV and II while maximum inter cluster distance was observed between clusters V and VI followed by cluster II and IV indicating wider genetic diversity between germplasm accessions.

Kumar *et al.* (2014) estimated genetic divergence in 134 rice germplasm and observed higher order of divergence between cluster IV and V followed by cluster II and V. The highest intra-cluster distance was observed among the genotypes, in cluster IV (12.19) followed by cluster III (11.03) and cluster V (10.90), indicating existence of wide genetic divergence among genotypes. Number of filled grains per panicle contributed highest towards divergence.

Kumar *et al.* (2014) carried out research with 29 genotypes of rice to study the nature and magnitude of genetic divergence using D2 statistics. Twenty nine genotypes were grouped into 6 clusters based on Euclidean cluster analysis with cluster-I containing maximum of 13 genotypes. Maximum intra-cluster distance was observed in cluster-V indicating greater genetic divergence among genotypes belonging to this cluster. Number of spikelets per panicle, plant height and days to 50% flowering contributed maximum towards genetic divergence. Maximum inter-cluster distance was observed between cluster-IV and VI followed by cluster-II and VI, cluster-I and VI and cluster-III and VI indicating wide genetic diversity between the clusters which may be used in rice hybridization programme(s) for improving grain yield through transgressive breeding. Hence, the crosses between HUR-3022 × Loha Chhad (cluster-IV × cluster-VI), Karahani × Loha Chhad (cluster-II × cluster-VI), Pusa Basmati-1 × Loha Chhad (cluster-I × cluster-VI), Pani Dhan × Loha Chhad (cluster-III × cluster-VI), BPT-5204 × Loha Chhad (cluster-IV × cluster-VI) and Karahani × BPT-5204 (cluster-II × cluster-V) could be suggested for the exploitation of transgressive segregants for both yield and yield traits.

Tuwar et al. (2014) estimated genetic components of variability by taking into consideration twenty nine genotypes of rice collected from diverse locations. Analysis revealed that plant height exhibited high estimates of GCV and PCV preceded by number of tillers and effective tillers per plant, number of spikelets and number of grains per panicle and grain weight per panicle. Heritability was higher for days to 50% flowering followed by days to maturity, plant height and panicle length which suggested that these traits would respond to selection owing to their high genetic variability and transmissibility. High heritability coupled with high genetic advance as per cent of mean was recorded for number of spikelets and number of grains per panicle and grain weight per panicle. These characters indicate the predominance of additive gene effects in their expression and would respond to selection effectively as they are least influenced by environment.

Bhati *et al.* (2015) investigated genetic divergence using D^2 studied in 52 rice genotypes for 17 yield and quality traits. All the genotypes were grouped into 8 clusters based on Euclidean cluster analysis with cluster IV containing maximum of 14 genotypes, among the traits number of spikelets/panicle, plant height, grains per panicle and grain yield per plant contributed maximum towards genetic divergence. Maximum inter cluster distance was observed between cluster II and cluster VII indicating wide genetic diversity between the cluster which may be used in future breeding programme for exploitation of transgressive segregantes.

Khare *et al.* (2015) clustered the rice accessions based on ten quantitative traits into seven groups, the cluster III contained highest 14 accessions, followed by cluster I comprising 11 accessions and cluster VI, VII, V, IV and II have 9,8,7,6, and 5 accessions, respectively. The first four principle components accounted for 77.13% of total variation of all the traits.

Umesh *et al.* (2016) studied genetic divergence among 24 genotypes of basmati rice and grouped them into five different clusters based on the interaction in genetic distances. The maximum intra cluster distance (D^2 -values) was found in cluster V (1047.879) followed by cluster I (877.138). The highest inter cluster distance was found between clusters IV and V (4604.881) whereas lowest distance was between clusters III and IV (1158.394), The highest mean genetic divergence was

observed between the clusters IV and V exhibiting wide diversity. Cluster I containing six genotypes was characterized by lowest mean value for spikelets/panicle (103.18).

Meena *et al.* (2017) studied genetic divergence of thirty eight maintainer lines was studied through Mohalanobis's D^2 and principal component analysis for eleven characters. Genotypes were grouped into five different clusters. Cluster IV comprised maximum number of genotypes (thirteen) followed by cluster V and III. The inter-cluster distance was maximum between clusters I and V indicating wide genetic diversity between these two clusters followed by the distance between cluster I and IV, cluster I and cluster III and cluster II and cluster V. The minimum inter-cluster distance was observed between cluster III and cluster IV followed by cluster IV and cluster V and cluster V and cluster III indicating that the genotypes of these clusters were genetically close. The intra cluster distance in the entire five clusters was more or less low indicated the genotypes within the same cluster were closely related. Among the characters number of panicles/m², number of grains per panicle, number of effective tillers and grain yield per plot contributed most for divergence in the studied genotypes.

Imran *et al.* (2017) evaluated the thirty seven genotypes of rice based on twelve important quantitative characters. All the genotypes were grouped into seven clusters with cluster VI containing the maximum of 9 genotypes followed by cluster III and IV each containing 7 genotypes. This suggested that the genotypes grouped within a particular cluster are high or less genetically similar to each other. The maximum intra cluster distance exhibited for cluster V while lowest intra cluster distance was recorded for cluster III. The maximum intra cluster distance was revealed between clusters V and V whereas, minimum inter cluster distance was between clusters III and IV. Cluster VII showed the highest mean values for number of productive tillers per plant, panicle length, biological yield per plant and grain yield per plant. Hence on the basis of higher cluster mean for almost all important component characters, cluster VII has been isolated as most divergent cluster containing genotypes.

MATERIAL AND METHODS

The present investigation on “**STUDY OF MORPHOLOGICAL AND MOLECULAR DIVERSITY IN DROUGHT TOLERANT RICE (*Oryza sativa* L.)**” was conducted during *Kharif* 2016 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP, under the project of Stress Tolerant Rice for Africa and South Asia (**STRASA**) IRRI Philippines. The whole study was divided into two parts i.e., field and laboratory experiments. The field experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, and laboratory experiments was conducted at molecular breeding laboratory (DBT), Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The present research work confined with 20 rice genotype (drought donors including checks).

3.1. Experimental site

The present investigation was carried out during *Kharif* 2016 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, which is situated at 25°18′ North latitude and 83°03′ East Longitude and at Altitude of 123.23 m from sea level. The experiment was laid out in randomized block design (RBD) with three replications. The nursery was sown on 20th June, 2016 on uniform raised beds applied with recommended fertilizer dose. Twenty one days old seedlings were transplanted in main research plot with one seedling per hill in RBD with three replications. Each plot consisted 3m x 0.6m size with spacing (20 × 15) cm. The recommended agronomic practices were followed to raise a good and healthy crop.

3.2. Experimental material

Table 3.1: List of 20 genotypes and their sources.

S. No.	Name of Genotype	Source
1	B 6149 F-MR-7	I.R.R.I., Philippines(S.A. Hub)
2	DZ 78	I.R.R.I. , Philippines(S.A. Hub)
3	E KHA KEHA	I.R.R.I. , Philippines(S.A. Hub)
4	E ZI 124	I.R.R.I. , Philippines(S.A. Hub)
5	GOPAL	I.R.R.I. , Philippines(S.A. Hub)
6	KAUKHMWE	I.R.R.I. , Philippines(S.A. Hub)
7	NP 125	I.R.R.I. , Philippines(S.A. Hub)
8	NS 252	I.R.R.I. , Philippines(S.A. Hub)
9	RTS 4	I.R.R.I. , Philippines(S.A. Hub)
10	SOLOI	I.R.R.I. , Philippines(S.A. Hub)
11	TCHAMPA	I.R.R.I. , Philippines(S.A. Hub)
12	VELLAI SEENETTI	I.R.R.I. , Philippines(S.A. Hub)
13	WANNI DAHANALA	I.R.R.I. , Philippines(S.A. Hub)
14	WAR 72-2-1-1	I.R.R.I. , Philippines(S.A. Hub)
15	XI NUO ZAO	I.R.R.I. , Philippines(S.A. Hub)
16	IR -74371-54-1-1	I.R.R.I. , Philippines(S.A. Hub)
17	IR -119	I.R.R.I. , Philippines(S.A. Hub)
18	IR-64	I.R.R.I. , Philippines(S.A. Hub)
19	SWARNA	ANGRAU, Hayderabad
20	Local check (NDR 359)	NDAUT, Faizabad

3.3 Observations recorded

The following observations were recorded.

3.3.1 Yield traits

The yield traits were recorded on five randomly selected plants from each replication and the arithmetic means of observations from each replication were used for the statistical analysis. Observations were recorded on the traits given below.

3.3.1.1 Days to 1stflowering

This was calculated as the number of days from the date of sowing (seeding) to the date at which any of the plant of the line/replication came to flowering.

3.3.1.2 Days to 50 per cent flowering

This was calculated as the number of days from the date of sowing (seeding) to the date at which 50 per cent of the plants of the line/replication came to flowering.

3.3.1.3 Days to maturity

This was recorded as the number of days from seeding to grain ripening (85 % of the grains in a panicle mature).

3.3.1.4 Plant height (cm)

The plant height was recorded as height of the plant from ground level to the tip of the tallest panicle (awn excluded).

3.3.1.5 Number of tillers per plant

This was recorded as number of panicle bearing tillers in a plant counted at maturity.

3.3.1.6 Number of effective tillers per plant

This was recorded as number of panicle bearing tillers in a plant excluding late tillers counted at maturity.

3.3.1.7 Panicle length (cm)

It was measured in cm from the collar to the tip of the panicle excluding awn.

3.3.1.8 Number of Spikelets per panicle

The total numbers of spikelets per panicle were counted at maturity.

3.3.1.9 Number of grains per panicle

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

3.3.1.10 Number of Sterile spikelets per panicle

The total numbers of sterile spikelets per panicle were counted at maturity.

3.3.1.11 Grain weight per panicle (g)

Total weight of filled grains per panicle was weighed on a precision balance.

3.3.1.12 1000- grain weight (g)

Weight of 1000 randomly selected well developed grains at 12-14% moisture content was recorded using electronic balance.

3.3.1.13 Grain yield per plant (g)

Mature panicles from single plants were harvested, threshed, cleaned and dried. The weight of paddy (rough rice) obtained from single plant was recorded using electronic balance at 12-14 per cent moisture content.

3.3.1.14 Grain yield per plot (g)

Mature panicles from all plants of one plot were harvested, threshed, cleaned and dried. The weight of paddy (rough rice) obtained from all plants of one plot were recorded using electronic balance at 12-14 per cent moisture content.

3.3.1.15 Biomass (kg/ha)

Mature plants were harvested and threshed. After threshing weight of paddy straw obtained and were recorded using electronic balance.

3.3.1.16 Harvest Index

Harvest index is defined as weight of grain divided by the total weight of above ground biomass (straw plus grain)

$$\text{Harvest Index} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

3.3.1.17 Grain quality characters

The grain quality traits were also recorded on randomly selected plants from each replication and the arithmetic means of observations from each replication were used for the statistical analysis. Observations were recorded on the traits given below.

3.3.1.18 Hulling recovery

The grains were dried to a moisture level ranging between 12-14 per cent before hulling. A sample of 100g was taken for hulling in a rice huller (Indosaw) and the weight of brown rice recorded. Hulling recovery on dry weight basis was calculated as:

$$\text{Hulling recovery} = \frac{\text{Weight of hulled kernel}}{\text{Weight of paddy}} \times 100$$

3.3.1.19 Milling recovery

The de-hulled kernels recovered after hulling were milled in a rice polisher (Indosaw) for one minute. The milled rice obtained was weighed in grams and milling value was calculated as the ratio of milled rice to initial weight of the paddy sample (100 g) expressed in percentage. Milling recovery on dry weight basis was calculated as:

$$\text{Milling recovery} = \frac{\text{Weight of polished kernel}}{\text{Weight of paddy}} \times 100$$

3.3.1.20 Kernel length (mm)

Length of 10 randomly selected, polished kernels from its base to tip was measured using an electronic dial caliper.

3.3.1.21 Kernel breadth (mm)

Breadth of 10 randomly selected, polished kernels were measured at the widest point using an electronic dial caliper.

3.3.1.22 Kernel L/B ratio

Average length of 10 kernels and breadth of ten kernels were taken in millimetres and length/breadth ratio was calculated by dividing length with the breadth.

3.3.1.23 Amylose content

The cooking and eating quality of rice depends on the ratio of two kinds of starches, amylose and amylopectin in the rice grain (Rao *et al.*, 1952). Amylose is linear, whereas amylopectin is branched starch. The formation of a helical complex between amylose and iodine gives rise to the typical deep blue colour of starch dispersions stained with iodine and forms the basis for quantitative determination of amylose content. In I_2 -KI solutions, polyiodide ions such as I_3^- and/or I_5^- interact with amylose forming single left-handed V-type helices. The formation of these complexes is determined by spectrophotometry (Juliano, 1971).

The simplified calorimetric method described by Juliano (1971) was followed for the estimation of amylose content. Fifty grains of milled rice were ground into a powder and 100 mg of rice powder was put into a 100 ml volumetric flask. One ml of 95 per cent ethanol and 9 ml of 1N sodium hydroxide were added to it. The contents were heated on a water bath at 100 °C to gelatinize the starch. After cooling for one hour, distilled water was added and volume made up to 100 ml. The contents were shaken well and five ml of starch solution was put in a 100 ml volumetric flask. One ml of 1N acetic acid and 2 ml of iodine solution (0.2 g iodine and 2 g of potassium iodide in 100 ml distilled water) were added and the volume was made up to 100 ml

with distilled water. Contents were shaken well and let undisturbed for 20 minutes in darkness as I-KI disintegrates on exposure to light. The solution was finally examined under a spectrophotometer at a wavelength of 620 nm for absorbance against a blank iodine solution.

For the standard curve, 40 mg of potato amylose were wet with 1 ml ethanol and 9 ml 1N sodium hydroxide. The contents were heated for 5-10 minutes in a boiling waterbath, cooled and made up to 100 ml volume with distilled water. 0, 1, 2, 3, 4, 5 ml of the solution were placed with a pipette in separate 100 ml volumetric flasks. The solution was acidified with 1N acetic acid (0.2, 0.4, 0.6, 0.8, and 1.0 ml, respectively) and treated as above. These standards including blank, respectively correspond to 0%, 4%, 8%, 12%, 16% and 20% of amylose. The absorbance values of standard samples were plotted at 620 nm, against the concentration of amylose. Amylose content of the sample to be examined was determined with reference to the standard curve and expressed on percent basis (Table 3.3.3c).

Table 3.2: Classification of rice genotypes on the basis of amylose content

Category	Amylose content (%)
Waxy	1-2
Very low	3-9
Low	10-19
Intermediate	20-25
High	26-30
Very High	>30

3.3.2 Drought characters

3.3.2.1 Canopy temperature depression (CTD)

It is measured in terms of °C. CTD is the difference in temperature between the canopy surface and the surrounding air. CTD is a highly integrating trait resulting from the effects of several biochemical and morpho-physiological features acting at

the root, stomata, leaf, and canopy levels. In the field, genotypes with a cooler canopy temperature under drought stress, or a higher CTD, use more of the available water in the soil to avoid excessive dehydration (Blum, 1988; Ludlow and Muchow, 1990; Reynolds et al., 2007, 2009).

CTD was measured directly in the field with the help of infrared thermometer just before heading.

3.3.2.2 Stomatal conductance (mmol/m²/s)

Low stomatal conductance helps to maintain higher leaf water potential in the genotypes under stress (Blum, 1982). The rate of stomatal conductance, or its inverse, stomatal resistance, is directly related to the boundary layer resistance of the leaf and the absolute concentration gradient of water vapour from the leaf to the atmosphere. It is under direct biological control of the leaf through the use of guard cells, which surround the stomatal pore (Taiz/Zeiger 1991). The turgor pressure and osmotic potential of guard cells is directly related to the stomatal conductance. Stomatal conductance is a function of stomatal density, stomatal aperture, and stomatal size. Stomatal conductance is integral to leaf level calculations of transpiration.

Stomatal Conductance was measured directly in the field with the help of leaf porometer just before heading.

3.3.2.3 Chlorophyll content (SPAD value)

Chlorophyll content of leaf was measured directly in the field with the help of SPAD Chlorophyll meter just before heading.

3.3.2.4 Proline content (µmol/g fresh weight)

A large body of data suggests a positive correlation between proline accumulation and plant stress. Proline, an amino acid, plays a highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule.

Free proline content in the leaves was determined by following the method of *Bates et al.* (1973).

Reagents used

a. Acid Ninhydrin Reagent

For the preparation of acid ninhydrin reagent 1.25 g of ninhydrin was dissolved in a mixture of warm 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid with agitation.

b. Sulphosalicylic Acid (3 per cent)

This was prepared by dissolving 3.0 g of sulphosalicylic acid in 100 mL of distilled water.

c. Glacial Acetic Acid (99.7 per cent)

d. Toluene (99.5 per cent)

50 mg dried seedlings of 25 DAS were taken into the mortar pestle separately in each replication, and for each treatment 3 replications were taken into consideration and crush with 3% sulphosalicylic acid and the volume was made up to 5 ml in centrifuge tube and the samples were centrifuged for 10 minutes at 5000rpm, then supernatant were taken out in test tubes and the volume was made up to 5ml by adding 3% sulphosalicylic acid, from this 2ml of supernatant were taken in separate test tube and 2 ml of acid ninhydrin & 2 ml of glacial acetic acid (GAA) were added to all the test tubes and kept in boiling water bath for 30 minutes then, it was cooled in running tap water. After that the solution was taken in separating funnel and it was shaken well and the lower toluene residue was discarded. Then the solution of pink colour was taken out in test tubes and the O.D was determined at 520 nm in spectrophotometer (SL 171 mini SPEC Spectrophotometer, ELICO). A blank was also prepared by using distilled water in place of sample aliquot. Standard curve was prepared by using Proline.

Standard curve for proline estimation

Proline (10 mg) was taken and dissolved in 3 per cent aqueous sulphosalicylic acid and then the solution was diluted to 100 mL. Then 0.2, 0.4, 0.6, 0.8 and 1.0 mL aliquots were taken in to different test tubes and the volume was raised to 2 mL by adding 3 per cent aqueous sulphosalicylic acid solution. Colour was developed in the same way as for samples and absorbance was determined with the help of spectrophotometer (Digispec 110D).

3.3.3 Laboratory experiments

3.3.3.1 DNA extraction

For DNA extraction young leaves of 15-20 days old from sixty five rice genotypes were clipped and stored in ice-box to carry it to the lab which is then stored in -80°C till DNA extraction. Genomic DNA was then extracted using CTAB method of Doyle and Doyle (1987).

3.3.3.1.1 Reagents required for DNA extraction

- Leaf samples
- Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer (100 ml)
- Chilled isopropanol
- Phenol : chloroform : isoamylalcohol in 25:24:1(v/v) ratio
- Ethanol
- RNase A: RNase A was dissolved in TE buffer and boiled for 15 minutes at 100 °C to destroy DNase and stored at -20 °C.

3.3.3.1.2 Preparation of solution

A. Extraction Buffer

- 100 ml CTAB extraction buffer [2% (w / v) CTAB, 1.4 M NaCl, 0.1% (v / v) 2-mercaptoethanol, 20 mM EDTA, 100 mM TrisHCl, pH 8.0].

- 20 ml of 10% CTAB (dissolved at 60°C in water bath for one hr)
- 35 ml of 4 M NaCl (autoclaved)
- 200 µl 2 b-Mercaptoethanol
- 4 ml of 0.5 M EDTA (autoclaved, pH adjusted to 8 using NaOH pellets)
- 10 ml of 1 M Tris (autoclaved, pH adjusted to 8 using conc. HCl)

The final volume was made upto 100 ml with sterile double distilled water to get extraction buffer concentration mentioned above.

B. Chloroform: Isoamyl alcohol

Mix chloroform and isoamyl alcohol in the ratio of 24:1 (v / v).

C. 70% ethanol

70 ml of absolute alcohol mixed with 30 ml of sterile double distilled water.

D. TE (Tris-EDTA) buffer

TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) was prepared by mixing 1 ml of Tris and 200 µl of 0.5 M EDTA and making up the final volume to 100 ml using sterile double distilled water. The solution was then autoclaved.

3.3.3.1.3 Protocol for DNA extraction

- a) Up to 100 mg of frozen leaf tissues were taken in autoclaved mortar pestle.
- b) The leaf tissues were ground in liquid nitrogen and extracted with 500 µl of CTAB buffer and transferred it to 2 ml centrifuge tube.
- c) Incubate at 65°C for at least one hour, mixing once after 15 minutes. They can stay in the water bath for a few hours if necessary.

- d) The tubes were removed from the water bath and equal volume of Phenol: chloroform: Isoamyl alcohol mixture [25:24:1 (v/v)] was added and mixed by using shaker for 15 min.
- e) It was centrifuged at 14,000 rpm for 10 minutes at room temperature.
- f) Obtained aqueous phase (top layer) was transferred into the 2 ml new labeled tube.
- g) 800 μ l of Chloroform: Isoamyl alcohol mixtures (24:1) were added, shaken for 10-15 minutes on shaker and centrifuged for 10 minutes at 14,000 rpm at room temperature.
- h) Supernatant was taken in 1.5 ml autoclaved and labelled centrifuge tube.
- i) 2/3 volume of chilled isopropanol was added and mixed gently by inversion and then kept in the deep freezer until DNA was precipitated out.
- j) It was then centrifuged at 14,000 rpm for 10 minutes at 4°C temperature.
- k) The supernatant was discarded and pellet was washed by using 200 μ l 70% of ethanol and kept it overnight at room temperature for drying.
- l) Then the DNA pellet was dissolved in 50 μ l of TE and stored at -20°C.

3.3.3.2 Purification of DNA

Major contaminations in crude DNA preparation are RNA, protein and polysaccharides. Inclusion of CTAB in extraction buffer helps elimination of polysaccharides. RNA was removed by RNase treatment and proteins were removed by phenol-chloroform extraction.

3.3.3.3 Quantification of DNA

DNA quantification is important to check the integrity of the DNA. Quantification can be done either by spectrophotometric measurements or by agarose gel electrophoresis. In the present study, quantification was done by agarose gel electrophoresis of the isolated DNA samples.

a) Preparation of gel

0.8% agarose gel was prepared by mixing 2 g agarose in 250 ml of 1X TAE solution. The solution was melted in a microwave oven, 20 μ l of ethidium bromide was then added to the agarose solution and poured into a gel casting tray set with combs. Once the gel was polymerised (about after an hour), combs were removed and gel was immersed in 1X TAE in the buffer tank for loading the samples.

b) Estimation of DNA quantity

2 μ l samples of genomic DNAs along with the gel loading dye were individually loaded onto the wells of the ethidium bromide stained agarose gel and resolved at a constant voltage of 70 V for 2-3hrs and the gel was visualized under UV light. A thick band without any smear on upper part of the gel was indicative of high molecular weight and good quality weight genomic DNA.

3.3.3.4 SSR primer selection

A total of twenty rice SSR markers reported to be linked to various rice sheath blight resistance QTLs (Channamallikarjuna *et al.*, 2010) were used for molecular diversity. List of markers along with their forward and backward sequences and chromosome number in which that marker is located is given in the Table 3.2.

Table 3.3. Details of the SSR primers used in present study.

S.No.	SSR Primer	Sequence	Chr. No.	Tm(°C)
1.	RM1 F	GCGAAAACACAATGCAAAA	1	54.7
	RM1 R	GCGTTGGTTGGACCTGAC		
2.	RM17 F	TGCCCTGTTATTTTCTTCTCTC	-	58.55
	RM17 R	GGTGATCCTTTCCCATTTCA		
3.	RM424 F	TTTGTGGCTCACCAGTTGAG	2	55.5
	RM424 R	TGGCGCATTCATGTCATC		
4.	RM16874 F	TAGCAAGCTTGGAGAAGTGATGG	4	61.8
	RM16874 R	CAGAAGAAGTCAGCTCTATGCTTGG		
5.	RM190 F	CTTTGTCTATCTCAAGACAC	6	54.5
	RM190 R	TTGCAGATGTTCTTCCTGATG		
6.	RM481 F	TAGCTAGCCGATTGAATGGC	7	57.3
	RM481 R	CTCCACCTCCTATGTTGTTG		
7.	RM434 F	GCCTCATCCCTCTAACCCTC	9	59.35
	RM434 R	CAAGAAAGATCAGTGCGTGG		
8.	RM216 F	GCATGGCCGATGGTAAAG	10	55.65
	RM216 R	TGTATAAAACCACACGGCCA		
9.	RM202 F	CAGATTGGAGATGAAGTCCTCC	11	57.6
	RM202 R	CCAGCAAGCATGTCAATGTA		
10.	RM174 F	AGCGACGCCAAGACAAGTCGGG	2	65.8
	RM174 R	TCCACGTCGATCGACACGACGG		
11.	RM 231 F	CCAGATTATTTCTGAGGTC	3	55.6
	RM231 R	CACTTGCATAGTTCTGCATTG		
12.	RM232 F	CCGGTATCCTTCGATATTGC	3	58.05

	RM232 R	CCGACTTTTCCTCCTGACG		
13.	RM263 F	CCCAGGCTAGCTCATGAACC	2	60.4
	RM 263 R	GCTACGTTTGAGCTACCACG		
14.	RM270 F	GGCCGTTGGTTCTAAAATC	12	57.95
	RM270 R	TGCGCAGTATCATCGGCGAG		
15.	RM 39 F	GCCTCTCTCGTCTCCTTCCT	5	57.55
	RM39 R	AATTCAAACCTGCGGTGGC		
16.	RM335 F	GTACACACCCACATCGAGAAG	4	59.8
	RM335 R	GCTCTATGCGAGTATCCATGG		
17.	RM528 F	GGCATCCAATTTTACCCCTC	6	57.3
	RM528 R	AAATGGAGCATGGAGGTCAC		
18.	RM551 F	AGCCCAGACTAGCATGATTG	4	58.35
	RM551 R	GAAGGCGAGAAGGATCACAG		
19.	RM84 F	TAAGGGTCCATCCACAAGATG	1	56.9
	RM84 R	TTGCAAATGCAGCTAGAGTAC		
20.	RM87 F	CCTCTCCGATACACCGTATG	5	58.35
	RM87 R	GCGAAGGTACGAAAGGAAAG		

3.3.3.5. Optimization of polymerase chain reaction (PCR)

PCR amplification using SSR primers was carried out using sterilized thin-walled PCR tubes (0.2 ml) according to the protocol of Channamallikarjunaet *al.* (2010) with minor modifications. After optimizing the reaction conditions, Polymerase Chain Reaction was carried out in 15 µl reaction mixture (Table 3.3) in a thermal cycler.

Table 3.4. List of various components of PCR master mixture (15 µl).

Sl no.	Reagent	Stock concentration	Required Concentration	Quantity (µl)
1	Assay buffer	10X	1X	1.50
2	dNTPs	25 Mm	0.3 Mm	0.20
3	MgCl ₂	10Mm	133 µM	0.20
4	Taq Polymerase	5 U	1 U	0.20
5	Primer (Forward)	10µl	1.25 µM	1.0
6	Primer (Reverse)	10µl	1.25 µM	1.0
7	Genomic DNA	15 ng/µl	15 ng/µl	1.0
8	HPLC water	-	-	9.90
Total				15.0

Appropriate volumes of all the reagents, excluding template DNA and sterile HPLC water, were pipetted into a sterile Eppendorf tube (1.5 ml). The appropriate volume for a reagent was estimated by multiplying the volume needed for one reaction by the number of reactions to be carried out. A small quantity of HPLC-grade water was added to this mixture to accommodate the pipetting errors, and it was mixed by vortexing. This master reaction mixture was distributed into individual PCR tubes at the rate of 14 µl/tube which already contains 1 µl of the template DNA. The quantities of the reagents for one reaction and the sequence of their addition are listed in Table 3.3. The tubes containing reaction mixture were placed in the wells of the thermal cycler block and amplification reaction was carried out with the temperature programme summarized in Table 3.4.

Table 3.5. The schedule of temperature and duration programmed for PCR amplification using SSR primers.

S.N.	Objective	Temperature (°C)	Duration	Number of Cycles
1	Initial denaturation	94	3 min	1
2	Denaturation	94	30 sec	39
3	Annealing	*T _m	30 sec	39
4	Extension	72	1 min	39
5	Final extension	72	5 min	1
6	Hold	15	-	Hold

For the PCR programming all the steps were kept as such except the annealing temperature. Annealing temperature (*T_m) was determined based on the GC content of the primer using the following formula:

$$*T_m = [2 \times (A + T) + 4 \times (G + C)] - 4$$

3.3.5.1. Separation of amplified products on agarose gel electrophoresis

3.3.5.1.1 Requirements

- 10x TAE (Tris Acetate EDTA buffer)
Tris Base 48.4 g
Acetic acid 11.42 ml
0.5M EDTA 20 ml
- (Dissolved in 800 ml of sterile water and made up to 1000 ml)
- Loading Dye
Glycerol 30% (v/v)
Bromophenol blue 0.5% (w/v)

3.3.5.1.2 Protocol

- Agarose gel of 2.5% concentration was prepared in 1x TAE buffer (electrophoresis buffer) and then heated in microwave to melt the agarose completely till the solution becomes clear.
- The agarose solution was cooled to 60°C and then ethidium bromide was added at a concentration of 0.5 µg/ml of gel and mixed well.
- The agarose solution was poured in gel casting tray slowly to avoid any air bubble formation.
- The gel was then allowed to set completely before removing the comb. The comb was removed carefully and the gel was placed in gel electrophoresis tank which is half filled with 1x TAE buffer.
- 2µl of 6x gel loading dye (Bromophenol blue 0.25%; Xylene cyanol 0.25% and Glycerol 30.0%) was added to 15µl of PCR products and mixed well before loading into the wells. Then the amplified product was loaded in each well using micropipette. Care was taken to prevent mixing of samples between the wells.
- After loading of all samples 4µl of 100bp DNA size ladder (Fermentas, India) was loaded in first well as a reference for molecular weight of amplified products.
- A constant voltage of 70V was given for a time period of three hours for separation of PCR fragments using a power pack (BIO RAD, USA).
- After the run, the amplified products were visualized and photographed under UV light source in a gel documentation system (Gel Doc TM XR+, BIO RAD, and USA) and image of band patterns were stored in a computer for future use.

3.3.5.2. Scoring of PCR amplified fragments

Band position in comparative SSR profile for each genotype and primer combination was scored from the respective gel images. SSR profile from only that genotype x primer combination, which gave consistent amplification for all the genotypes and without any blank lane/unclear bands, was included in this study. The amplified fragments were scored as “1” for the presence and “0” for the absence of a band generating the 0 and 1 matrix. These binary data matrix was then utilized to generate genetic similarity data among the 20 rice genotypes.

3.4 Statistical Analysis

The experimental data were compiled by taking mean value over randomly selected plants for all the three replication and subjected to the following statistical analysis:

3.5.1. Analysis of variance for the design of experiment.

3.5.2 Selection parameters:

- A. Mean and range.
- B. Phenotypic and genotypic coefficient of variability.
- C. Heritability and genetic advance.

3.5.3 Genetic divergence (D^2 analysis).

3.4.1 Analysis of variance

Analysis of variance is used for testing whether there is significant difference between the treatment or not. It was carried out by following the procedure of randomized block design (RBD) analysis (Panse and Sukhatme, 1967) for each of the sixty five germplasm accession. The variance was partitioned in to three sources of variance, viz. replication, treatment and error.

The model of ANOVA is given bellow:-

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

Where, Y_{ij} = Phenotypic observation in the i^{th} Genotype in the j^{th} Block

μ = Grand Mean

g_i = Effect of i^{th} Genotype

r_j = Effect of j^{th} Replication

e_{ij} = Random Error

Here, $i = 1, 2, 3, \dots, 65$.

$J = 1, 2, 3$.

Table 3.6. Analysis of variance (ANOVA)

Source	df	SS	MSS	F value (Calculated)
Replication	$r-1$	SSr	$\frac{SSr}{(r-1)} = MSR$	$\frac{MST}{MSE}$
Treatment	$t-1$	SSt	$\frac{SSt}{(t-1)} = MST$	
Error	$(r-1)(t-1)$	SSe	$\frac{SSe}{(r-1)(t-1)} = MSE$	
Total	$(rt-1)$	TSS		

Where,

r = Number of replications

t = Number of treatments

df = Degree of freedom

SSr = Replication sum of square

SSt = Treatment sum of square

SSe = Error sum of square

TSS = Total sum of square

MST = Treatment mean sum of square

MSR = Replication mean sum of square

MSE = Error mean sum of square

The significance of difference among the treatments was tested by comparing the calculated 'F' value with table value of 'F' at 5 and 1 per cent level of significance at (t-1) and (r-1) (t-1) degrees of freedoms, respectively.

3.4.2 Selection parameters

A. Mean and range

Mean:

The mean value of each character was determined by summing up all the observations and dividing them by corresponding number of observations.

$$\bar{X} = \frac{\sum_{i=1}^N X_i}{N}$$

Where,

$\bar{X}$ = mean,

$\sum_{i=1}^N X_i$ = Sum of all observations

N = Number of observations

Range:

The lowest and highest values for each character were taken as the range.

B. Phenotypic and genotypic variances

These estimates were obtained as below-

Error variance, $\sigma^2_E = M_E$

Genotypic variance, $\sigma^2_g = \frac{M_T - M_E}{r}$

Phenotypic variance, $\sigma^2_p = \sigma^2_g + \sigma^2_e$

Standard error of difference between two means [S.E.d.(M)]

S.E.d.(M) was calculated with the help of error mean square from ANOVA table.

$$S.E.d.(M) = \sqrt{\frac{2M.S.E.}{r}}$$

Where,

r = Number of replications

MSE = Mean sum of square due to error

B. Coefficient of variation (CV)

Phenotypic and genotypic coefficient of variation was calculated by the method suggested by Burton and Devane (1953):

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

$$= \frac{\sigma_p}{\bar{X}} \times 100$$

$$\text{Genotypic coefficient of variation (PCV)} = \frac{\text{Genotypic standard deviation}}{\text{General mean}} \times 100$$

$$= \frac{\sigma_g}{\bar{X}} \times 100$$

C. Heritability and genetic advance:

Heritability

It was calculated by the formula given by Allard (1960) which is given below:

$$H = \frac{\sigma_g^2}{\sigma_p^2}$$

Where,

H = Heritability in broad sense

$$\sigma_g^2 = \text{Genotypic variance} = \frac{\text{MST} - \text{MSE}}{r}$$

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

Genetic Advance (Expected)

The genetic advance i.e. expected genetic gain resulting from selecting five per cent superior plants was estimated by the following formula suggested by Allard (1960):

$$\text{Genetic advance (Expected)} = H \times \sqrt{\sigma_p^2} \times k$$

Where,

H = Heritability coefficient

$$\sqrt{\sigma_p^2} = \text{Phenotypic standard deviation}$$

K = Selection differential in standard units which is 2.06 at 5% selection intensity.

Genetic advance as percentage of mean was calculated by the following formula:

$$\text{Genetic advance as percentage of mean} = \left(\frac{GA}{\bar{X}} \right) \times 100$$

Where,

GA = Expected genetic advance

$\bar{X}$ = General mean of the character in the population

3.4.3 Genetic divergence by Mahalanobis' D²-statistics

Genetic divergence amongst different genotypes is assessed based on the estimated *inter-se* genetic distances amongst the genotypes. D²-statistics of Mahalanobis (1928) is one of the most effective tools to measure the genetic distance between genotypes as measured by allelic frequencies at a sample of loci. Genetic similarity is defined as the converse of the genetic divergence i.e. the extent of gene similarity among the genotypes.

3.4.3.1 Testing difference in the population

From the variance analyses, when the null hypothesis of no treatment differences fails with regards to individual characters, a dispersion table was prepared. Using V statistic, which intern utilize Wilk's criterion (Δ), a simultaneous test of differences between mean values of a number of correlated variables was done (Rao, 1948)

$$\text{Wilk's criterion } (\Delta) = \frac{\text{Determinants of error variance and covariance matrix (E)}}{\text{Determinants of genotypic + error variance and covariance matrix}}$$

$$V \text{ statistic} = -m \log_e \Lambda = - \left(n - \frac{p + q + 1}{2} \right) \log \Lambda$$

Where,

$$m = n - \frac{p + q + 1}{2}$$

p = Number of variables or characters

q = Degrees of freedom for population (i.e., number of genotypes-1)

n = Degrees of freedom for error + genotypes

$$\log_e \Lambda = 2.3026 \log_{10} \Lambda$$

‘V’ statistic is distributed as χ^2 with pq degrees of freedom. The test of significance of ‘V’ statistic showed that the differences between the means in respect of the pooled effect of p characters between different populations were significant. Hence further analyses were made to estimate D²-values.

3.4.3.2 Mahalanobis’ D²-statistics

In the present investigation genetic divergence was estimated based on Mahalanobis’ generalized distance as described by Rao (1952). Original variable means were transformed to un-correlated variables by the pivotal condensation method of inversion matrix.

The D²-values between the genotypes were obtained as the sum of squares of differences of the values of the corresponding transformed variables.

For each pair of combinations the mean deviation i.e.

$d_i = Y_i^1 - Y_i^2$, where Y_i denotes the transformed variables ($i = 1, 2, 3, 4, 5, \dots, p$) were calculated and the D² was then calculated as sum of the squares of those deviations, i.e.

$$D^2 = \sum (Y_i^1 - Y_i^2)^2$$

Where, p = Number of characters.

The significance of D²-values was tested by treating them as chi-square (χ^2) at p degrees of freedom where p is the number of characters considered.

3.4.3.3 Grouping of genotypes by Tocher's method

After arranging the D^2 -values of all combinations of one genotype with the others in ascending order of magnitudes, the genotypes were grouped into a number of clusters by Tocher's method described by Rao (1952). The criterion used in this method was that any two varieties belonging to the same cluster, at least on an average, show a smaller D^2 -value than those belonging to two different clusters. Then inter and intra-cluster distances were calculated and their relationships were diagrammatically represented.

3.4.3.4 Contribution of individual characters towards divergence

In all the combinations of genotypes, each character was ranked on the basis of $d_i = Y_i^j - Y_i^k$ values. Rank 1 was given to the highest mean difference and rank p to the lowest mean difference, where, p represented the total number of characters. The per cent contribution was calculated by considering total number of combinations as 100 per cent.

3.4.4 Correlation

3.4.4.1 Correlation coefficient:

The simple phenotypic correlation coefficients among pairs of characters were calculated according to the formula suggested by Searle (1961).

$$r(X_1X_2) = \frac{\text{Cov}(X_1X_2)}{\sqrt{V(X_1) \cdot V(X_2)}}$$

Where,

X_1 = Character 1

X_2 = Character 2

$r(X_1X_2)$ = Correlation between characters X_1 and X_2

$\text{Cov } X_1X_2$ = Covariance between X_1 and X_2

$V(X_1)$ = Variance of X_1

$V(X_2)$ = Variance of X_2

In the estimation of phenotypic correlation coefficients, phenotypic covariance and variance are considered for calculation.

To test the significance of correlation coefficients, the estimated values were compared with the table value (statistical table by Fisher and Yates, 1963) at $n-2$ degrees of freedom (where n denotes the number of genotypes tested) at 5% and 1% level of significance.

3.5 Statistical analysis of molecular data:

3.5.1 Standardization of quantitative data

The effects of different scales of measurement for different quantitative traits were minimized by standardizing the data for each trait separately prior to cluster analysis. Standardization was done by dividing the deviation of mean for a line from the mean for sixty five lines with the standard deviation for the given trait; the STAND module of NTSYS (Rohlf, 1997) software was used to furnish the same.

3.5.2 Genetic dissimilarity

The binary data matrix generated by polymorphic SSR markers were subjected to further analysis using NTSYS-pc version 2.11W (Rohlf, 1997). The SIMQUAL programme was used to calculate the Jackard' dissimilarity coefficient.

3.5.3 Cluster analysis based on UPGMA

The dissimilarity matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using SAHN module of NTSYSpc for dendrogram construction. In Unweighted pair-group average (UPGMA) clusters are joined based on the average distance between all members in the two groups.

3.5.3.1 Polymorphic information content (PIC)

PIC for SSR markers was calculated as per the formula: $PIC = 1 - \sum p_{ij}^2$, where PIC_i is the polymorphic information content of a marker i and the summation extends over n patterns. PCA was also done to check the result of UPGMA base clustering using EIGEN module of NTSYSpc.

3.5.3.2 Principal component analysis (PCA)

In principal component analysis (PCA), the total variance of original characters is divided into a limited number of uncorrelated new variables known as principal components (PCs). The first step in PCA is to calculate Eigen values, which define the amount of total variation that is displayed on PC axes. The first PC summarizes most of the variability not summarized by, and uncorrelated with, the first PC, and so on. PCs were used for 2-dimensional (2-D) and 3-dimensional (3-D) plotting, respectively, against each other using module PROJ and MXPLOT of NTSYSpc.

EXPERIMENTAL FINDINGS

A field experiment comprising of 20 rice genotypes was laid out in a Randomized Block Design with three replications during *Kharif 2016*. The extent of genetic variability, association between yield and yield components, and genetic diversity (D^2) was studied. Total observations were recorded on twenty seven characters. Apart from these attributes, marker based analysis was also done in the laboratory to study the genetic diversity using SSR markers.

The experimental results obtained from the present study conducted during *kharif 2016* for twenty rice genotypes are presented in the following heads:

4.1 Analysis of variance.

4.2 Variability, heritability and genetic advance.

4.3 Genetic divergence.

4.4 Molecular diversity.

4.1 Analysis of variance (ANOVA)

The analysis of 27 traits was carried out to partition the total variation into genotypic variation and variation due to other sources. Analysis of variance was based on the mean values of eleven quantitative traits in 20 rice genotypes. The mean values are presented in Appendix I.

4.1.1 Analysis of Mean performance and Range

Mean of replication is calculated by taking the average of five randomly selected plants of each genotype. Similarly mean of the other two replications were also considered and a final mean was calculated from these three data. Performance of

individual genotypes for different traits is mentioned in Appendix I and the mean performance and range of all the characters under study among 20 genotypes are presented in Table 4.1.

4.1.1.1 Days to 1st flowering

Days to first flowering ranged from 79.33 to 108.33 days. The average mean for this trait was 89.63 days. The early flowering was seen in three genotypes namely XI NUO ZAO (79.33), WANNI DAHANALA (80.67) and SOLO I (80.67) while genotype SWARNA (108.33) exhibited late days to first flowering followed by IR 119(97.67), GOPAL (96.33) and KAUKHMWE (95.67) .

4.1.1.2 Days to 50% flowering

Days to 50% flowering ranged from 84 to 112.33 days. The average mean for this trait was 94.55days. The early flowering was seen in three genotypes namely XINUO ZAO (84), WANNI DAHANALA (85) and SOLO I (85) while genotype SWARNA (112.33) exhibited late days to 50% flowering followed by IR 119(101.67), GOPAL (101.33) and KAUKHMWE (100).

4.1.1.3 Days to maturity

Days to maturity varied from 106 to 141days with a mean value of 117.37. The highest days to maturity was observed in genotype SWARNA (141.33) followed by GOPAL (123.67), NDR359 (123.67) and IR 119 (123.33) while, lowest in DZ 78 (106.67) followed by XINUO ZAO (108.33), WANNI DAHANALA (109.33) and E ZI 124(109.33). The average mean for this trait was 117.36 days.

4.1.1.4 Plant height

Plant height ranged from 93.13 cm to 206.31 cm with a mean value of 157.19 cm. Among all genotypes SWARNA was the shortest followed by IR 64 (106.48) whereas, GOPAL (206.31) was having highest plant height followed by KAUKHMWE (196.19) and NP 252 (185.11).

4.1.1.5 Number of tillers per plant

Total no. of tillers per plant varied from 4 to 11 with general mean 6.35. SWARNA (11.67) showed maximum tillers followed by IR 64 (9.67), IR 119 (7.67) and RTS 4 (7.33) minimum tillers was showed by DZ 78 (4.33) followed by TCHAMPA (5), NS 252 (5), GOPAL (5) and EZI 124 (5).

4.1.1.6 Number of effective tillers per plant

The mean values of genotypes ranged from 3 to 8 with a general mean of 4.95. The highest number of effective tillers per plant was observed in genotype IR 64(8.33) followed by SWARNA (7.33), IR -119 (6.33) and VELLAI SEENETTI (6.67) whereas lowest value was registered in three genotypes i.e. DZ 78(3.33), TCHAMPA (3.33) and NS 252 (3.33).

4.1.1.7 Panicle length (cm)

Panicle length ranged from 23.3 cm to 30.06 cm with a mean value of 27.01 cm. The longest panicle length was observed in NP 125 (30.07) followed by TCHAMPA (30.07), NS 252 (29.67) while the shortest panicle was found in genotype VELLAI SEENETTI (23.3) followed by SWARNA (24.30) and XI NUO ZAO (24.93).

4.1.1.8 Number of Spikelets per panicle

The mean values of genotypes ranged from 105 to 186 with a general mean of 141.47. The highest number of spikelets per panicle was observed in genotype NDR 359 (186) followed by WAR 72-2-1-1 (180) and RTS 4 (169.67) whereas lowest value was registered in three genotypes E KHA KEHA (105.67), E ZI 124 (110.33) and NP 125 (121.33)

4.1.1.9 Number of grains per panicle

Number of grains per panicle ranged from 84.33to 158.33. The highest number of grains per panicle was recorded in RTS 4 (158) and second highest was in NDR 359 (local check) (144) followed by B 6149 F-MR-7 (138), lowest value was

found in E ZI 124 (84.33) followed by E KHA KEHA (90) and IR-64 (90.66). The average number of filled grains per panicle was observed in 116.95.

4.1.1.10 Number of Sterile Spikelets per panicle

Number of Sterile Spikelets per panicle was ranged from 11.33 to 84.66. The less number of Sterile Spikelets per panicle was observed in genotype RTS 4 (11.33) followed by KAUKHMWE (13.33) and GOPAL (14.33) while high in WAR 72-2-1-1 (84.66) followed by Local check (NDR 359) (41.67) and NS 252 (38).

4.1.1.11 Spikelets fertility%

Spikelets fertility % ranged from 52.92 to 93.38. The average spikelets sterility was observed was 84.79. . The highest spikelets fertility % was recorded in RTS 4 (93.38) and second highest was in GOPAL (92.97) followed by KAUKHMWE (92.42) lowest value was found in WAR 72-2-1-1 (52.92) followed by IR-64 (73.03) and Local check (NDR 359) (77.06).

4.1.1.12 Grain weight per panicle (g)

Grain weight per panicle ranged from 1.74 to 3.69. The highest grain weight was observed in genotype RTS 4 (3.69) followed by NS 252 (3.51), Local check (NDR 359) (3.46) and B 6149 F-MR-7 (3.45) lowest in E ZI 124 (1.74). The mean value for this trait was 2.70.

4.1.1.13 1000-grain weight (g)

This value ranged from 18.04 to 30.26. The highest mean value for 1000 grain weight was registered in genotype NS 252 (30.26) followed by KAUKHMWE (26.10) and WAR 72-2-1-1 (26.03) whereas lowest grain weight was observed in genotype SWARNA (18.03) followed by IR-64 (20.02) and VELLAI SEENETTI (20.73). The mean of 1000-grain weight was 23.63 g.

4.1.1.14 Grain yield per plant (g)

Data for **Grain yield per plant** ranged from 4.56g to 16.17 g. Highest grain yield was observed in genotype IR -119 (16.17) followed by RTS 4 (14.31) and SWARNA (14.32) whereas lowest grain yield was observed in genotype E KHA KEHA (4.57) , WANNI DAHANALA (5.13) and E ZI 124 (5.56). The average mean for this trait was 9.39g.

4.1.1.15 Grain yield per plot (kg)

The value for grain yield per plot was ranged from 0.21 to 0.82 kg with mean value of 0.45 kg. The upper value for this trait was observed in genotype IR 119 (0.82) followed by NS 252 (0.79) and SWARNA (0.76) lower was observed in WANNI DAHANALA (0.21) followed by E KHA KEHA (0.22) and E ZI 124 (.26).

4.1.1.16 Hulling recovery percent

The range of hulling recovery per cent was observed from 62.19% to 92.51% . Highest hulling recovery percent was observed in genotype SWARNA (92.51) followed by WANNI DAHANALA (91.42), XI NUO ZAO (90.81) and IR -74371-54-1-1 (90.73) whereas lowest grain yield was observed in genotype KAUKHMWE (62.19) followed by E ZI 124 (76.23) and NS 252 (76.67). The average mean for this trait was 81.49.

4.1.1.17 Milling recovery percent

In terms of milling recovery per cent, the range was observed from 50.89% (IR -119) to 89.01% (SWARNA) with a mean value of 71.50%. Highest milling recovery was observed in genotype SWARNA (89.01) followed by IR -74371-54-1-1 (87.20), WANNI DAHANALA (85.04) and GOPAL (81.11) whereas lowest grain yield was observed in genotype IR -119 (50.89) followed by SOLOI (62.96) and IR-64 (63.61).

4.1.1.18 Kernel length (mm)

Kernel length ranged from 5.29 to 7.89 with a mean value of 6.37. The highest kernel length was observed in genotype RTS 4 (7.89) followed by SOLOI (7.14) and E ZI 124 (7.02) whereas lowest kernel length was observed in genotype E KHA KEHA (5.29) followed by XI NUO ZAO (5.61) and SWARNA (5.80).

4.1.1.19 Kernel breadth (mm)

Kernel breadth ranged from 1.89 to 2.84 with a mean value of 2.30. The highest kernel breadth was observed in genotype DZ 78 (2.84) followed by NS 252 (2.84) and WANNI DAHANALA (2.68) whereas lowest kernel breadth was found in genotype E ZI 124 (1.89) followed by RTS 4 (1.90) and IR-64 (1.93).

4.1.1.20 Kernel L/B ratio

Kernel L/B ratio ranged from 2.04 to 4.15 with a mean value of 2.85. Highest kernel L/B ratio was observed in genotype RTS 4 (4.15) followed by E ZI 124 (3.71) and SWARNA (3.63) whereas lowest kernel L/B ratio was observed in genotype DZ 78 (2.04) followed by E KHA KEHA (2.14) and WANNI DAHANALA (2.31).

4.1.1.21 Amylose content percent

Amylose content varied from 21.16 to 24.87 with a mean of 23.94. Highest amylose content was observed in genotype IR-119 (24.87) followed by WANNI DAHANALA (24.70) and NS 252 (24.69) whereas lowest amylose content was observed in genotype XI NUO ZAO (21.16) followed by GOPAL (21.35) and VELLAI SEENETTI (21.40).

4.1.1.22 Canopy temperature depression (CTD)

CTD value varied from 28.70°C to 30.23°C with a mean of 29.26°C. Highest CTD was observed in genotype GOPAL (30.23) followed by WAR 72-2-1-1 (29.77) and DZ 78 (29.67) whereas lowest CTD was observed in genotype B 6149 F-MR-7 (28.70) followed by E ZI 124 (28.73) and NP 125 (28.80).

4.1.1.23 Stomatal conductance (mmol/m²/s)

Stomatal Conductance varied from 527.16 to 988.40. the highest value for stomatal conductance was exhibited by genotype TCHAMPA and lowest by genotype B 6149 F-MR-7 with a mean of 751.40.

4.1.1.24 Chlorophyll content (SPAD value)

Chlorophyll content ranged from 38.60 to 46.76. Highest chlorophyll content was observed in genotype Danteshwari (46.76) followed by WANNI DAHANALA (45.03) and SWARNA (43.93) whereas lowest grain yield was observed in genotype WAR-72-2-1-1 (38.60) followed by XI NUO ZAO (40.67) and TCHAMPA (41.07). The average mean for this trait was 42.71.0

4.1.1.25 Proline content (µmol/g fresh weight)

Proline content ranged from 9.05 to 25.61. The highest proline content was registered in genotypes SWARNA (25.61) followed by Local check (NDR 359)

(22.17) and GOPAL (21.28) lowest proline content was found in genotype TCHAMPA (9.05) followed by NS 252 (10.83) and NP 125 (11.83). The average mean for this trait was 17.33.

4.1.1.26 Biomass (kg/ha)

The value for biomass varied from 1.14 to 1.90. The highest biomass was registered in genotype IR 119 and lowest was E KHA KEHA. The mean for this trait was value with 15.62.

4.1.1.27 Harvest index %

The value for HI was ranged from 16.76 to 45.03. The highest harvest index was registered in genotype IR 119 and lowest was E KHA KEHA. The mean for this trait was value with 28.52.

4.1 Genetic Variability and Heritability and Genetic advance parameters

The results pertaining to phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic advance expressed as percent of mean for all the characters under study are presented in Table 4.1.

4.1.1 Phenotypic and Genotypic coefficient of variation

Table 4.1 revealed considerable variation over the traits under study exhibiting a wide range of phenotypic as well as genotypic coefficient of variation. In general, the values of phenotypic coefficient of variance were higher than those of genotypic coefficient of variance. The relative magnitudes of the phenotypic as well as genotypic variances between the traits were compared based on the phenotypic and genotypic coefficient of variation. PCV was recorded highest for sterile spikelets per panicle (67.48) followed by grains yield per plot (43.24) and grain yield per plant (38.31). Low magnitude of PCV was exhibited by canopy temperature depression (1.98) followed by chlorophyll content (5.32) and amylose content (5.37). Rest other traits exhibited medium values of PCV.

Similarly, GCV was also high for sterile spikelets per panicle (65.94) followed by grains yield per plot (42.22) and grain yield per plant (38.21). Whereas, low

magnitude of GCV was exhibited by canopy temperature depression (.96) followed by chlorophyll content (3.2) and days to maturity (7.03). The differences between the values of PCV and GCV were small for almost all the traits indicating less influence of environment in expression of these traits. However, the differences was comparatively greater in case of stomatal conductance (5.29) followed by effective tillers per plant (4.42) and tillers per plant (2.44).

4.1.2 Heritability

In the present study, heritability (broad sense) ranged from 36% to 99%. The highest heritability was found in days to maturity, plant height , grain weight per panicle, grain yield per panicle, grain yield per plant, kernel breadth, kernel L/B ratio, proline content (99%) followed by days to 50% flowering, days to first flowering, panicle length (98%) and 1000grain weight, kernel length (97%). Lowest heritability was observed in canopy temperature depression (24%) followed by chlorophyll content (36%) and stomatal conductance (53%). Other traits showed intermediate heritability.

4.1.3 Genetic advance

Genetic advance as percent of mean (5%) was realized highest for sterile spikelets per panicle (132.75) followed by grain yield per plot (84.92) and grain yield per plant (78.49). Lowest value was observed in canopy temperature depression (.97) followed by chlorophyll content (3.97) and amylose content (10.11).

4.2 Genetic Divergence

χ^2 test applied to 'V' statistic indicated significant difference between the means in respect of pooled effect of 27 traits under study and between different populations. Hence, further analysis was carried out for estimating D^2 values to study genetic divergence. D^2 statistics is a numerical approach for measuring genetic divergence in the genotype collections. The composition of different clusters obtained from the D^2 analysis has been presented in the Table 4.2. Twenty rice genotypes were grouped into five different clusters based on the *inter-cluster* genetic distances. This indicated presence of considerably diverse rice genotype in the set of material under study.

Detail insight into the diversity is therefore, important in order to select the desirable genotypes to be utilized in the breeding programs.

The genotypes were grouped into five clusters. Clustering pattern indicated that 13 out of 20 genotypes belong to the same cluster i.e. cluster I. On the other hand, four genotypes belongs to cluster II and III, IV consists of one genotype each. D^2 values among five clusters have been presented in the Table 4.3 and intra and inter-cluster distances i.e. D-values have been presented in the Table 4.4. The diagrammatic representation of clusters with intra and inter cluster D values has been presented in figure 4.1. The highest intra-cluster distance was observed in the cluster I (41.42) which comprised of 13 genotypes. The highest inter-cluster distance (105.62) was found between cluster II and V followed by cluster I and V (79.20) and cluster III and V (70.08). The smallest inter-cluster distance (46.61) was observed between I and III followed by cluster III and IV (49.27) and between cluster II and IV (54.99).

Mean values for different traits for different clusters have been presented in Table 4.5. The cluster V was comprised with the highest numbers of traits for their extreme mean values viz. days to first flowering (108.33), days to 50% flowering (112.33), days to maturity (141.33), tillers/ plant (11.67), spikelets/ panicle (150.67), grains/ panicle (127.33), grain yield/ plant (14.32), hulling recovery (92.51), milling recovery (89.01), kernel l/b ratio (3.63), chlorophyll content (43.93), proline content (25.61), grain yield/ plot (0.76), biomass (1.69), harvest index (45.03) and lowest mean value of plant height (93.13), panicle length (24.30) and 1000- grain weight (18.04). Cluster IV was having the highest mean values of traits like effective tillers/ plant (8.33), sterile spikelets/ panicle (33.33), kernel length (672), canopy temperature (29.57) and lowest mean value for traits viz. spikelet fertility (73.03),milling recovery (63.61),kernel breadth (1.93) and amylose content stomatal conductance (22.53). The traits with their highest mean values spikelet fertility (88.44), kernel breadth (2.47) and amylose content (24.50) were felled in cluster III which had lowest mean value of effective tillers/ plant (4.33), spikelets/ panicle (105.67), grains/ panicle (90.00), sterile spikelets/ panicle (15.67), grain weight/ panicle (1.87), grain yield/ plant (4.57), kernel length (5.30), kernel l/b ratio (2.14), grain yield/ plot (0.22), biomass (1.15) and harvest index (19.40) . Cluster II showed extreme mean values of plant

height (165.67), panicle length (27.83), stomatal conductance (868.18) and lower mean value of days to first flowering (83.83), days to 50% flowering (88.58), days to maturity (111.75), Tillers/ Plant (5.50), hulling recovery (75.36), chlorophyll content (42.34) and proline content (14.50). Only two traits i.e., grain weight/ panicle (2.92) and 1000- grain weight (24.71) were felled in cluster I with highest mean value and lowest mean value of canopy temperature (29.22).

4.2.1 Relative contribution of characters towards diversity

The relative contribution of twenty seven characters towards diversity is presented in Table 4.6 and fig. 4.

The characters appeared in first rank more it contributed towards diversity. Among all the characters, Biomass (kg/ha) contributed the maximum (31.05%) to the diversity by taking first rank in 59 times, followed by Grain Yield/ Plant (g) (16.84% with 32 times ranked first), Harvest Index (11.58% with 22 times ranked first), Grain Yield/ Plot(kg) (8.95 with 17) and Kernel Breadth (mm) (7.89% with 15 times ranked first).

Other characters viz., Milling Recovery%, Amylose Content, Kernel L/B Ratio, Canopy Temperature Depres, contributed 2.11, 1.58, 1.05, and 0.53 percent, respectively to the genetic divergence in decreasing order. On contrary rest of the traits have very little contribution towards genetic divergence.

4.3.2 Molecular diversity using SSR primers

4.3.3 Polymorphism and marker efficiency

Twenty rice genotypes were subjected to SSR marker assay to assess the molecular diversity. Out of 10 primers used, 10 produced reproducible and polymorphic pattern. The 10 polymorphic primers yielded a total of 33 fragments (amplified products). Maximum fragments were produced by primers RM152 (six fragments), RM408 and RM25 which yielded four fragments each and an average of 3.3 fragments was produced per primer which showed polymorphic amplification. Gel images showing SSR banding profile obtained by primers RM259, RM154, RM283,

RM431, RM316, RM105, RM215, RM215, RM152, RM25 and RM408 are presented in figure.

The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability. The PIC value is an evidence of allele diversity and frequency among varieties. The PIC value of SSR markers ranged from 0.346 to 0.775 with a mean PIC of 0.498. Markers RM152, RM408, RM259 and RM25 were the most informative primers on the basis of highest PIC of 0.775, 0.667, 0.643 and 0.602 respectively. SSR marker RM215, RM316, RM105 and RM154 showed least PIC value of 0.346, 0.346, 0.375 and 0.375 respectively (Table 4.7).

4.3.4 Dendrogram analysis:

A dendrogram (figure 3) based on Jackard's dissimilarity coefficient was constructed using UPGMA. The genetic divergence was studied based on D^2 statistics. The twenty rice genotypes fall in a major cluster i.e., cluster I. Cluster I was further sub-divided into two sub groups IA and IB. Sub group IA was further divided in sub cluster IA-1 and 1A-2, Cluster IB was subdivided into clusters IB-1 and 1B-2. The sub cluster IA-2 was further partitioned into IA-2a and IA-2b.

On the basis of dendrogram, the highest similarity was observed between E KHA KEHA and B-6149FMR-7 with dissimilarity coefficient of 0.20 followed by DZ 78 and SOLOI (0.429), NP 252 and TCHAMPA (0.50), TCHAMPA and VELLAISEENETTI (0.50), IR-64 and IR-119 (0.875), KAUKHMWE and TCHAMPA (0.50). The most diverse cultivars having dissimilarity coefficient of 1.00 were SWARNA and XINUOZAO (1.00), SWARNA and DZ 78 (1.00), VELLAISEENETTI and XINUOZAO (1.00), XINUOZAO and SOLOI (1.00), Local Check NDR359 and WANNIADAHANALA (1.00). Apart from these, most of the genotypes were showing diversity which are presented in Table 4.8. The genotypes were grouped into five sub-clusters. Cluster indicated that six genotypes (IR-119, KAUKHMWE, DZ 78, SOLOI, XI NUO ZAO, WANNIADAHANALA), out of twenty belong to the cluster IA-2a followed by cluster IA-1 which has five genotypes (NP 125, VELLAISEENETTI, NP252 and SWARNA) and cluster IB-1

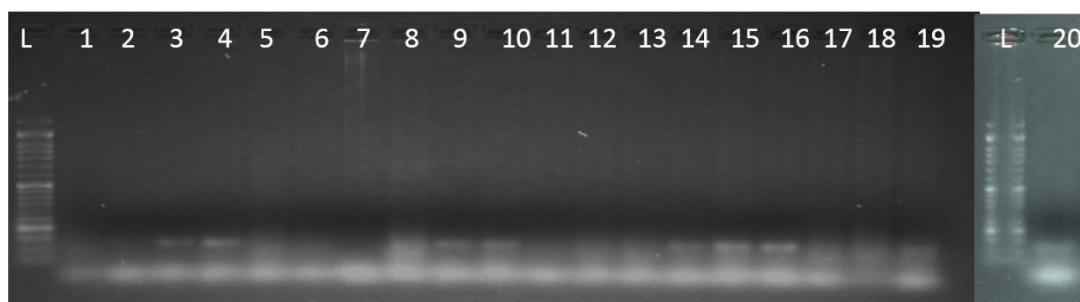
with five genotypes (IR-64, GOPAL, WAR 72-2-1-1, IR -74371-54-1-1 and E ZI 124). Clusters IB-2 comprises of three genotypes (B 6149 F-MR-7, E KHA KEHA and Local check (NDR 359), and cluster IA-2b have only one genotypes i.e. RTS 4.

4.3.5 Jackard dissimilarity coefficient

The dissimilarity coefficient varies from one to zero, close to one shows high dissimilarity while close to zero shows high similarity. The dissimilarity coefficient varied from the largest value 1.00 between the most of the cultivar like SWARNA and XINUOZAO, SWARNA and DZ 78, VELLAISEENETTI and XINUOZAO, XINUOZAO and SOLOI, Local Check NDR359 and WANNIADAHANALA which are furnished in Table 4.8. which showing that they are highly divers from each other. The lowest value 0.20 was observed for E KHA KEHA and B-6149FMR-7 followed by DZ 78 and SOLOI, NP 252 and TCHAMPA, TCHAMPA and VELLAISEENETTI, IR-64 and IR-119, KAUKHMWE and TCHAMPA shows high similarity between them and it may be expected that both of them may have arouse from the same parents.

Figure 4.1: Banding pattern obtained using four SSR primers between 20 genotypes .

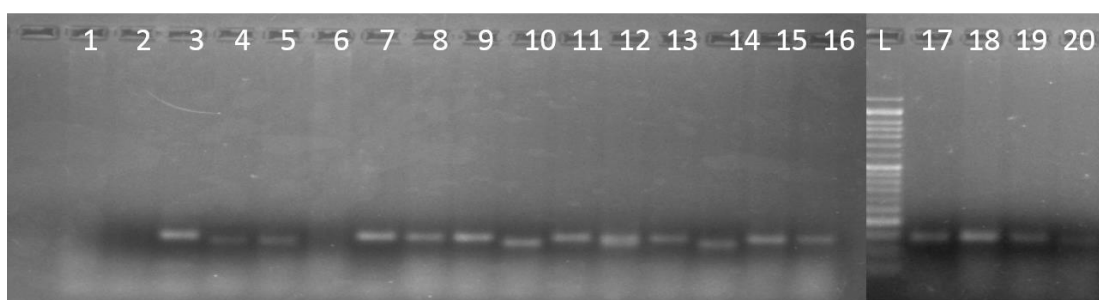
PRIMER-RM408



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.2: Banding pattern obtained using four SSR primers between 20 genotypes .

PRIMER-RM25



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.3: Banding pattern obtained using four SSR primers between 20 genotypes .

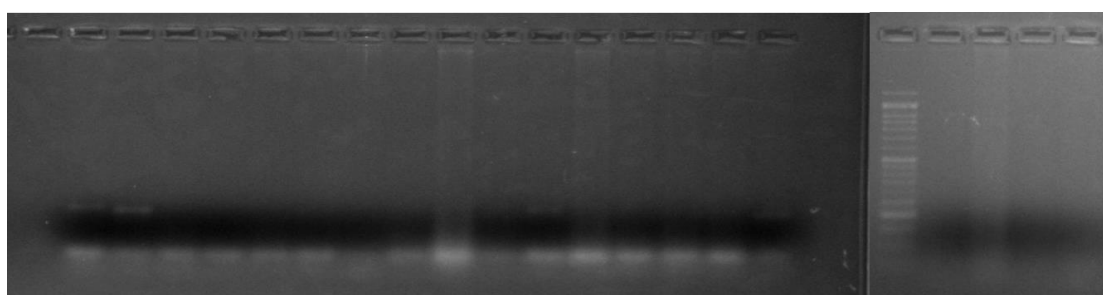
PRIMER-RM316



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.4: Banding pattern obtained using four SSR primers between 20 genotypes .

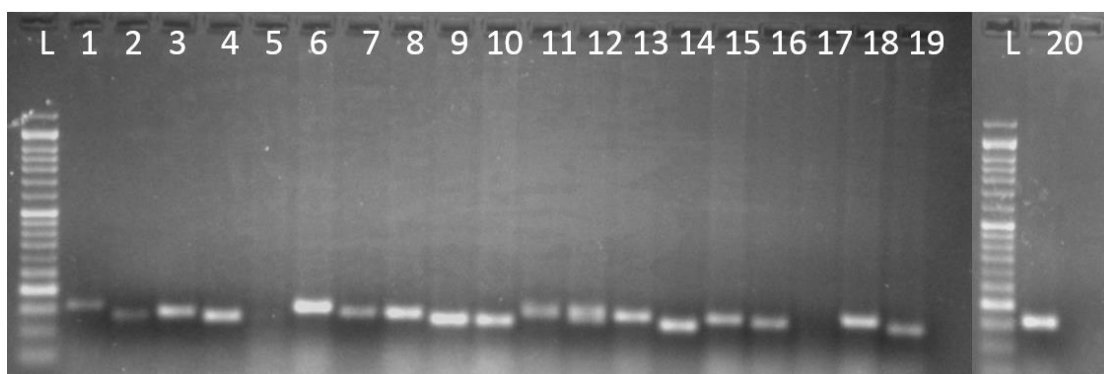
PRIMER-RM154



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.5: Banding pattern obtained using four SSR primers between 20 genotypes .

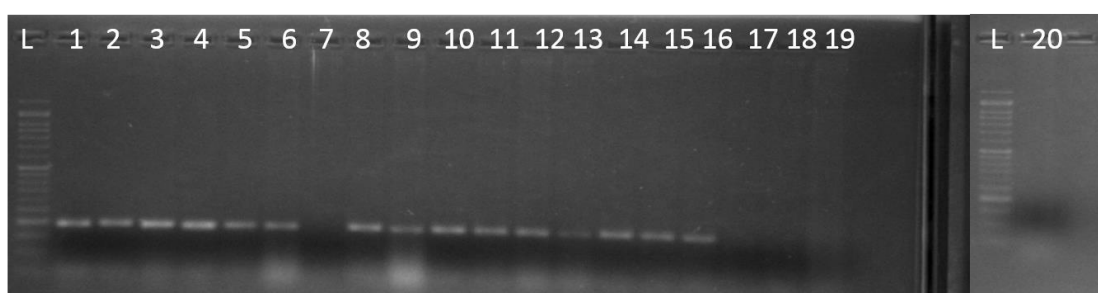
PRIMER-RM152



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64,13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.6: Banding pattern obtained using four SSR primers between 20 genotypes .

PRIMER-RM259



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64,13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.7: Banding pattern obtained using four SSR primers between 20 genotypes .

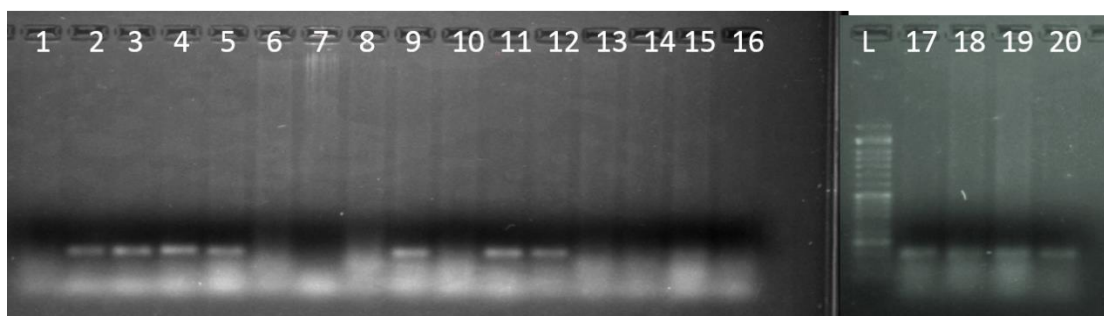
PRIMER-RM431



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.8: Banding pattern obtained using four SSR primers between 20 genotypes .

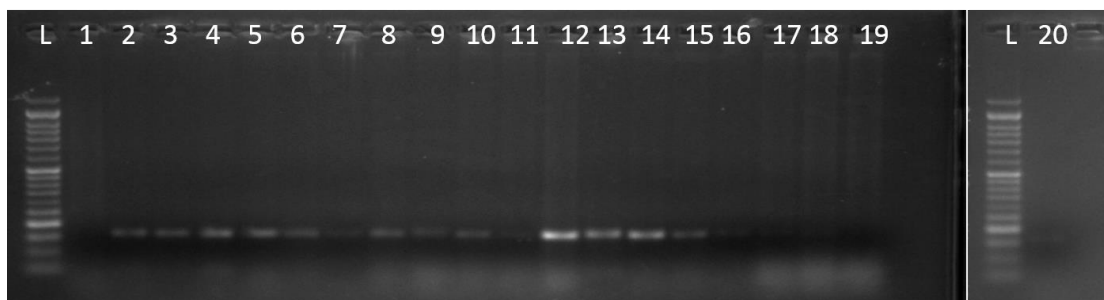
PRIMER-RM215



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.9: Banding pattern obtained using four SSR primers between 20 genotypes .

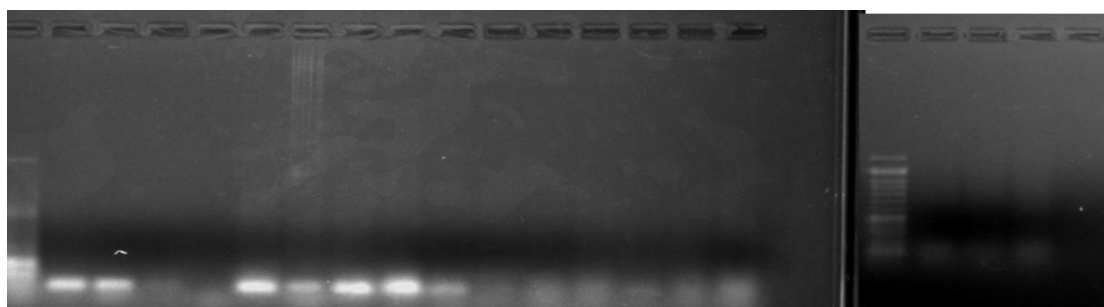
PRIMER-RM283



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Figure 4.10: Banding pattern obtained using four SSR primers between 20 genotypes .

PRIMER-RM105



L-MARKER (100bp) 1-SWARNA,2- NP 125,3- NP-252,4- TCHAMPA,5- VELLAISEENETTI,6- IR-119, 7-XINUOZAO,8- KAUKHMWE,9- DZ 78, 10- SOLOI,11- WANNIADAHANALA,12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7,16- E KHA KEHA,17- WAR 72-2-1-1,18- IR-74371-54-1-1, 19- E ZI 124,20-RTS-4.

Table: 4.1 ANOVA of 20 rice genotypes for twenty seven yield and yield attributing trait

	Days to First Flowering	Days to 50% Flowering	Days to Maturity	Plant Height cm	Tillers/Plant	Effective Tillers/Plant	Panicle Length (cm)	Spikelets/Panicle	Grains/Panicle	Sterile Spikelets/Panicle	Spikelet Fertility %	Grain Weight/Panicle (g)	1000-Grain Yield (g)	Grain Yield/Plant (g)	Hulling Recovery %	Milling Recovery%	Kernel Length (mm)	Kernel Breadth (mm)	Kernel/LB Ratio	Amlyose Content	Canopy Temperature Depression	Stomatal Conductance (mmolM ⁻¹ S)	Chlorophyll Content (spad Value)	Proline Content (µmol/g Fresh Weight)	Grain Yield (Plot/kg)	Grain Biomass (kg/ha)	Harvest Index
GCV	9.08	8.46	7.03	18.11	26.60	25.67	7.86	15.03	16.26	65.94	10.79	21.26	10.91	38.21	9.25	12.55	9.41	12.75	19.88	5.13	0.96	14.03	3.20	22.92	42.22	13.49	34.32
PCV	9.19	8.53	7.08	18.23	29.03	30.09	7.95	15.42	16.56	67.48	11.01	21.38	11.06	38.31	9.51	13.04	9.55	12.81	20.00	5.37	1.98	19.33	5.32	23.02	43.24	14.52	34.95
r ² (Broad Sense)	0.98	0.98	0.99	0.99	0.94	0.73	0.98	0.95	0.96	0.96	0.96	0.99	0.97	0.99	0.96	0.93	0.97	0.99	0.99	0.91	0.24	0.53	0.36	0.99	0.95	0.86	0.96
Gen. Adv as % of Mean 5%	18.48	17.28	14.39	37.05	50.19	45.12	16.02	30.16	32.90	132.75	21.80	43.56	22.16	78.49	18.53	24.88	19.06	26.15	40.69	10.11	0.97	20.99	3.97	47.01	84.92	25.81	69.42
General Mean	89.63	94.55	117.37	157.19	6.35	4.95	27.01	141.53	116.95	24.55	84.80	2.70	23.63	9.39	81.49	71.51	6.37	2.31	2.86	23.95	29.27	751.41	42.71	17.34	0.46	1.56	28.53
Exp Mean next Generation	106.20	110.89	134.26	215.43	9.54	7.18	31.34	184.22	156.42	57.14	103.28	3.88	28.87	16.76	96.59	89.30	7.59	2.91	4.02	26.37	29.55	909.14	44.41	25.69	0.84	1.97	48.33
Range Lowest	79.33	84.00	106.67	93.13	4.33	3.33	23.30	105.67	84.33	11.33	52.93	1.74	18.04	4.57	62.20	50.89	5.29	1.89	2.04	21.16	28.70	527.17	38.60	9.06	0.21	1.15	16.77
Range Highest	108.33	112.33	141.33	206.31	11.67	8.33	30.07	186.00	158.33	84.67	93.38	3.69	30.26	16.17	92.51	89.01	7.89	2.84	4.15	24.88	30.24	988.40	46.77	25.81	0.82	1.90	45.03

Table 4.2 Grouping of 20 rice germplasm into five clusters (by Tocher method)

Clusters	Germplasm	Number
I	B 6149 F-MR-7, Local check (NDR 359), DZ 78, GOPAL, IR -74371-54-1-1, WANNI DAHANALA, NP 125	7
II	SOLOI, TCHAMPA, XI NUO ZAO, E ZI 124, VELLAI SEENETTI, KAUKHMWE, IR -119 E KHA KEHA, RTS 4, WAR 72-2-1-1	10
III	NS 252	1
IV	IR-64	1
V	SWARNA	1

Table 4.3 : Inter and intra cluster distance of D² value of 20 rice genotype

	I	II	III	IV	V
I	1715.825	3507.736	2172.594	3052.358	6273.167
II		1674.107	3431.723	3023.518	11155.591
III			0.000	2427.430	5195.264
IV				0.000	4670.376
V					0.000

Table 4.4 : Inter and intra cluster distance of D value of 20 rice genotype

	I	II	III	IV	V
I	41.42	59.23	46.61	55.25	79.20
II		40.92	58.58	54.99	105.62
III			0.000	49.27	70.08
IV				0.000	68.34
V					0.000

Table 4.5 : Cluster mean of 20 rice genotypes

	Days to First Flowerin g	Days to 50% Flowerin g	Days to Maturit y	Plant Height cm	Tillers / Plant	Effectiv e Tillers/ Plant	Panicle Lengt h (cm)	Spikelet s/ Panicle	Grains / Panicle	Sterile s/ Panicle	Spikele t Fertilit y %	Grain Weight / Panicle e (g)	1000- Grain Weight Plant (g)	Grain Yield/ Recover y %	Hulling Recover y %	Milling Recovery %	Kerne l Lengt h (mm)	Kernel Breadt h (mm)	Kerne l/LB Ratio	Amylos e Content	Canopy Temperatur e Depression	Stomatal Conductanc e (mmol/m ² /s)	Chlorophy ll Content (spad Value)	Proline Conten t (µmol/ g Fresh Weight)	Grain Yield/ Plot(kg)	Biomass (kg/ha)	Harves t Index
1 Cluster	89.462	94.462	117.00 0	163.15 2	6.026	4.667	27.226	147.718	121.15 4	26.487	84.624	2.917	24.711	9.860	82.411	72.252	6.432	2.399	2.729	24.082	29.221	718.610	42.710	17.398	0.469	1.607	28.779
2 Cluster	83.633	88.583	111.75 0	165.67 5	5.500	4.583	27.483	132.500	114.00 0	18.583	87.444	2.428	22.742	6.952	75.363	66.783	6.509	2.129	3.099	23.806	29.237	868.175	42.342	14.499	0.347	1.487	23.008
3 Cluster	96.000	101.000	123.33 3	160.46 7	5.333	4.333	25.667	105.667	90.000	15.667	88.437	1.867	22.367	4.567	79.730	71.090	5.290	2.473	2.140	24.503	29.550	742.567	42.533	20.407	0.223	1.147	19.400
4 Cluster	90.000	95.333	114.66 7	106.47 7	9.667	8.333	26.433	124.000	90.667	33.333	73.033	1.877	20.017	13.08 3	84.763	63.610	6.723	1.927	3.490	22.533	29.567	716.000	43.167	16.573	0.628	1.570	39.967
5 Cluster	108.333	112.333	141.33 3	93.133	11.66 7	7.333	24.300	150.667	127.33 3	23.333	84.543	2.630	18.037	14.32 0	92.510	89.013	5.800	2.017	3.630	23.593	29.400	754.933	43.933	25.613	0.762	1.693	45.033

Table 4.6: Relative contribution of 27 characters to genetic diversity in 20 genotypes of rice

Source	Contribution %	Times Ranked 1st %
1 Days to First Flowering	0.01	0.000
2 Days to 50% Flowering	0.01	0.000
3 Days to Maturity	0.01	0.000
4 Plant Height cm	0.01	0.000
5 Tillers/ Plant	0.01	0.000
6 Effective Tillers/ Plant	0.01	0.000
7 Panicle Length (cm)	0.01	0.000
8 Spikelets/ Panicle	0.01	0.000
9 Grains/ Panicle	0.01	0.000
10 Sterile Spikelets/ Panicle	0.01	0.000
11 Spikelet Fertility %	0.53	1.000
12 Grain Weight/ Panicle (g)	0.53	1.000
13 1000- Grain Weight (g)	0.01	0.000
14 Grain Yield/ Plant (g)	16.84	32.000
15 Hulling Recovery %	8.95	17.000
16 Milling Recovery%	2.11	4.000
17 Kernel Length (mm)	0.53	1.000
18 Kernel Breadth (mm)	7.89	15.000
19 Kernel LB Ratio	1.05	2.000
20 Amylose Content	1.58	3.000
21 Canopy Temperature Depres	0.53	1.000
22 Stomatal Conductance (mmo	0.01	0.000
23 Chlorophyll Content (spad	0.01	0.000
24 Proline Content (µmol/g F	7.89	15.000
25 Grain Yield/ Plot(kg)	8.95	17.000
26 Biomass (kg/ha)	31.05	59.000
27 Harvest Index	11.58	22.000

Table 4.7: Pic value of 10 polymorphic markers

Sl.No.	Locus	Allels	PIC
1	RM152	6.000	0.775
2	RM 408	4.000	0.667
3	RM25	4.000	0.547
4	RM259	3.000	0.567
5	RM 431	3.000	0.535
6	RM283	3.000	0.449
7	RM154	3.000	0.375
8	RM 316	3.000	0.346
9	RM105	2.000	0.375
10	RM215	2.000	0.346

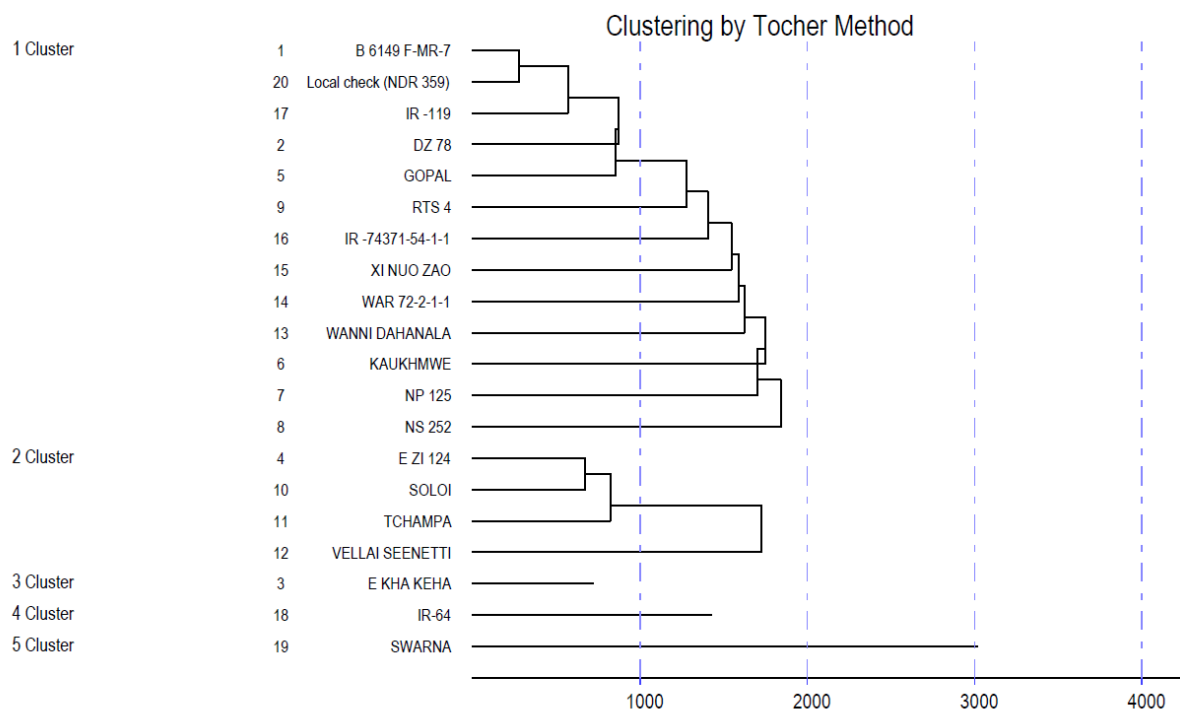
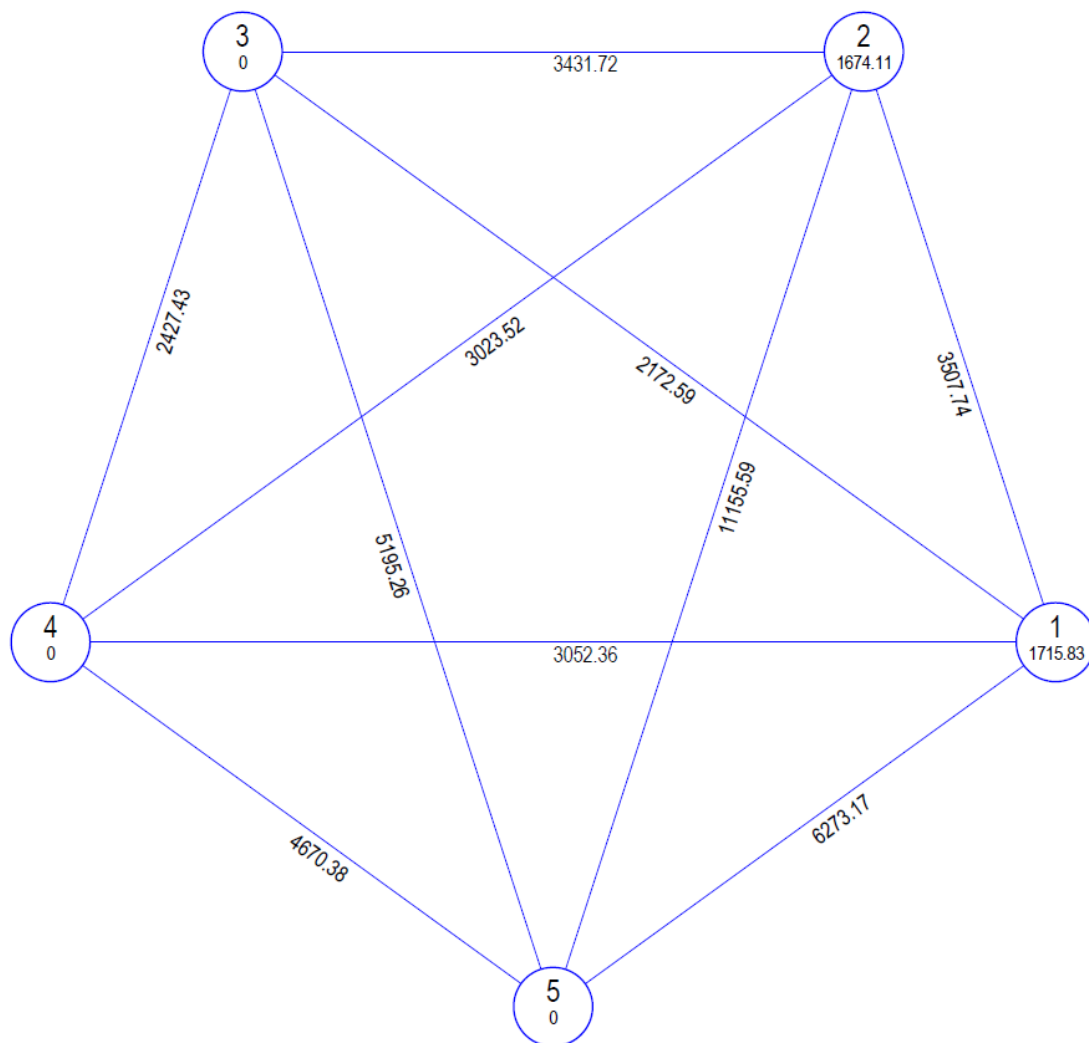


Fig. 1 : Clustering of 20 rice genotype

Tocher Method



Mahalanobis Euclidean Distance (Not to the Scale)

Fig. 2 : Cluster by tocher method

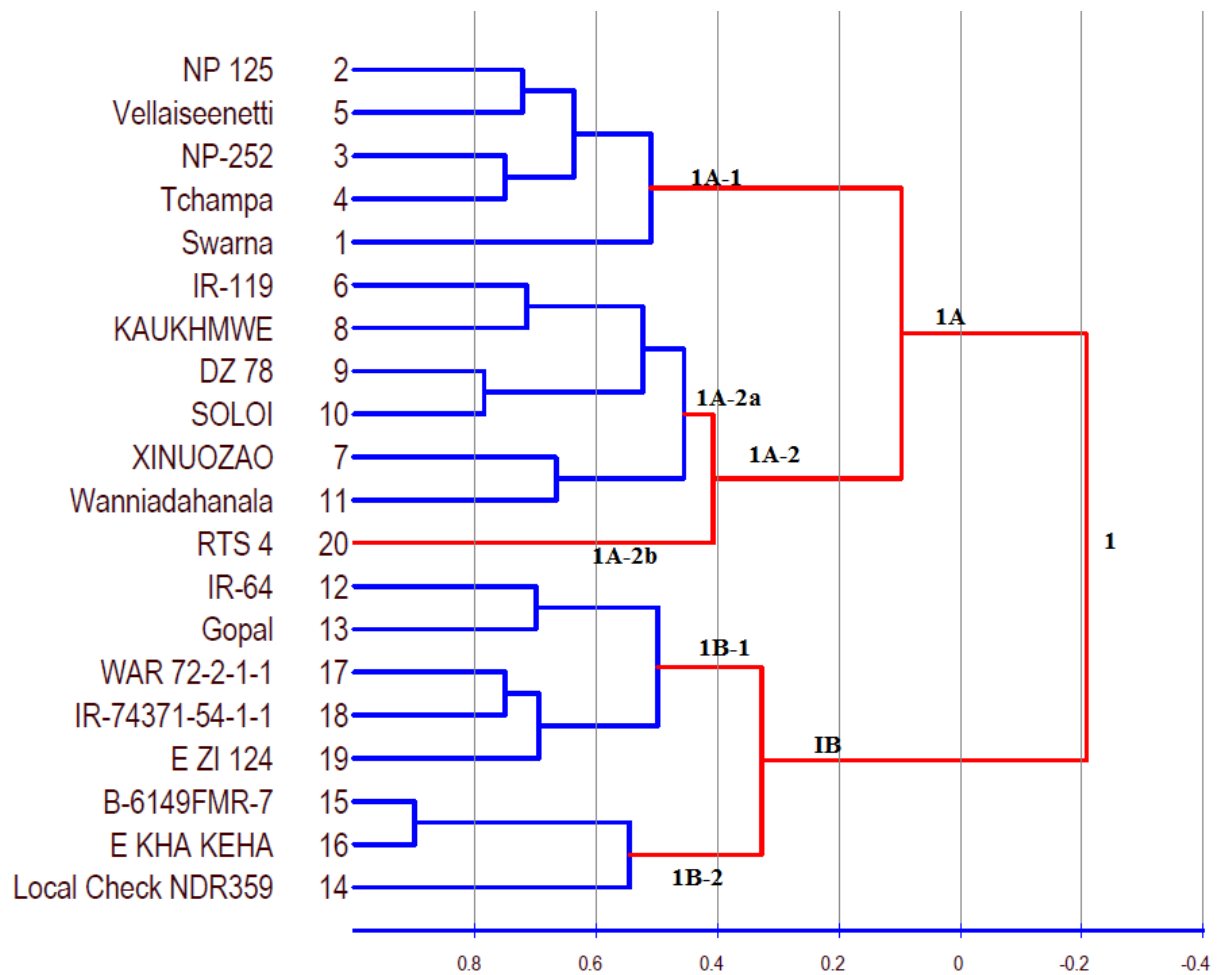


Fig 3. Dendrogram

Jackard IJ Distance

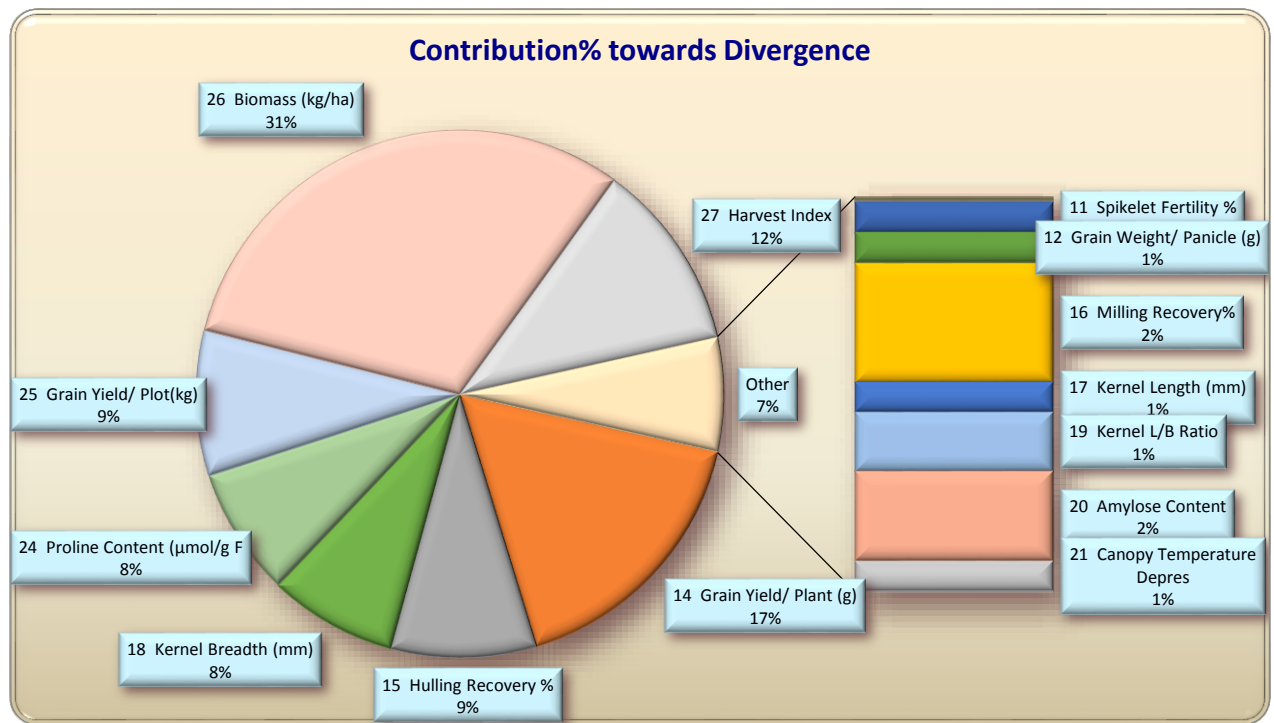


Fig 4 : Character contribution of 27 traits of 20 genotypes of rice

DISCUSSION

Information regarding genetic variation for drought attributes their heritability and genetic advance coupled with that of association of different component traits among themselves and with grain yield are of immense help to breeder for selection of parents in hybridization programme. Likewise study encompassing different genotypes may lead to identification of best crosses with wide genetic divergence which can be utilized in further breeding programme. In present study, an attempt has been made to extract information on genetic variation and divergence.

Assessing diversity in a crop is a prerequisite for improvement through breeding programme. Phenotypic variation does not directly indicate its usefulness for selection in order to obtain genetic improvement unless the genetic fraction of variation is known. Therefore, it is important to partition out the genotypic component of total variation to arrive at reliable conclusion about the exploitable (genetic) variability in a set of genotypes. The basic objective of most of the crop improvement programmes is to realize a marked improvement in crop yield. Yield as such is a complex trait influenced by various traits. Thus, information on association of yield attributes and their direct and indirect effects on grain yield are of paramount significance.

Information on the genetic distance among genotypes plays a vital role in hybridization programme. More diverse the parents, more is the expected amount of variability generated in the segregating generations. In the present investigation genetic divergence was estimated by employing Mahalanobis' D^2 -statistics to examine the diversity for drought donor including checks among different genotype.

The information on genetic diversity which helps in choosing parents for generation of new varieties needs continuous evaluation of genotype for useful

characters. Breeding strategies are chiefly influenced by the genotype. Wrong choice of genotype to initiate the selection process results not only in wastage of resources and labor but also the time of breeder. It is well known that all breeding programme involves selection at one stage or the other. The results derived from analysis of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially grown varieties. SSRs were chosen for the analysis of genetic diversity of rice cultivars because several works have showed that these markers are very powerful for differentiating individual genotype accessions, particularly when they are closely related (Bligh *et al.* 1999; Xu *et al.* 2004; Jeunget *et al.* 2005). Additionally, SSRs show a series of advantages when compared with other DNA-based markers, such as abundance in the genome, high level of polymorphism, repeatability, co-dominance and cost-effectiveness (Ni *et al.* 2002).

The results obtained from the present investigation of twenty genotype with respect to twenty seven traits have been discussed here in the light of observations made by earlier rice workers in related aspects under the following heads:

Analysis of variance

The studies of variance among 20 genotypes for twenty seven characters revealed that the genotypes differed significantly for all the characters which suggested that the materials selected for the studies might be of diverse origin. Several workers have reported the presence of variability in and amongst the genotypes of rice for different traits. These findings are in accordance with the findings of Shobha *et al.* (2001), Yadav (2002), Vivek *et al.* (2005), Rahman (2012) and Paikhomba *et al.* (2014). The early flowering and maturity was seen in three genotypes namely XI NUO ZAO, WANNI DAHANALA and SOLO I while genotype SWARNA exhibited late flowering and maturity suggesting that drought donars are short duration genotypes like XI NUO ZAO, WANNI DAHANALA and SOLO I so these genotype can be used as a drought donor for evolving early maturity or short duration rice. Several workers Viraktamath (1987) also reported positive as well as negative value for earliness in rice genotypes. The negative significant value for plant height is

desirable because dwarf plant stature is essential to develop semi-dwarf high yielding hybrid which is believed to be lodging resistant. The highest mean performance for plant height was observed in genotype GOPAL and lowest in SWARNA. Similar observations for plant height were also reported by Mitra (1962), Srivastava and Seshu (1982), Tseng and Huang (1987), Young and Virmani (1990), Khoyumthem *et al.* (2005), Gawas *et al.* (2007), Sharma *et al.* (2013) and Singh *et al.* (2013) in rice. Higher number of tillers per plant contributes to higher grain yield. Genotype SWARNA had highest mean performance for total no. of tillers but highest effective tillers in genotype IR64 and lowest mean performance for total no. of tillers in genotype DZ78 and effective tillers in genotypes DZ 78, TCHAMPA NS 252. NP125 had highest mean performance for panicle length and lowest in genotype VELLAI SEENETTI. Number of spikelets and number of grain per panicle is one of the important traits which contribute directly to the yield enhancement. highest mean performance for number of spikelets per panicle in genotype NDR 359 and number of grain per panicle in genotypes RTS 4 followed by NDR359. Number of Sterile Spikelets per panicle were less in genotype RTS 4 while high in WAR 72-2-1-1. Highest Spikelets fertility % was observed in genotype RTS 4. The grain weight per panicle is one of the important yield related traits. Genotype RTS4 revealed highest mean performance for grain weight. The highest 1000 grain weight was registered in genotype NS 252. Grain yield per plant in positive direction is desirable as higher grain yield is the main objective for almost all the breeding programmes. Highest grain yield per plant was observed in genotype IR -74371-54-1-1 whereas lowest grain yield was observed in genotype E KHA KEHA. The highest mean performance for grain yield per plot was observed in genotype IR 119 and lowest in WANNI DAHANALA. Adequate number of fertile grains per panicle and heavy grains are important traits which should be considered in selection for high yield (Satyanarayana *et al.*, 2005). Hulling recovery and milling recovery are an important factor deciding the amount of marketable produce. Highest hulling recovery percent was observed in genotype SWARNA followed by WANNI DAHANALA, XI NUO ZAO and IR -74371-54-1-1. Many workers Roy *et al.* (2009), Priyanka *et al.* (2014), Venkanna *et al.* (2014) and Singh *et al.* (2013) have reported significant positive as well as negative heterosis for hulling recovery in rice. Highest mean performance for milling

recovery was observed in genotype SWARNA followed by IR -74371-54-1-1, WANNI DAHANALA and GOPAL whereas lowest grain yield was observed in genotype IR -119 followed by SOLOI and IR-64. Pandey and Tripathi (2006), Sarawgi *et al.* (2000), Babu *et al.* (2013), Sharma *et al.* (2013) and Singh *et al.* (2013) have also reported the similar observations for milling recovery in their genotypes. The highest biomass was registered in genotype IR 119 and lowest was E KHA KEHA, the highest harvest index was also registered for same.

Quality traits

Assessment of physico-chemical properties of grain is an important aspect in rice crop, as it is directly related to the price differentials. Kernel length, kernel breadth, kernel length breadth ratio (L/B), and amylose content are the important physical quality characters which determine the appearance and market value of the rice grain. Long slender type is more preferred by the consumers. The highest kernel length was revealed genotype RTS 4 followed by SOLOI and E ZI 124 Reddy *et al.* (1991), Vivekanandan and Giridharan (1996), Reddy *et al.* (2012), Sharma *et al.* (2013) and Singh *et al.* (2013) also reported significant positive value for kernel length.

Least value of kernel breadth enhances grain fineness. The highest kernel breadth was observed in genotype DZ 78 followed by NS 252 and WANNI DAHANALA whereas lowest kernel breadth was found in genotype E ZI 124 followed by RTS 4 and IR-64. Sarawgi *et al.* (2000), Binodh *et al.* (2006), Reddy *et al.* (2012) Sharma *et al.* (2013) and Singh *et al.* (2013) also reported similar results.

Highest kernel L/B ratio was observed in genotype RTS 4 followed by E ZI 124 and SWARNA. The studies of earlier workers, Vivekanandan and Giridharan (1996), Binodh *et al.* (2006), Reddy *et al.* (2012), Sharma *et al.* (2013) and Singh *et al.* (2013) Venkanna *et al.* (2014) also support the present findings.

The amylose content of rice is the main parameter of cooking and eating qualities (Juliano, 1972) that determines the hardness or stickiness of cooked rice. Rice with low amylose content is waxy, sticky and remains firm after cooking. Rice

varieties having 20-25% (intermediate) amylose give soft and flaky cooked rice (Dipti *et al.*, 2002). The highest mean performance was recorded for amylose content in genotype was observed in genotype IR-119 followed by WANNI DAHANALA and NS 252 whereas lowest amylose content was observed in genotype XI NUO ZAO followed by GOPAL and VELLAI SEENETTI .

Drought related traits

The positive value for Canopy temperature depression (CTD) is desirable from stress point of view. In the field, genotypes with a cooler canopy temperature under drought stress, or a higher CTD, use more of the available water in the soil to avoid excessive dehydration (Blum, 1988; Ludlow and Muchow, 1990; Reynolds *et al.*, 2007, 2009). Genotype GOPAL had highest mean performance for Canopy temperature depression (CTD) and lowest in genotype B 6149 F-MR-7 .

The lowest value for Stomatal conductance is desirable from stress point of view because, low stomatal conductance helps to maintain higher leaf water potential in the genotypes under stress due to low transpiration (Blum, 1982). The highest stomatal conductance was exhibited by genotype TCHAMPA and lowest by genotype B 6149 F-MR-7.

Chlorophyll content is one of the important traits which generally contribute directly to the yield enhancement. Genotype Danteshwari had high mean performance for chlorophyll content.

The highest amount for proline content is desirable from drought stress point of view. The highest proline content was registered in genotypes SWARNA and lowest proline content was found in genotype TCHAMPA. The significant positive value for proline content have also been reported by Gopikannan *et al.* (2013).

The present study showed that superior performance for all the characters was not expressed in a single genotype. However, different genotypes were found to be superior for various characters.

Genetic variability, heritability and genetic advance

The magnitude of genetic variability decides the effectiveness of selection. It is an established fact that greater the variability among the genotypes better is the chance for further improvement in the crop. But this variability can be utilized better if it is heritable. The heritable portion of the overall observed variation can be ascertained by studying the components of variation such as GCV, PCV, heritability and predicted genetic advance. In this study, the estimates of PCV were higher than their corresponding GCV for all the traits studied. These findings were similar to the findings of Souroushet *et al.* (2004), Singh *et al.* (2013) and Paikhomba *et al.* (2014). The highest PCV and GCV were high recorded for sterile spikelets per panicle followed by grains yield per plot and grains yield per plant indicating that these traits were under the major influence of genetic control and less variable due to environmental factors. Therefore, such traits are important for further improvement. These findings are in close agreement with the researchers Anjaneyulu *et al.* (2010) and Singh *et al.* (2013). In the present study traits such as canopy temperature depression followed by chlorophyll content, days of maturity had low estimates of PCV and GCV indicating that selection for these traits will be less effective in comparison to remaining traits. The GCV provides a measure of comparison of variability and sometimes give some indication regarding validity of traits for selection. However, it does not provide clean picture of the extent of genetic gain to be expected from selection of phenotypic traits, unless heritable fraction of variation (heritability) is known. (Burton, 1952). The difference between the values of PCV and GCV were small for almost all the traits indicating less influence of environment in expression of these traits suggesting phenotypic differences may be considered as genetic difference among genotypes for selection. However, the difference was comparatively greater in case of stomatal conductance followed by effective tillers and tiller per plant. This cautions that *per-se* performance of these traits should not be taken directly as the basis of selection other variability parameter for these traits such as heritability may also be taken into consideration.

The relative magnitude of genotypic and phenotypic variances for the traits is the broad sense heritability and it is used as analytical role in selection procedures. In

the present investigation, high heritability was recorded for most of the characters except spikelet fertility per cent and number of effective tillers. Days to 50% flowering and days to maturity exhibited highest heritability followed by panicle length and total grains per panicle. Similar results were obtained by Verma *et al.* (2000), Mahto *et al.* (2003), Aktar *et al.* (2004), Singh *et al.* (2007), Chouhan *et al.* (2014) and Lingaiah (2015) in rice genotype they studied. This indicated that selection of these traits would be more effective as compared to others.

High heritability does not always indicate high genetic gain. Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone. It is not necessary that a character showing high heritability will also exhibit high genetic advance. The breeder should be cautious in making selection based on heritability as it indicates both additive and non-additive gene action. Thus, heritability values coupled with genetic advance would be more reliable and useful in formulating selection procedure as it indicates that most likely the heritability is due to additive gene effects. In the present set of materials, high heritability coupled with high genetic advance as percent was recorded for panicle weight, total grains per panicle and filled grains per panicle indicating effectiveness of selection for the improvement of these traits while high heritability coupled with low genetic advance as percent of mean were observed for panicle length, days to maturity and days to 50% flowering which is indicative of non-additive gene action. High heritability coupled with high genetic advance may be attributed to additive gene action (Khan, 1990). The high heritability is being exhibited due to favorable influence of environment rather than genotype and selection for such traits may not be rewarding. These results are in conformity with the findings of Krishna *et al.* (2010), Singh *et al.* (2012) and Sawarkar and Senapati (2014).

Genetic diversity analysis

Assessment of diversity among the set of genotypes is generally the first step before proceeding for any breeding programme. Mahalanobis' D^2 -statistics is supposed to be sound enough to assess this. Much work has been done using D^2 -

statistics to relate genetic divergence with the geographical diversity. Earlier studies in this regard have shown that genetic diversity does not necessarily related with geographical diversity (Shivakumar *et al.* 1989; Vivekanand *et al.* 1993). However, it provides the means of finding out similar and dissimilar groups of genotype and divergence between and among such groups of genotype (Banumathy *et al.* 2010; Vennila *et al.* 2011; Ovung *et al.* 2012; Islam *et al.* 2014; Sarawagi *et al.* 2015; The force of differentiation is measured at two levels, i.e., intra and inter-cluster levels.

In the present investigation, D^2 analysis revealed the presence of considerable diversity in the set of twenty genotypes under study. The genotypes were observed to be distributed in five different clusters. The intra-cluster distance for clusters III, IV and V was observed to be zero since these contains only one genotype in each cluster. Cluster I showed the maximum intra-cluster distance with 7 genotypes followed by cluster II with 10 genotypes. Genotypes included in the same cluster indicated their close relationship among themselves as compared to genotypes in other clusters. Therefore, it could be expected that genotypes grouped together in one cluster are less divergent than those which are placed in different cluster. The maximum inter-cluster distance was observed between cluster II and V indicating that the hybridization between the most diverse genotypes would yield desirable segregates with the accumulation of favorable genes in the segregating generations. The next two diverse clusters were cluster I and V and cluster III and V. The clustering pattern indicated wide diversity between different groups of genotypes. The greater the distance between two clusters, wider is the expected genetic advance between them. Therefore, indication of the genetically diverse genotypes would help in selecting parents for hybridizing programme. However, while selecting parents for hybridization programmes their yield potential should not be overlooked (Singh *et al.* 1987).

Molecular Diversity

The level of polymorphism among rice genotypes was evaluated by calculating allelic number and PIC values for each of the ten polymorphic SSR markers. A total of 33 alleles were detected by 10 polymorphic markers across 20 rice accessions with an average of 3.3 alleles per polymorphic marker. Among the

polymorphic markers, 2 produced 2 alleles each, 2 markers have produced 4 alleles each, 5 markers have produced 3 alleles each and 1 of them produced a maximum of 6 alleles. The PIC value observed in the present investigation ranged from 0.345 to 0.775 with a mean PIC of 0.498, comparable to three previous estimates of microsatellite analysis in rice viz., 0.26 to 0.65 with an average of 0.47 (Singhet *et al.*, 2015), 0.28-0.50 with a mean of 0.45 (Umadevi *et al.*, 2014) and 0.239 to 0.765 with an average of 0.508 (Hossain *et al.*, 2012). The higher the PIC value of a locus, the higher the number of alleles detected. RM152 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.775.

The dendrogram showed the twenty rice genotypes fall in a major cluster i.e., cluster I. Cluster I was further sub-divided into two sub groups IA and IB. Sub group IA was further divided in sub cluster IA-1 and 1A-2, Cluster IB was subdivided into clusters IB-1 and 1B-2. The sub cluster IA-2 was further partitioned into IA-2a and IA-2b. In the dendrogram cluster indicated that six genotypes (IR 119, KAUKHMWE, DZ 78, SOLOI, XI NUO ZAO, WANNIADAHANALA), out of twenty belong to the cluster IA-2a followed by cluster IA-1 which has five genotypes (NP 125, Vellaiseenetti, NP 252, TCHAMPA, SWARNA), and cluster IB-1 with five genotypes (IR-64, GOPAL, WAR 72-2-1-1, IR -74371-54-1-1, E ZI 124). Clusters IB-2 comprises of three genotypes (B 6149 F-MR-7, E KHA KEHA, Local check (NDR 359), and cluster IA-2b have only one genotypes i.e. RTS 4. On the basis of dendrogram the highest similarity observed between cultivar (B-6149FMR-7 and E KHA KEHA) followed by DZ 78 and SOLOI, TCHAMPA, TCHAMPA and VELLAISEENETTI, WAR 72-2-1-1 and IR-74371-54-1-1, WAR 72-2-1-1 and E ZI 124, E KHA KEHA and IR-74371-54-1-1, NP 125 and VELLAISEENETTI. Similar result was found also by Singh *et al.* (2015) where the accessions were grouped in two major groups and 14 sub-groups. The dissimilarity coefficient was calculated by Jackard IJ distance analysis, and result showed value ranged from 0 to 1. According to this dissimilarity coefficient we can understand the dendrogram and their relatedness. So, highest diverse genotypes can be used as parents in breeding programme.

SUMMARY AND CONCLUSION

The present investigation entitled “**STUDY OF MORPHOLOGICAL AND MOLECULAR DIVERSITY IN DROUGHT TOLERANT RICE (*Oryza sativa* L.)**” was undertaken using 20 diverse rice genotypes with the objectives to assess direct selection parameters (variability, heritability and genetic advance), estimation of genetic diversity using Mahalanobis D² statistics and molecular diversity based on polymorphic SSR markers. The experiment was carried out on the project of Stress Tolerant Rice for Africa and South Asia (**STRASA**) IRRI Philippines. The experiment was carried out at the Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during the *kharif* 2015. The experiment was carried out in randomized block design with three replications. Observations were recorded on randomly selected five plants for the twenty seven grain yield and its related traits viz., 50% flowering, days to maturity, plant height, number of effective tillers per plant, number of seeds per panicle, number of tillers per plant, number of spikelets per panicle, length, kernel length, kernel breadth, grain yield per plant, grain yield per plot, grain yield per plant number of sterile spikelets per panicle, spikelets fertility%, grain weight per panicle, 1000 grain weight, hulling recovery%, milling recovery%, , kernel L/B ratio, amylose content, canopy temperature depression, stomatal conductance, chlorophyll content, proline content, biomass and harvest index%. Apart from these yield attributes, marker based analysis was also done in the laboratory to study the genetic diversity using random SSR markers.

Analysis of variance revealed the highly significant differences among the genotypes for all the characters under study. The genotypes exhibited a wide range of variability for most of the traits. This indicated that there is ample scope for selection of promising genotypes from present set of genotypes for yield improvement. On the basis of *per se* performance, genotypes viz., RTS4, SWARNA, NS252, IR-74371-54-

1-1 and IR 119 were found to be the best for yield and yield contributing traits. Therefore, these can be successfully utilized as parents in future breeding programme. Genotype MGD 1206 was earliest in flowering and maturity suggesting that this genotype can be used as a donor in hybridization programme for evolving early maturing rice variety.

The high estimates of genotypic coefficient of variation and phenotypic coefficient of variation were observed for traits viz. sterile spikelets per panicle followed by grains yield per plot and grain yield per plant. Low magnitude of GCV and PCV was exhibited by canopy temperature depression followed by chlorophyll content and amylose content, rest other traits exhibited medium values of PCV.

Further, high heritability coupled with high expected genetic advance as percent of mean was also observed for the traits viz. panicle, grain yield per plant, kernel breadth, kernel L/B ratio, proline content(99%) followed by days to 50% flowering, days to first flowering, panicle length (98%) and 1000grain weight, kernel length (97%). Lowest heritability was observed in canopy temperature depression (24%) followed by chlorophyll content (36%) and stomatal conductance (53%). Other traits showed intermediate heritability.

. D^2 analysis was carried out in order to assess the genetic divergence amongst the genotypes. Based on the *inter-se* genetic distance, 20 rice genotypes could be grouped into six clusters. Clustering pattern indicated that 13 out of 20 genotypes belong to the same cluster i.e. cluster I. On the other hand, four genotypes belongs to cluster II and III, IV consists of one genotype each, indicating their distinctness among themselves and also from other genotypes under investigation. The highest intra-cluster distance was observed in the cluster I which comprised of 13 genotypes. The highest inter-cluster distance was found between cluster II and V followed by cluster I and V and cluster III and V. The smallest inter-cluster distance was observed between I and III followed by cluster III and IV and between cluster II and IV.

To access the molecular diversity among 20 rice genotypes, ten random SSR markers were used out of which all were polymorphic. A total of 33 alleles were detected by 10 polymorphic markers across 20 rice genotypes with an average of 3.3

alleles per polymorphic marker. PIC value ranged from 0.345 to 0.775 and marker RM152 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.775. The genetic divergence study grouped 20 rice genotypes into single major clusters and five sub cluster in which cluster IA-2a had maximum six genotypes followed by cluster IA-1 and cluster IB-1. On the basis of dendrogram the highest similarity observed between cultivar E KHA KEHA and B-6149FMR-7, followed by DZ 78 and SOLOI NP 252 and TCHAMPA, TCHAMPA and VELLAISEENETTI. The most diverse cultivar was SWARNA and XINUOZAO, SWARNA and DZ 78, Local Check NDR359 and WANNIADAHANALA and most of the genotypes showing high diversity between them.

In conclusion, the present study has revealed valuable information on different yield traits in rice improvement. Genotypes SWARNA, RTS4 E KHA KEHA, IR 119, IR 64 LC-5 and GK 5022 were found to be the promising genotypes for yield and yield contributing traits. Hence these genotypes can be utilized in future breeding programme to obtain potential transgressive segregants.

REFERENCES

- Akagi, H., Yokozeki, Y., Inagaki, A. and Fujimura, T. (1997). Highly polymorphic microsatellites of rice consist of AT repeats and a classification of closely related cultivars with these microsatellite loci TAG. *Theoretical and Applied Genetics*, **94**(1): 61-67.
- Akkaya, M.S. and Bal, B. E.B. (2004). Assessment of genetic variation of bread wheat varieties using microsatellite markers. *Euphytica*, **135**(2):179-185.
- Akter, K., Iftakharuddaula, K. M., Bashir, M. K., Kabir, M. H. and Sarkar, M. Z. A. (2004). Genetic variability, correlation and path analysis in irrigated hybrid rice. *Journal of Subtropical Agricultural Research and Development* **2**(1): 17-23.
- Ali, S. S., Jafri, S. J. H., Zaman, T.U. Khan, Mahamood, A. and Butt, M. A. (2000). Heritability of yield and yield components of rice *Pakistan Journal of Agricultural Research*, **16**(2): 89-91.
- Alvarez, A., Fuentes, J. L., Puldón, V., Gómez, P. J., Mora, L., Duque, M. C. and Tohme, J. M. (2007). Genetic diversity analysis of Cuban traditional rice (*Oryza sativa* L.) varieties based on microsatellite markers. *Genetics and Molecular Biology*, **30**(4): 1109-1117.
- Anbanandan, V., Saravanan, K. and Sabesan, T. (2009). Variability, heritability and genetic advance in rice (*Oryza sativa* L.). *International Journal of Plant Science*, **3**(2): 61-63.
- Anjaneyulu, M., Reddy, D. R. and Reddy, K. H. P. (2010). Genetic variability, heritability and genetic advance in rice (*Oryza sativa* L.). *Research on Crops*. **11**(2): 415-416.
- Babu, S., Anbumalaramati, J., Yogameenakshi, P., Sheebha, A. and Rangaswami. (2003). Genetic diversity studies in rice (*Oryza sativa* L.). *Crop Research*, **25**(2): 280-286.
- Babu, V.R., Shreya, K., Dangi, K.S., Usharani, G. and Nagesh, P. (2013). Evaluation of popular rice (*Oryza sativa* L.) hybrids for quantitative, qualitative and nutritional aspects. *International Journal of Scientific and Research Publications*, **3**(1): 1-8.
- Banumathy S., Manimaran R., Sheeba A., Manivannan N., Ramya B., Kumar D. and Ramasubramanian G.V. (2010). Genetic diversity analysis of rice germplasm lines for yield attributing traits. *Electronic Journal of Plant Breeding*, **1**(4): 500-504.
- Banumathy, S., Manimaran, R., Sheeba, A., Manivannan, N., Ramya, B., Kumar, D. and Ramasubramanian, G.V. (2010). Genetic diversity analysis of rice

- germplasm lines for yield attributing traits. *Electronic Journal of Plant Breeding*, **1**(4): 500-504.
- Bhinda, M.S., bKarnwal, M.K. and Kumar, M.C. (2017). Estimates of genetic variability, heritability and genetic advance for yield contributing and quality traits in advance breeding lines of rice (*Oryza sativa* L.). *International Journal of Advanced Biological Research*, **7**(2): 229-233.
- Binodh, A.K., Kalaiyarasi, R., Thiagarajan, K. and Manonmani, S. (2006). Physiochemical cooking quality characteristics of promising varieties and hybrids in rice (*Oryza sativa* L.). *Indian Journal of Genetics*, **66**(2): 107-112.
- Blair, M.W., Hedetale, V. and McCouch, S.R. (2002). Fluorescent-labeled microsatellite panels useful for detecting allelic diversity in cultivated rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **105**(2): 449-457.
- Bligh, H.F.J., Blackhall, N.W., Edwards, K.J. and McClung, A.M. (1999). Using amplified length polymorphisms and simple sequence length polymorphisms to identify cultivars of brown and white milled rice. *Crop Science*, **39**(6): 1715 – 1721.
- Burton, G. W. and Devane, E. H. (1953). Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal*, **45**(10), 478-481.
- Candit, R. and Hubbel, S.P. (1991). Abundance and DNA sequence of two base repeat regions in tropical genomes. *Genome*, **34**(1): 66-71.
- Chandra, B.S., Reddy, T.D. and Ansari, N.A. (2007). Genetic divergence in rice (*Oryza sativa* L.). *Research on Crops*, **8**(3): 600-603.
- Chase, M. R., Moller, C., Kesseli, R. and Bawa, K. S. (1996). Distant gene flow in tropical trees. *Nature*, **383**(6): 399-599.
- Chattopadhyay, T., Biswas, T., Chatterjee, M., Mandal, N. and Bhattacharya, S. (2008). Biochemical and SSR marker based characterization of some Bengal land races of rice. *Indian Journal of Genetics*, **68**(1): 15-20.
- Chaturvedi, H.P. And Maurya, D.M. (2005). Genetic divergence analysis in rice (*Oryza sativa* L.). *Advances in Plant Sciences*, **18**(1): 349-353.
- Che, K., Zhan, Q., Xing, Q., Wang, Z., Jin, D., He, D. and Wang, B. (2003). Tagging and mapping of rice sheath blight resistant gene. *Theoretical and Applied Genetics*, **106**(2): 293-297.
- Chen, X., Temnykh, S., Xu, Y., Cho, Y and McCouch, S.R. 1997. Development of microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **95**(4): 556-567.

- Chen, X., Temnykh, S., Xu, Y., Cho, Y.G and Mc Couch, S.R. (1997). Development of microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **95**(4): 553-557.
- Chikkalingaiah, S., Lingaraju, S and Radhakrishna, R.M. (1999). Genetic variability of plant and quality traits in promising genotypes of scented rice (*Oryza sativa* L.). *Mysore Journal of Agricultural Sciences*, **33**(4): 338-431.
- Cho, Y. G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., McCOUCH, S. R., and Cartinhour, S., (2000). Diversity of microsatellites derived from genomic libraries and Gene Bank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **100**(5): 713-722.
- Chouhan, S. K., Singh, A. K., Aparajita, S., Ram, M., Singh, P. K. and Singh, N. K. (2014). Characterization and evaluation of *Oryza nivara* and *Oryza rufipogon*. *The Bioscan*, **9**(2): 853-858.
- Chouhan, S. K., Singh, A. K., Aparajita, S., Ram, M., Singh, P. K. and Singh, N. K. (2014). Characterization and evaluation of *Oryza nivara* and *Oryza rufipogon*. *The Bioscan*, **9**(2): 853-858.
- Chouhan, S.K., Singh A.K., Singh, A., Singh, N.K., Yadav, S.K. and Singh, P.K. (2014). Genetic variability and association analysis in wild rice (*Oryza nivara* and *Oryza rufipogon*). *Annals of Plant and Soil Research*, **16**(3): 219-223.
- Chuang, H. Y., Huu-Shen, L. U. R., Kae-Kang, H. W. U. and Chang, M. C. (2011). Authentication of domestic Taiwan rice varieties based on fingerprinting analysis of microsatellite DNA markers. *Botanical Studies*, **52**(4): 393-405.
- Coburn, J.R., Temnykh, S.V., Paul, E.M. and McCouch, S.R. (2002). Design and application of microsatellite markers panels for semi automated genotyping of rice. (*Oryza sativa* L.). *Crop Science*, **42**(6): 2092-2099.
- Commodity Profile for Rice- March, 2017 (20-3-2017)
- Corderio, G.M., Christopher, M.J., Henry, R.J and Reinke, R.F. (2002). Identification of microsatellite markers for fragrance in rice by analysis of rice genome sequence. *Molecular Breeding*, **9**(4): 245-250.
- Courtois, B., Ahmadi, N., Khowaja, F., Price, A. H., Rami, J. F., Frouin, J. and Ruiz, M., (2009). Rice root genetic architecture: meta-analysis from a drought QTL database. *Rice*, **2**(2-3): 115-128.
- Das, P., Kundu, A., Mandal, N. and Dana, I. (2004). Genetic divergence in land race collections of rice (*Oryza sativa* L.). *Journal of Interacademia*, **8**(4): 488-494.
- DeWoody, J.A., Honeycutt, R.L. and Skow, L.C. (1995). Microsatellite markers in White tailed deer. *Journal of Heredity*, **86**(4): 317-319.

- Dey, M. M. and Upadhyaya, H. K. (1996). Yield loss due to drought, cold and submergence in Asia. *Rice research in Asia: progress and priorities*. CAB International, Wallingford, **3**(1): 291-303.
- El Rabey, H., Salem, K. F., & Mattar, M. Z. (2013). The genetic diversity and relatedness of eight rice (*Oryza sativa* L.) cultivars as revealed by AFLP and SSRs markers. *Life Science Journal*, **10**(1): 295-302.
- Enoki, H., Sato, H. and Kolhuma, K. (2002). SSR analysis of genetic diversity among maize inbred lines adapted for cold regions of Japan. *Theoretical and Applied Genetics*, **104**(8): 1270-1277.
- Gangashetty, P. I., Salimath, P. M. and Hanamaratti, N. G. (2013). Genetic variability studies in genetically diverse non-basmati local aromatic genotypes of rice (*Oryza sativa* (L.)). *Rice genomics and genetics*, **4**(1): 4-8.
- Garg, P., Pandey, D. P. and Kaushik, R. P. (2011). Genetic divergence for yield and quality traits in rice (*Oryza sativa* L.). *Journal of Rice Research*, **4**(1): 1-5.
- Garland, S. H., Lewin, L., Abedinia, M., Henry, R. and Blakeney, A. (1999). The use of microsatellite polymorphisms for the identification of Australian breeding lines of rice (*Oryza sativa* L.). *Euphytica*, **108**(1): 53-63.
- Garland, S.H., Lewin, L., Abedinia, M., Henry, R and Blakeney, A. (1999). The use of microsatellite polymorphism for the identification of Australian breeding lines of rice (*Oryza sativa* L.). *Euphytica*, **108**(1): 53-63.
- Gawas, Y. K., Bhawe, S.G., Bendale, V.W., Sawant, S.S. and Jadhav, B.B. (2007). Nature and magnitude of heterosis for growth yield and yield components in hybrid rice. *Journal of Maharashtra Agricultural University*, **32**(1): 52-56.
- Ghneim Herrera, T., Posso Duque, D., Pérez Almeida, I., Torrealba Núñez, G., Pieters, A. J., Martinez, C. P. and Tohme, J. M. (2008). Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electronic Journal of Biotechnology*, **11**(5): 3-4.
- Giovannoni, J.J., Wing, R.A., Ganai, M.W. and Tanksley, S. (1991). Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. *Nucleic Acids Research*, **19**(23): 6553-6558.
- Gopikannan, M. and Ganesh, S.K. (2013). Investigation on combining ability and heterosis for sodicity tolerance in rice (*Oryza sativa* L.). *African Journal of Agricultural Research*, **8**(32): 4326-4333.
- Gouda, P.K., Varma, C. M. K., Saikumar, S., Kiran, B., Shenoy, V. and Shashidhar, H. E. (2012). Direct selection for grain yield under moisture stress in *Oryza sativa* cv. IR58025B x *Oryza meridionalis* population. *Crop science*, **52**(2): 644-653.

- Hedge, S.G. and Patil, C.S. (2000). Genetic divergence in rainfed rice. *Karnataka Journal of Agricultural Science*, **13**(3): 533-549.
- Hee Chung, J. I., Young Il, K. I. M., Hee Bong, L. E. E., Jin–Woong, C. H. O., Sung, S. E. O., and Yamakawa, T. (2007). Genetic diversity of colored rice lines based on botanical characteristics and simple sequence repeat (SSR) markers. *Journal of the Faculty of Agriculture, Kyushu University*, **52**(2): 287-293.
- Hossain M.M., Islam M.M., Hossain H., Ali M.S., Jamie A., Komamine A. and Prodhan S.H. (2012). Genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.) by microsatellite markers. *Genes, Genomes and Genomics* **6**(1): 42-47.
- International. Journal of Science Environmental and Technology*, **3**(3): 910-916.
- Innan, H., Terauchi, R. and Miyashita, N. T. (1997). Microsatellite polymorphism in natural populations of the wild plant *Arabidopsis thaliana*. *Genetics*, **146**(4): 1441-1452.
- IRRI (2016). Standard evaluation system for rice. IRRI, Los Banos, Philippines. DES 3rd Advance Estimate. Source: Lok Sabha Unstarred Question No. 2633, dated on 02.08.2016.
- Jackson, M. T. and Huggan, R. (1993). Sharing the diversity of rice to feed the world. *Diversity*, **9**(3): 22-25.
- Jacob, H.J., Lindpainter, K., Lincoln, S.E., Kusumi, K., Bunker, R.K., Yi P. M., Ganten, D., Dzau, V.J and Lander, E.S. (1991). Genetic mapping of a gene causing hypertension in the stroke- prone spontaneously hypertensive rat. *Cell*, **67**(1): 213-224.
- Jadhav, P. V., and Dudhare, M. S. (2008). Study of variability, heritability and genetic advance in seedling growth and yield component traits in indigenous and exotic rice. *Annals of plant Physiology*, **22**(1): 77-80.
- Jaiswal, U.H.K., Sravan, T. and Waza, S.A. (2016). Genetic divergence analysis for yield and quality traits in some indigenous genotypes of basmati rice (*Oryza sativa* L.). *Green Farming*, **7**(2): 345-348.
- Jalaluddin, M., Nakai, H and Yamanato, T. (2007). Genetic diversity and DNA fingerprinting of some modern *indica* and *japonica* rice. *SABRAO Journal of Breeding and Genetics*, **39**(1): 43-52.
- Jayamani, P., Negrao, S., Martins, M., Macas, B., & Oliveira, M. M. (2007). Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. *Crop Science*, **47**(2): 879-884.
- Jayasudha, S. and Sharma, S. (2010). Genetic parameters of variability, correlation and path-coefficient for grain yield and physiological traits in rice (*Oryza*

- sativa* L.) under shallow lowland situation. *Electronic Journal of Plant Breeding*, **1**(5): 1332-1338.
- Jeung, J.U., Hwang, H.G., Moon, H.P. and Jena, K.K. (2005). Fingerprinting temperate japonica and tropical indica rice genotypes by comparative analysis of DNA markers. *Euphytica*, **146**(3): 239 – 251.
- Jongdee, S., Mitchell, J. H. and Fukai, S. (1997). Modelling approach for estimation of rice yield reduction due to drought in Thailand. In Australian Centre for International Agricultural Research (ACIAR) *Proceedings*, **77**(2): 65-76.
- Junhua, P. and Shoujun, T. (1991). Analysis of heterotic relationships among quantitative characters of hybrid rice. *International Rice Research Notes*, **16**(4): 6-7.
- Kamoshita, A., Babu, R. C., Boopathi, N. M. and Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Research*, **109**(1): 1-23.
- Karande, S. S., Thaware, B. L., Bhawe, S. G. and Devmore, J. P. Genetic Variability and Character Association Studies on Some Exotic Germplasm Lines in Kharif Rice (*Oryza sativa* L.). *Advanced Agricultural Research and Technology Journal*, **1**(1): 110-114.
- Khare R., Singh, A.K., Eram, S. and Singh, P.K. (2015). Genetic variability, association and diversity analysis in upland rice (*Oryza sativa* L.). *SAARC Journal of Agriculture* **12**(2): 40-51.
- Khoyumthem, P., Sharma, P.R., Singh, N.B. and Singh, M.R.K. (2005). Heterosis for grain yield and its component characters in rice (*Oryza sativa* L.). *Environment and Ecology*, **23**(4): 687-691.
- Kibria, K., Islam, M.M and Begum, S.N. (2008). Screening of aromatic rice lines by phenotypic and molecular markers. *Bangladesh Journal of Botany*, **37**(2): 141-152.
- Kole, P. C. and Hasib, K. M. (2008). Correlation and regression analysis in scented rice. *Madras Agricultural Journal*, **95**(1-6): 178-182.
- Krishna, T., Kavita, A. and Pushpalata, T. (2010). Genetic variability, heritability and genetic advance for quantitative traits in rice (*Oryza sativa* L.) accession. *Agricultural and Biological Research*, **26**(1): 13-19.
- Kuchanur, P. H., Naresh, D. and Vijayakumar, A. G. (2009). Genetic variability and divergence in 'New Plant Type' rice genotypes. *Crop Improvement*, **36**(1): 20-24.
- Kumar N., Singh, P.K., Vaishampayan, A., Saini, R., MukhRam, Singh, A. and Singh, N.K. (2014). Genetic Divergence Analysis in Rice under Irrigated Conditions. *Indian Journal of Plant Genetic Resource*, **27**(3): 246-250.

- Kumar, A., Dixit, S., Ram, T., Yadaw, R. B., Mishra, K. K. and Mandal, N. P. (2014). Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. *Journal of Experimental Botany*, **65**(21): 6265-6278.
- Kumar, A., Singh, S. K., Sharma, A., Bhati, P. K. and Dhurai, S. Y. (2014). Genetic divergence for quantitative traits in rice germplasm. *Research Journal of Agricultural Sciences*, **5**(2): 249-253.
- Kumar, R. and Singh, A. K. (2012). Evaluation of genetic diversity in rice using simple sequence repeats (SSR) markers. *African Journal of Biotechnology*, **11**(84): 14988-14995.
- Lafitte, H. R., Li, Z. K., Vijayakumar, C. H. M., Gao, Y. M., Shi, Y., Xu, J. L. and Maghirang, R. (2006). Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. *Field Crops Research*, **97**(1): 77-86.
- Lander, E. S. and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, **121**(1): 185-199.
- Lapitan, V. C., Brar, D. S., Abe, T. and Redoña, E. D. (2007). Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breeding science*, **57**(4): 263-270.
- Li, G., Hu, W., Jin, R., Tan, G., Zhu, L. and He, G. (2007). Simple sequence repeat analysis of interspecific hybrids and MAAL of *Oryza officinalis* and *Oryza sativa*. *Genetica*, **34**(2): 169-180.
- Lingaiah, N. (2015). Genetic variability, heritability and genetic advance in rice (*Oryza sativa* L.). *Asian Journal of Environmental Science*, **10**(1): 110-112.
- Litt, M. and Luty, J. A. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, **44**(3): 397-401.
- Liu, X. C. and Wu, J. L. (1998). SSR heterogenic patterns of parents for marking and predicting heterosis in rice breeding. *Molecular Breeding*, **4**(3): 263-268.
- Lush, J. L. (1949). Heritability of quantitative characters in farm animals. *Hereditas*, **35**(1): 356-375.
- Mackill, D. J. (1995). Classifying japonica rice cultivars with RAPD markers. *Crop Science*, **35**(3): 889-894.
- Mackill, D. J., Zhang, Z., Redona, E. D. and Colowit, P. M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome*, **39**(5): 969-977.
- MacMillan, K., Emrich, K., Piepho, H. P., Mullins, C. E. and Price, A. H. (2006). Assessing the importance of genotype x environment interaction for root traits

- in rice using a mapping population. I: a soil-filled box screen. *Theoretical and Applied Genetics*, **113**(6): 977-986.
- Madhavalatha, L., Sekhar, M. R., Suneetha, Y. and Srinivas, T. (2005). Genetic variability, correlation and path analysis for yield and quality traits in rice (*Oryza sativa* L.). *Research on Crops*, **6**(3): 527-534.
- Madhavalatha, L., Sekhar, M.R., Suneetha, Y. and Srinivas, T. 2005. Studies on genetic divergence and isozyme variability in rice (*Oryza sativa* L.). *Research on Crops*, **6**(3): 520-526.
- Mahto, R. N., Yadav, M. S. and Mohan, K. S. (2003). Genetic variation, character association and path analysis in rainfed upland rice. *Indian Journal of Dryland Agricultural Research and Development*, **18**(2): 196-198.
- Mahto, R.N., Yadav, M.S. and Mohan, K.S. (2003). Genetic variation, character association and path analysis in rainfed upland rice. *Indian Journal of Dryland Agriculture Research and Development*, **18**(2): 196-198.
- Manickavelu, A., Nadarajan, N., Ganesh, S. K., Gnanamalar, R. P. , Chandra Babu, R. (2006). Drought tolerance in rice: morphological and molecular genetic consideration. *Plant Growth Regulation*, **50**(2-3): 121–138.
- McCouch, S. R., Cho, Y. G., Yano, M., Paul, E., Blinstrub, M., Morishima, H., and Kinoshita, T., (1997). Report on QTL nomenclature. *Rice Genet Newsl*, **14**(11): 11-131.
- McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y., Zhang, Q., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D. and Stein, L. (2002). *DNA Research*, **9**(6): 199–207.
- Ministry of Agriculture and Farmers Welfare, Govt. of India. 15.02.2017
- Mishu, F. M., Rahman, W., Azad, M. A. K., Biswas, B. K., Talukder, A. I., Kayers, O., Islam, R. and Alam, R. (2016). Study on genetic variability and character association of aromatic rice (*Oryza sativa* L.) cultivar. *International Journal of Plant and Soil Science* **9**(1): 1-8.
- Mitra, G.N. (1962). Hybrid vigour and inheritance of height in rice. *Nature*, **194**(2): 707-708.
- Mustafa, M. A. and Elsheikh, M. A. Y. (2007). Variability, correlation and path coefficient analysis for yield and its components in rice. *African Crop Science Journal*, **15**(4): 183-189.
- Muthuramu, S. and Sakthivel, S. (2016). Genetic Variability Studies in Rainfed Rice (*Oryza sativa* L.). *Journal of Rice Research*, **9**(2): 20-23.

- Naik, D., Sao, A., Sarawgi, A. K. and Singh, P. (2006). Genetic divergence studies in some indigenous scented rice (*Oryza sativa* L.) accessions of Central India. *Asian Journal of Plant Sciences*, **5**(2): 197-200.
- Neeraja, C. N., Hariprasad, A. S., Malathi, S. and Siddiq, E. A. (2005). Characterization of tall landraces of rice (*Oryza sativa* L.) using gene-derived simple sequence repeats. *Current Science*, **88**(1): 149-152.
- Ni, J., Colowit, P. M. and Mackill, D. J. (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science*, **42**(2): 601-607.
- Olufowote, J.O., Xu, Y., Chen, X., Park, W.D., Beachel, H.M., Dilday, R.H., Goto, M and McCouch, S.R. (1997).Comparative evolution of within cultivar variation of rice (*Oryza sativa*. L) usng microsatellite and RAPD markers. *Genome*, **40**(3): 370- 378.
- Osman, K. A., Mustafa, A. M., Ali, F., Yonglain, Z., and Fazhan, Q. (2012). Genetic variability for yield and related attributes of upland rice genotypes in semi-arid zone (Sudan). *African Journal of Agricultural Research*, **7**(33): 4613-4619.
- Ovung C.Y., Lal G.M. and Rai P.K. (2012). Studies on genetic diversity in rice (*Oryza sativa* L.). *International Journal of Agricultural Technology*, **8**(3): 1059-1065.
- Ovung C.Y., Lal, G.M. and Rai, P.K. (2012). Studies on genetic diversity in rice (*Oryza sativa* L.). *International Journal of Agricultural Technology*, **8**(3): 1059-1065.
- Padmaja, D., Radhika, K., Subba Rao, L.V and Padma, V. (2011). Correlation and path analysis in rice germplasm. *Oryza*, **48**(1): 69-72.
- Paikhomba, N., Kuma,r A., Chaurasia, A.K. and Rai, P.K. (2014).Assessment of Genetic Parameters for Yield and Yield Components in Hybrid Rice and Parents. *Journal of Rice Research*, **2**(1): 117.
- Pal, G., Verma, O. P., Verma, G. P., Pratap, N., Kumar, M., Chaudhary, R. K. and Singh, K. (2011). Genetic variability, heritability and divergence studies in rice (*Oryza sativa* L.) Under Sodic Soil. *Environment and Ecology*, **29**(3): 1597-1600.
- Pal, G., Verma, O.P., Pratap, N., Kumar, M., Chaudhary, R. K. and Singh, K. (2011). Genetic variability, heritability and divergence studies in rice (*Oryza sativa* L.) under sodic soil. *Environment and Ecology*, **29**(3): 1597-1600.
- Panaud, O., Chen, X. and McCouch, S.R. (1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Molecular and General Genetics*, **252**(5): 597-607.

- Pandey, S., Bhandari, H., Ding, S., Prapertchob, P., Sharan, R., Naik, D. and Sastri, A. (2007). Coping with drought in rice farming in Asia: insights from a cross- country comparative study. *Agricultural Economics*, **37**(1): 213-224.
- Pandey, V. K. and Awasthi, L. P. (2002). Studies on genetic variability on yield contributing traits aromatic rice. *Crop Research*, **23**(2): 214-218.
- Pandya, R. and Tripathy, R.S. (2006). Heterosis breeding in hybrid rice. *Oryza*, **43**(2): 87-93.
- Parimalan R., Joel, A.J. and Vanniarajan, C. (2008). Genotypes clustering in rice (*Oryza sativa* L.). *Crop Improvement*, **35**(2): 115-118.
- Pervaiz, Z.H., Rabbani, M.A., Pearce, S.R. and Malik, S.A. (2009). Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. *African Journal of Biotechnology*, **8**(21): 5641-5651.
- Priyanka, K., Jaiswal, H.K. and Waza, S.A. (2014). Combining ability and heterosis for yield, its component traits and some grain quality parameters in rice (*Oryza sativa* L.). *Journal of Applied and Natural Science*, **6**(2): 495-506.
- Provan, J.G., Corbott, J.W., Mc Niol and Powell, W. (1997). Chloroplast DNA variability in wild and cultivated rice (*Oryza sativa* L.) revealed by polymorphic chloroplast simple sequence repeats. *Genome*, **40**(1): 104-110.
- Qian, W., Ge, S. and Hong, D. Y. (2001). Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *TAG Theoretical and Applied Genetics*, **102**(2): 440-449.
- Rabbani, M.A., Hassan, Z., Pervaiz, M and Shahid, M. (2008). Genetic analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic Journal of Biotechnology*, **11**(3): 1-10.
- Rahman, M. M., Syed, M. A., Adil, M., Ahmad, H. and Rashid, M. M. (2012). Genetic variability, correlation and path coefficient analysis of some physiological traits of transplanted Aman rice (*Oryza sativa* L.). *Middle East Journal of Scientific Research*, **11**(5): 563-566.
- Rahman, M., Acharya, B., Sukla, S. N. and Pande, K. (1997). Genetic divergence in low land rice genotypes. *Oryza*, **34**(3): 209-212.
- Ram, S.G., Thiruvengada, V. and Vinod, K.K. (2007). Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *Journal of Applied Genetics*, **48**(4): 337-345.
- Ram, S.G., Thiruvengadan, V. and Vinod, K.K. 2005. Genetic diversity among cultivars, landraces and wild relatives as revealed by microsatellite markers. *Journal of Applied Genetics*. **48**(4): 337-345.
- Ramya, K. and Senthilkumar (2008). Genetic divergence in rice (*Oryza sativa* L.). *Crop Improvement*, **35**(2): 119-121.

- Rani, N. S., Prasad, A. S., Prasad, G. S. V., Reddy, P. B., and Veni, B. K. (2001). Genetic variability for yield components in aromatic and quality rice germplasm. *Indian Journal of Plant Genetic Resources*, **14**(2): 206-209.
- Rather, A.G., Mir, G.N. and Sheikh, F.A. (1998). Genetic parameters for some quantitative parameters for some quantitative traits in rice. *Advances in Plant Science*, **121**(2): 163-166.
- Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M. (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*, **133**(2): 243-252.
- Reddy, C.D.R., Nerkar, Y.S. and Lakshi, G.D. (1991). Heterosis and inbreeding depression for grain size and yield in rice under direct seeding. *Indian Journal of Agricultural Science*, **61**(6): 416-419.
- Reddy, J.N. and De, R.N. (1996). Study of genetic variability, heritability and genetic advance for panicle characters in rice. *Oryza*, **35**(1): 19-21.
- Reddy, K.H.P. and Agrawal, B.D. (1992). Estimation of genetic variation in an improved population of maize. *Madras Agricultural Journal*, **79**(12): 714-719.
- Reddy, M.R., Raju, C.H.S., Sarvani, T., Reddy, D., Reddy, N., (2012). Heterosis for yield and kernal size in aromatic rice (*Oryza Sativa. L*) *Annals of Biological Research*, **3**(6): 2662-2666.
- Reynolds, M., Manes, Y., Izanloo, A. and Langridge, P. (2009). Phenotyping approaches for physiological breeding and gene discovery in wheat. *Annals of Applied Biology*, **155**(3): 309-320.
- Roa, A. C., Chavarriaga-Aguirre, P., Duque, M. C., Maya, M. M., Bonierbale, M. W., Iglesias, C. and Tohme, J. (2000). Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. *American Journal of Botany*, **87**(11): 1647-1655.
- Rouf, M., Shikari, A.B., Singh, S.G. and Parray, G.A. (2009). Genetic divergence studies in high altitude temperate rice (*Oryza sativa* L.). *Asian Journal of Experimental Sciences*, **23**(3): 487-490.
- Roy, S.K., B.K. Senapati, S.P. Sinhamahapatra and K.K. Sarkar. (2009). Heterosis for yield and quality traits in rice. *Oryza*, **46**(2): 87-93.
- Sabesan, T., Saravanan, K. and Anandan, A. (2009). Genetic divergence analysis for certain yield and quality traits in rice (*Oryza sativa* L.) grown in irrigated saline low land of Annamalaiagar, South India. *Journal of Central European Agriculture*, **10**(4): 405-410.
- Saini, N., Jain, N., Jain, S. and Jai, R.K. (2004). Assessment of genetic diversity within and among basmati and non-basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica*, **140**(3): 133-146.

- Sarawgi, A. K. and Rastogi, N. K. (2000). Genetic diversity in traditional aromatic rice accessions from Madhya Pradesh. *Indian Journal of Plant Genetic Resources*, **13**(2): 138-146.
- Sarawgi, A.K., Rastogi, N.K. and Munhot, M.K. (2000). Heterosis among line x tester crosses for grain yield and quality components in rice. *Tropical Agricultural Research and Extension*, **3**(2): 2000.
- Sarla, N., Bobba, S. and Siddiq, E.A. (2003). ISSR and SSR markers based on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. *Current Science*, **84**(5): 683-690.
- Satyanarayana, P. V., Srinivas, T., Reddy, P. R., Madhaviatha, L. and Suneetha, Y. (2005). Studies on variability, correlation and path coefficient analysis for restorer lines in rice (*Oryza sativa* L.). *Research on Crops*, **6**(1): 80-84.
- Sawarkar, A. and Senapati, B.K. (2014). Polygenic variations and cause effect relationship in some photo-insensitive recombinant inbred lines (RILs) of Basmati derivative. *African Journal of Biotechnology*, **13**(1): 112-118.
- Seetharam, K., Thirumeni, S and Paramasivam, K. 2008. Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. *African journal of Biotechnology*, **8**(10): 2050-2059.
- Semagan, K., Ndjiondjop, M.N. and Cissoko, M. (2006). Microsatellites and agronomic traits for assessing genetic relationships among 18 new rice for Africa (NERICA) varieties. *African Journal of Biotechnology*, **5**(10): 800-810.
- Shanthi, P. and Singh, J. (2001). Variability studies in induced mutants of Mahsuri rice (*Oryza sativa* L.). *Madras Agriculture Journal*, **88**(12): 707-709.
- Sharma, A., Yadav, D. V., Singh, A. K., Yadav, G., Gulia, S., Gupta, K. R., Singh, R. and Deepak, Prem. (2002). Genetic divergence in aromatic rice (*Oryza sativa* L.). *National Journal of Plant Improvement*, **4**(2): 46-49.
- Sharma, S.K., Singh, S.K., Nandan, R., Amita Sharma, Ravinder Kumar, Kumar, V., Singh, M.K. (2013). Estimation of heterosis and inbreeding depression for yield and yield related traits in rice (*Oryza sativa* L.). *Molecular Plant Breeding*, **29**(4): 238-246.
- Sheethal, Y., Poonam, R., Navinder, S., Sunitha, J. and Rajinder, J.K. (2008). Assessment of genetic diversity among rice genotypes with differential adaptations to salinity using physio-morphological and molecular markers. *Journal of Plant Biochemistry and Biotechnology*, **17**(1): 1-8.
- Singh A., Saini R., Singh J., Arya M., Mukh Ram, Pallavi, Mukul and Singh P.K. (2015). Genetic diversity studies in rice (*Oryza Sativa* L.) using microsatellite markers. *International Journal of Agriculture, Environment and Biotechnology*, **8**(1): 143-152.

- Singh A.K., Singh P.K., Arya M., Singh N.K. and Singh U.S. (2015). Molecular screening of blast resistance genes in rice using SSR markers. *The Plant Pathology Journal*, **31**(1): 12
- Singh, A.K., Nandan, R. and Singh, P.K. (2013). Genetic variability and association analysis in rice germplasm under rainfed conditions. *Crops Research*, **47**(1-3): 7-11.
- Singh, A.K., Sharma, P. and Singh, P.K. (2013). Studies on Genetic Characteristic of Upland Rice (*Oryza sativa* L.). *International Journal of Agriculture, Environment and Biotechnology*, **6**(4): 515-520.
- Singh, A.K., Singh, P.K. and Rao, M. (2011). Diversity analysis in rice under rainfed condition. *Annals of plant and soil research*, **13**(1): 58-61.
- Singh, B., Singh, S.P. and Kumar, J. (2011). Assessment of genetic diversity of aromatic rices (*Oryza sativa* L.) using morphological, physiochemical and SSR markers. *Indian Journal of Genetics and Plant Breeding*, **71**(3): 214-222.
- Singh, M., Kumar, K. and Singh, R. P. (2007). Study of co-efficient of variation, heritability and genetic advance in hybrid rice. *Oryza*, **44**(2): 160-162
- Singh, P.K., Mishra, M.N., Hore, D.K. and Verma, M.R. (2006). Genetic divergence in lowland rice of north eastern region of India. *Communications in Biometry and Crop Science*, **1**(1): 35-40.
- Singh, R. P., Kumar, M. S. and Madhavilatha, L. (2007). Variability and relationship studies of yield and yield attributing traits in diverse lines of international irrigated observational nursery of rice (*Oryza sativa* L.). *Journal of Research (Acharya N G Ranga University of Agriculture)*, **35**(2): 16-22.
- Singh, R.K., Sharma, R.K., Singh, A.K., Singh, V.P., Singh, N.K., Tiwari, S.P. and Mahopatra, T. (2004). Suitability of mapped sequence tagged microsatellite site markers for establishing distances, Uniformity and stability in aromatic rice. *Euphytica*, **135**(2): 135-143.
- Singh, S., Sahu, R., Singh, P., Vennela, P., Verva, S. R., Kumar, D., and rathan, N. (2017). Identification of restorers and maintainers for the development of drought tolerant rice hybrids for eastern Uttar Pradesh. *SABRAO Journal of Breeding & Genetics*, **49**(1): 77-82.
- Singh, S.K., Vikash Sahu, Amita Sharma and Pradeep Kumar Bhati, (2013). Heterosis for yield and yield components in rice (*Oryza sativa* L.). *Bioinfolet*, **10**(2): 752-761.
- Siva, R., Kumar, K., & Rajasekaran, C. (2013). Genetic diversity study of important Indian rice genotypes using biochemical and molecular markers. *African Journal of Biotechnology*, **12**(10).

- Song, Z. P., Xu, X., Wang, B., Chen, J. K. and Lu, B. R. (2003). Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theoretical and Applied Genetics*, **107**(8): 1492-1499.
- Soo-Jin, K., Sang-Nag, A. and Huhn-Pal, M. (1999). Evaluation of genetic relationship and fingerprinting of rice varieties using microsatellite and RAPD markers. *Korean Journal of Crop Science*, **44**(2): 112-116.
- Souroush, H. R., Mesbah, M., Hossainzadeh, A. and Bozorgipour, R. (2004). Genetic and phenotypic variability and cluster analysis for quantitative and qualitative traits of rice. *Seed and Plant*, **20**(2): 167-182.
- Srivastava, M.N. and Seshu, D.V. 1982. Heterosis in rice involving parents with resistance for various stresses. *Oryza*, **19**(1):172-177.
- Srividhya, A., Vemireddy, L. R., Sridhar, S., Jayaprada, M., Ramanarao, P. V., Hariprasad, A. S. and Siddiq, E. (2011). Molecular mapping of QTLs for yield and its components under two water supply conditions in rice (*Oryza sativa* L.). *Journal of Crop Science and Biotechnology*, **14**(1): 45-56.
- Suman, A., Shankar, V.G., Rao, L.V.S. and Sreedhar, N. (2005). Analysis of genetic divergence in rice (*Oryza sativa* L.) germplasm. *Research on Crops*, **6**(3): 487-491.
- Sun, C.Q., Wang, X.K., Yoshimura, A. and Iwata, N. (2000). A study of the genetic diversity of common wild rice (*O. rufipogon* Griff.) and cultivated rice (*Oryza sativa* L.) by RFLP analysis. *Yi Chuan Xue Bao*, **27**(3): 227-34.
- Tang, A., Neng, S.G., Ai, J.G., Ju, L., Li, W.J., Qing, T.S. and Song, H.P. (2009). Comparative assessment of SSR diversity in aromatic rice germplasm. *Hereditas*, **31**(4): 412-419.
- Temnykh, S., Declerck, G., Lukshova, A., Lipovich, L., Cartinhour and McCouch, S.R. (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.), frequency, length variation, transposon associations and genetic marker potential. *Genome Research*, **11**(8): 1441-1452.
- Temnykh, S., Park, W. D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, L. and McCouch, S. R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *TAG Theoretical and Applied Genetics*, **100**(5): 697-712.
- Tuwar, A. K., Singh, S. K., Sharma, A. and Bhati, P. K. (2013). Appraisal of genetic variability for yield and its component characters in rice (*Oryza sativa* L.). *Biolife*, **1**(3): 84-89.
- Umadevi M., Veerabadhiran P. and Manomani S. (2014). Assessment of genetic diversity of rice (*Oryza sativa*) cultivars using simple sequence repeat (SSR) markers. *African Journal of Biotechnology*, **13**(35): 3547-3552.

- Umadevi M., Veerabhadhiran P. and Manomani S. (2014). Assessment of genetic diversity of rice (*Oryza sativa*) cultivars using simple sequence repeat (SSR) markers. *African Journal of Biotechnology* **13**(35): 3547-3552.
- Umadevi, M., Veerabhadhiran, P., Shanmugasundaram, P. and Manonmani, S. (2010). Genetic diversity analysis in rice (*Oryza sativa* L.) based on morphological traits and microsatellite markers. *Crop Improvement*, **37**(1): 1-8.
- Upadhyay, P., Neeraja, C. N., Kole, C. and Singh, V. K. (2012). Population structure and genetic diversity in popular rice varieties of India as evidenced from SSR analysis. *Biochemical genetics*, **50**(9-10): 770-783.
- Vanaja, T., Randhawa, G.J. and Mammooty, K.P. (2006). Pedigree evaluation and molecular diversity of some true breeding rice (*Oryza sativa* L.) genotypes of Kerala. *Journal of Tropical Agriculture*, **44**(2): 42-47.
- Veerabhadhiran, P., Umadevi, M., Pushpam, R. (2009). Genetic variability, heritability and genetic advance of grain quality in hybrid rice. *Madras Agricultural Journal*, **96**(1-6): 95-99.
- Venkannan, V., Raju, Ch. S., Lingaiuh, N. and Roa V.T. (2014). Studies on heterosis and inbreeding depression for grain yield and grain quality traits in rice (*Oryza sativa* L.).
- Venkannan, V., Raju, Ch. S., Lingaiuh, N. and Roa V.T. (2014). Studies on heterosis and inbreeding depression for grain yield and grain quality traits in rice (*Oryza sativa* L.). *International Journal of Science, Environment and Technology*, **3**(3): 910-916.
- Vivek, S., Singh, S., Singh, S.K. and Singh, H. (2005). Estimation of genetic variability, heritability and genetic advance in rice (*Oryza sativa* L.). *Agricultural Science Digest*, **25**(3): 207-209.
- Vivek, S., Singh, S., Singh, S.K. and Singh, H. (2005). Estimation of genetic variability, heritability and genetic advance in rice (*Oryza sativa* L.). *Agricultural Science Digest*, **25**(3): 207-209.
- Vivekanandan P. and Subramanian S. (1993). Genetic diversity in rainfed rice. *Oryza*, **30**(2): 60-62.
- Vivekanandan, P. and Giridharan, S. (1996). Heterosis and inbreeding depression for kernel traits in rice. *Madras Agricultural Journal*, **83**(12): 773-774.
- Waghmare, C.R., Arti, G., Kulkarni, A.A. and Khobragade, A.M. (2008). Genetic divergence studies in some early rainfed rice genotypes. *Journal of Soils and Crops*, **18**(2): 371-374.
- Wang Sheng-jun¹, Zuomei, L.U and Wan Jian-min¹. (2006). Genetic diversity among parents of hybrid rice based on cluster analysis of morphological traits and simple sequence repeat markers. *Rice Science*, **13**(3): 155-160.

- Wang, P., Chen, H. X., Zhou, H. J., Li, Q. P., Wang, C. R. and Zhang, Q. F., (2011). A major QTL, Ghd8, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. *Molecular Plant*, **4**(2): 319-330.
- Weber, J. L. and May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American journal of human genetics*, **44**(3): 388.
- Wu, K.S. and Tanksley, S.D. (1993). Abundance of polymorphism and genetic mapping of microsatellites in rice. *Molecular General Genetics*, **241**(1): 225-235.
- Wu, Kun-Sheng, and Steven, D., Tanksley (1993). "Genetic and physical mapping of telomeres and macrosatellites of rice." *Plant molecular biology*, **22**(5): 861-872.
- Xu, K., Deb, R. and Mackill, D.J. (2004). A microsatellite markers and a co-dominant PCR-based marker for marker-assisted selection of submergence tolerance in rice. *Crop Science*, **44**(1): 248-253.
- Yadav, P.N., Chauhan, M.P. and Singh, R.S. (2002). Genetic variability, heritability and expected genetic advance for certain qualitative characters in rice. *New Agriculture*, **13**(112): 84-94.
- Yaqoob, M., Hussain, N. and Rashid, A. (2012). Assessment of genetic variability in rice (*oryza sativa* L.) genotypes under rainfed conditions. *Journal of Agricultural Research*, **50**(3): 311-321.
- Yashitola, J., Thirumurugan, T., Sundaram, R.M., Naseerullah, M.K., Ramesha, M.S., Sarma, N.P. and Sonti, R.V. (2002). Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Science*, **42**(2): 1369-1373.
- Zhenjin, X., Weinju, C., Rong, M.D., Yingna, L., Qing, Z.S. and Lixia, L. (2004). Correlation between rice grain, shape and main quantitative characters. *Acta Agronomica Sinica*, **30**(9): 894-900.

No	Character	Days to First Flowering	Days to 50% Flowering	Days to Maturity	Plant Height cm	Tillers/Plant	Effective Tillers/Plant	Panicle Length (cm)	Spikelets/Panicle	Grains/Panicle	Sterile Spikelets/Panicle	Spikelet Fertility %	Grain Weight/Panicle (g)	1000-Grain Weight (g)	Grain Yield/Plant (g)	Hulling Recovery %	Milling Recovery%	Kernel Length (mm)	Kernel Breadth (mm)	Kernel L/B Ratio	Amylose Content	Canopy Temperature Depression	Stomatal Conductance (mmol/m ² /s)	Chlorophyll Content (spad Value)	Proline Content (μmol/g Fresh Weight)	Grain Yield/Plot(kg)	Biomass (t/gha)	Harvest Index
1	B 6149 F-MR-7	93.00	99.67	119.33	153.88	5.33	4.33	25.50	158.67	138.00	20.67	86.92	3.45	25.83	12.63	78.97	69.55	6.15	2.45	2.51	24.59	28.70	527.17	42.43	18.89	0.58	1.60	36.17
2	D2 78	80.67	86.00	106.67	163.84	4.33	3.33	26.53	143.00	122.33	20.67	88.74	2.83	23.45	10.59	84.14	72.34	5.80	2.84	2.04	24.47	29.67	794.53	44.57	16.24	0.36	1.25	28.63
3	E KHA KEHA	96.00	101.00	123.33	160.47	5.33	4.33	25.67	105.67	90.00	15.67	88.44	1.87	22.37	4.57	79.73	71.09	5.29	2.47	2.14	24.50	29.56	742.57	42.53	20.41	0.22	1.15	19.40
4	E 21 124	81.67	86.33	109.33	175.02	5.00	4.33	29.30	110.33	84.33	26.00	80.07	1.74	23.13	5.56	76.22	70.18	7.02	1.89	3.71	24.55	28.73	863.20	43.73	14.35	0.26	1.30	20.30
5	5GCPAL	96.33	101.33	123.67	206.31	5.00	4.33	26.21	146.33	132.00	14.33	92.97	3.19	23.18	9.11	88.74	81.12	6.63	2.53	2.63	21.35	30.24	701.60	46.77	21.28	0.41	1.77	23.13
6	6CAUKHME	95.67	100.00	123.00	196.19	5.33	4.33	28.27	130.67	117.33	13.33	92.42	3.02	25.60	10.29	62.20	70.88	6.71	2.29	2.94	24.57	29.00	555.30	42.53	19.35	0.49	1.45	34.03
7	7NP 125	81.00	87.00	111.00	152.26	6.33	4.33	30.07	121.33	104.33	17.00	88.20	2.64	25.60	7.07	77.09	64.59	6.14	2.55	2.41	24.62	28.80	712.10	42.70	11.83	0.35	1.55	22.20
8	8MS 252	96.67	101.00	123.00	185.11	5.00	3.33	29.67	155.33	117.33	38.00	80.88	3.51	30.26	7.83	76.67	67.75	6.63	2.84	2.33	24.69	29.37	787.67	43.80	10.83	0.36	1.47	24.30
9	9RTS 4	85.33	91.00	114.67	180.81	7.33	6.33	28.20	169.67	158.33	11.33	93.38	3.69	24.50	14.31	80.41	67.47	7.89	1.90	4.15	24.37	29.30	725.70	42.37	20.15	0.79	1.90	41.40
10	10SOLOI	80.67	85.00	109.67	153.21	5.33	4.33	27.27	142.33	126.67	16.00	88.98	2.87	23.40	7.10	72.79	62.66	7.14	2.14	3.33	24.61	29.23	801.13	41.77	18.07	0.33	1.51	22.20
11	11CHAMPA	80.33	85.33	109.33	168.26	5.00	3.33	30.07	147.33	130.00	17.33	92.27	2.83	23.70	5.60	75.25	69.48	6.09	2.05	2.97	24.67	29.62	988.40	41.07	9.06	0.26	1.49	17.77
12	12VELLASENETTI	92.67	97.67	118.67	166.20	6.67	6.33	23.30	130.00	115.00	15.00	88.46	2.27	20.73	9.54	77.19	64.51	5.80	2.43	2.38	21.40	29.37	819.97	42.80	16.52	0.52	1.64	31.77
13	13WANNI DAHANALA	80.33	85.00	109.33	167.06	6.33	4.33	29.60	121.33	105.67	15.67	89.81	2.15	21.81	5.13	91.43	85.04	6.20	2.68	2.31	24.70	28.83	731.57	45.03	17.42	0.21	1.24	16.77
14	14WARR 2-2-1-1	94.33	99.33	119.33	173.81	6.33	5.33	25.23	180.00	95.00	84.67	52.93	2.43	26.03	7.19	78.49	72.09	6.10	2.01	3.04	24.65	29.77	885.50	38.60	18.07	0.45	1.81	24.67
15	15KINUO ZAO	79.33	84.00	108.67	147.00	6.67	5.33	24.93	127.00	103.67	23.33	82.94	2.23	22.61	7.11	90.81	72.39	5.61	2.18	2.57	21.16	29.33	822.07	40.67	14.19	0.33	1.79	18.17
16	16IR-74371-54-1-1	87.00	91.33	115.33	125.23	6.33	4.33	29.30	144.00	120.33	23.67	87.94	2.57	22.67	6.51	90.74	87.20	6.36	2.36	2.68	24.36	28.87	638.63	41.97	19.57	0.30	1.60	18.57
17	17IR-119	97.67	101.67	123.33	148.57	7.67	6.33	25.23	137.00	116.67	20.00	85.39	2.76	24.83	16.17	87.27	50.89	6.66	2.33	2.86	24.88	28.83	908.40	42.33	16.18	0.82	1.90	43.47
18	18IR-64	90.00	95.33	114.67	106.48	9.67	8.33	26.43	124.00	90.67	33.33	73.03	1.88	20.02	13.08	84.76	63.61	6.72	1.93	3.49	22.53	29.57	716.00	43.17	16.57	0.63	1.57	39.97
19	19SWARNA	108.33	112.33	141.33	93.13	11.67	7.33	24.30	150.67	127.33	23.33	84.54	2.63	18.04	14.32	92.51	89.01	5.80	2.02	3.63	23.59	29.40	754.93	43.93	25.61	0.76	1.69	45.03
20	20 Local check (NDR 359)	95.67	100.67	123.67	120.91	6.33	4.67	25.20	186.00	144.00	41.67	77.60	3.46	24.37	14.11	84.41	77.98	6.71	2.23	3.01	24.65	29.17	551.70	41.47	22.17	0.67	1.56	42.63
	Mean	89.63	94.55	117.37	157.19	6.35	4.95	27.01	141.53	116.95	24.55	84.80	2.70	23.63	9.39	81.49	71.51	6.37	2.31	2.86	23.95	29.27	751.41	42.71	17.34	0.46	1.56	28.53
	C.V.	1.40	1.10	0.81	2.11	11.64	15.69	1.16	3.47	3.13	14.31	2.16	2.23	1.83	2.80	2.21	3.53	1.67	1.23	2.24	1.57	1.72	13.29	4.25	2.15	9.34	5.38	6.62
	F ratio	127.08	177.79	224.82	221.46	16.66	9.03	137.63	57.21	82.11	64.70	75.93	273.49	107.46	561.19	53.51	38.88	96.30	323.67	237.08	33.06	1.94	0.04	0.00	0.00	0.00	0.00	0.00
	F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
	S.E.	0.72	0.60	0.55	1.92	0.43	0.45	0.18	2.84	2.11	2.03	1.06	0.03	0.25	0.15	1.04	1.46	0.06	0.02	0.04	0.22	0.29	57.64	1.05	0.22	0.02	0.05	1.09
	C.D. 5%	2.07	1.72	1.58	5.49	1.22	1.28	0.52	8.12	6.05	5.81	3.03	0.10	0.72	0.43	2.98	4.17	0.18	0.05	0.11	0.62	0.83	165.03	3.00	0.62	0.07	0.14	3.12
	C.D. 1%	2.78	2.31	2.12	7.35	1.64	1.72	0.70	10.88	8.10	7.78	4.05	0.13	0.96	0.58	3.99	5.59	0.24	0.06	0.14	0.83	1.12	221.05	4.02	0.83	0.09	0.19	4.18
	Range Lowest	79.33	84.00	106.67	93.13	4.33	3.33	23.30	105.67	84.33	11.33	52.93	1.74	18.04	4.57	62.20	50.89	5.29	1.89	2.04	21.16	28.70	527.17	38.60	9.06	0.21	1.15	16.77
	Range Highest	108.33	112.33	141.33	206.31	11.67	8.33	30.07	186.00	158.33	84.67	93.38	3.69	30.26	16.17	92.51	89.01	7.89	2.84	4.15	24.88	30.24	988.40	46.77	25.61	0.82	1.90	45.03

Appendix I : mean of 27 traits of 20 genotypes