GENOTYPING OF *Rf* (RESTORING FERTILITY) LOCI OF RICE VARIETIES OF KERALA USING MOLECULAR MARKERS.

by

RAJIB DAS

(2013 - 21 - 107)

THESIS

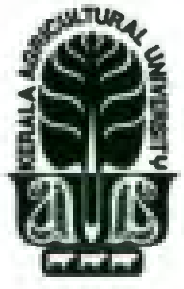
Submitted in partial fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University



DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2017

DECLARATION

I, hereby declare that this thesis entitled **"GENOTYPING OF *Rf* (RESTORING FERTILITY) LOCI OF RICE VARIETIES OF KERALA USING MOLECULAR MARKERS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,

Date: 29-08-2017

Rajib Das

(2013 -21-107)

CERTIFICATE

Certified that this thesis entitled **"GENOTYPING OF *Rf* (RESTORING FERTILITY) LOCI OF RICE VARIETIES OF KERALA USING MOLECULAR MARKERS"** is a record of research work done independently by **Mr. Rajib Das** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellayani,

Date: 29-08-2017

Dr. Jayalekshmy V. G.
(Chairman, Advisory Committee)
Professor
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani.

CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Rajib Das, a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in Plant Breeding and Genetics, agree that the thesis entitled **"GENOTYPING OF *Rf* (RESTORING FERTILITY) LOCI OF RICE VARIETIES OF KERALA USING MOLECULAR MARKERS"** may be submitted by Mr. Rajib Das in partial fulfillment of the requirement for the degree.

Dr. Jayalekshmy V. G.
(Chairman, Advisory Committee)
Professor
Department of Plant Breeding and Genetics
College of Agriculture Vellayani,

Dr. Arya K.
(Member, Advisory Committee)
Professor & Head
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani.

Dr. Leena Kumari S.
(Member, Advisory Committee)
The Registrar
Kerala Agricultural University
Thrsissur

Dr. Vijayaraghavakumar
(Member, Advisory Committee)
Professor & Head
Department of Agricultural Statistic
College of Agriculture, Vellayani.

Dr. Swapna Alex
(Member, Advisory Committee)
Professor & Head
Department of Plant Biotechnology
College of Agriculture, Vellayani

EXTERNAL EXAMINER

ACKNOWLEDGEMENTS

It is my heart's turn to express my deepest sense of gratitude to all of those who directly and indirectly helped me in this endeavour.

At the very outset, I fell inadequacy of words to express my profound indebtness and deep sense of gratitude to my esteemed Chairman of Advisory Committee, Dr. Jayalekshmy V.G, Professor, College of Agriculture, Vellayani, Trivandrum for her scholastic guidance, unceasing interest, constructive criticism, technical guidance, scientific view, cherishabe conselling, warmer affection, caring nature, moral support and also her help in bringing out this thesis.

I feel a great pleasure in expressing my whole hearted sense of gratitude to the Co-Chairman of the Advisory Committee, Dr. Vijayaraghavakumar, Professor and Head, Department of Agricultural Statistics, College of Agriculture, Vellayani and Dr. Leenakumari S, Registrar, Kerala Agricultural University, Thrissur for designing this beguiling piece of work for my thesis, sincere technical guidance, learned counsel, unstinted attention during the entire period of study. Their keen interest, patience hearing, indomitable quest for science and constructive criticism have installed in me the spirit of confidence to successfully complete the task.

I deem it my privilege in expressing my fidelity to Member of Advisory Committee, Dr. Arya K, Professor and Head, Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Dr. Swapna Alex, Professor and Head, Department of Plant Biotechnology, College of Agriculture, Vellayani for their constant encouraging, constructive suggestion, sustained interest for his constant encouragement, constructive suggestions, fruitful advice and all necessary support during the execution of thesis work.

I express my sincere gratitude to Dean, College of Agriculture, Vellayani for providing the facilities and all necessary support during the execution of this thesis work.

I feel a great privilege in placing on record my profound thanks to Dr. Manju P, Dr. Maya Devi, Dr. Lekharani C , Dr. Meren Abraham and Dr.Beena Thomas for their moral support and timely help in successful completion of my course.

My diction is poor to translate into words the sense of gratitude, heartfelt respect and affection to my mother Smt. Amita Das, my brother Sujit Das, My sisters Smt. Smriti Das, Smt Rupali Das, My brother inlaws and my relatives for their blessing, moral support, boundless love and building unshakable confidence in me which motivated as a force to fulfill this long cherished ambition.

I feel to express my heartfelt, sincere thanks to Remya chechi, Anchu chechi, Sumina, Reshma, Beena chichi and Joly chechi for their immense help, friendly co-operation and constant support during my work, which will be remembered by me everlastingly.

It is rather difficult to express in words my deep sense of boundless affection, I would heartly acknowledge the constant encouragement, assistance and constructive suggestions given by seniors Dr. Chandan Roy, Gogineni Srinivas and Gangadhar K. I fondly thank my juniors Akshay, Darshan, Vinay, Shiddhesh, Bibhishan, Harikrisha, Brunda, Pritin, Sai, Atul, Satish, Manu, Asha, Jeena, Nikitha and Saranya and all others for all their help.

I use this opportunity to sincerely thank my dearest classmates Ramaling, Kranti, Ashish and Narmada for their lovely friendship, help and care and for making the four year study very much enjoyable and memorable.

I am grateful to the authorities of KAU, Thrissur for having provided the opportunity to study in the university.

Above all, my whole hearted prostrations to the Almighty for sprinkling his blessings upon me.

RAJIB DAS

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	-	per cent
μ	-	Mean
μl	-	Micro litre
μM	-	Micro molar
^{0}C	-	Degree Celsius
χ2	-	Chi-Square
ANOVA	-	Analysis of Variance
BC_1F_1	-	First filial back cross generation
bp	-	base pairs
BP	-	Better parent
BSA	-	Bulk Segregant Analysis
CD	-	Critical difference
cm	-	centimeter
CMS	-	Cytoplasmic male sterility
CGMS	-	Cytoplasmic genetic male sterility
CRD	-	Cely randomized Designomplet
d.f	-	Degrees of freedom
dNTP		Deoxyribonucleoside triphosphate
EDTA	-	Ethylene diamine tetra acetic acid
et al.	-	and co-workers/co-authors
F_1	-	First filial generation
F_2	-	Second filial generation
GCA	-	General combining ability
Fig.	-	Figure
g	-	gram
i.e.	-	that is
ISSR	-	Inter-simple sequence repeats

kg	-	kilogram
m	-	Meter
m.ha	-	Million hactares
M	-	Molar
mg	-	milligram
min	-	minutes
mM	-	Milli molar
cM	-	Centimorgan
MP	-	Mid parent
Nacl	-	Sodium chloride
ng	-	Nanogram
PCR	-	Polymerase Chain Reaction
PTB	-	Pattambi
QTL	-	Quantitative Trait Loci
RFLP	-	Restriction Fragment Length Polymorphism
RAPD	-	Random amplified polymorphic marker
RBD		Randomized Block Design
Rf	-	Restoring fertility
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
RPG	-	Recurrent parent genome
rpm	-	revolutions per minute
S.E(d)	-	Standard Error deviation
SE	-	Standard Error
spp.	-	Species
SCA	-	Specific combining ability
SSR	-	simple sequence repeat
SH	-	Standard heterosis
TAE		Tris –Acetate- EDTA

Taq	-	*Thermus aquaticus*
Tm	-	Annealing Temperature
v/v	-	Volume/ volume
Viz.,	-	namely
WA	-	Wild Abortive
w/v	-	weight/volume

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) member of the poaceae family is the major staple food crop in the world. Today, rice provides 50 to 80% of calorie intake of over 75% of Asian population and more than three billion of world population (Khush, 2005). India is the largest rice growing country and accounts for about 43 per cent of total food grain production and 46 % of total cereal production in the world. In 2016-2017 rice was cultivated in an area around 43.5 m.ha with a production of 105 m.tonnes (Singh, 2016). However to ensure food supply to ever increasing population there is a need to enhance the current annual rice production to 120 m.tonnes by the year 2020. Kerala produced around 5.6 lakh tonnes (2011-12) of rice (Government of Kerala, 2013), where as in the year 2016 the production reduced to 4.2 lakh tonnes, while the estimated requirement of rice for the state is around 35-40 lakh tonnes/year.

The challenge of increasing the annual rice production of the country calls for both short and long term planning encompassing genetic as well as crop management options. Among the innovative genetic options available, hybrid rice technology is practically feasible and readily adoptable (Viraktamath *et al.*, 2010). Hybrid rice technology aims to increase the yield potential of rice beyond the level of high yielding varieties (HYVs) by exploiting hybrid vigour or heterosis. This technology has been successfully developed and widely adapted by farmers in China during the past 25 years. Currently, about 15 m.ha out of a total of 30 m.ha of rice area is covered with hybrid rice in China, producing 103.5 m. tonnes (MT) (17% of world paddy production) - *i.e.* 22.5 m.tonnes of extra paddy every year. During 2006, hybrids were cultivated in India in around 1 m.ha, and the National Food Security Mission (NFSM) has set a target of expanding the hybrid rice cultivation to 3 m.ha by 2011-12. Earlier in 2012, it was estimated that by 2016 hybrid rice would cover acreage of 5 m.ha. China was the first country where yield barrier in semi – dwarf inbred rice was broken by successful development of rice hybrids which yielded about 20% more than conventional inbred rice (Virmani *et al.*, 1982). Commercial success of hybrid rice in China has

clearly demonstrated the potential of this technology to meet the ever-increasing demands for rice world over. In India during the year 2011-12, hybrid rice had occupied an area of 1.3 m.ha and an additional rice production of 1.5 to 2.5 m. tonnes was added to our food basket through this technology.

Hybrid rice technology particularly utilizing the Cytoplasm Genic Male Sterility (CGMS) has now been widely adopted across several countries in Asia and USA. The most desirable requirements in hybrid rice breeding programme are highly heterotic parents for developing high yielding hybrids. The cause of male sterility in WA (Wild abortive) cytoplasm is governed by its plasma gene and its counteracting fertility restorer genes are present in the nuclear genome. Different cytoplasm in rice have different mechanism for the cause of male sterility and they have different fertility restorer genes governed by nuclear genome. Anandakumar and Subramaniam (1992) reported that a major dominant gene controls fertility restoration of WA-cytoplasm. However most of the genetic studies of fertility restoration for the WA CMS system have suggested that fertility restoration is governed by multiple genes namely *Rf3, Rf4, Rf5, Rf6 and Rf7* (Yao *et al.*, 1997; Zhang *et al.,* 1997; Ahmadikhah and Karlov, 2006; Bazrkar *et al.,* 2008 and Ahmadikhah and Alavi, 2009).

To increase rice productivity and production, the scientists opted to develop and disseminate hybrid rice technology which was initiated by Yuan in 1966. For an efficient hybrid rice breeding programme, identification of restorers, maintainer lines and evaluation of parental lines and development of promising maintainer lines into CMS lines forms an integral part of hybrid rice technology. Yuan and Fu (1995) detailed all the stages that should be followed to obtain male-sterile (A) lines, restorers (R) and maintainers (B). Hybrid rice produced by using these three lines is known as three-line system, where one line would have cytoplasmic male sterility; the second line would be responsible for maintaining the sterility and a third one would be used as restorer parent for the hybrid with the responsibility of restoring the fertility. Nuclear genes are required to restore pollen fertility to CMS lines. Restorer line carrying the restorer gene (*Rf*) to

restore fertility is indispensable for the development of hybrid varieties (Virmani *et al.*, 2003). The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype based screening (Ahmadikhah and Alavi, 2009). Restorers for different cytosterile sources will increase the cytoplasmic diversification, which in turn can prevent genetic vulnerability due to the use of single CMS source (Pradhan *et al.*, 1992).

The concept of combining ability is a landmark in the hybridization programme. Knowledge on the nicking ability of genotypes in hybrid combination is of paramount importance, since the combining ability of parents and hybrids does not always depend on the *per* se performance. The Line x Tester analysis gives reliable information about the nature and magnitude of gene action and combining ability effects present in the genetic material. Combining ability analysis is useful in selecting the parents and desirable cross combinations to be used in any breeding programme to produce superior hybrid. It also gives an idea about the relative magnitude of additive and non-additive types of gene actions in the expression of a trait. The potentiality of a strain to be used as a parent in hybridization, or in a cross to be used as a commercial hybrid, may be judged by comparing the *per* se performance of the parents, the F_1 value (heterosis) and the combining ability effects (Venkateshwaralu and Singh, 1982). To exploit maximum heterosis using cytoplasmic male sterile (CMS) technique in the hybrid programme, we must know the combining ability of different male sterile and restorer lines.

Heterosis is expressed in three ways, depending on the criteria used to compare the performance of a hybrid (Gupta, 2000). These three ways are average or relative or midparents heterosis (the performance of a hybrid compared with the average performance of its parents), better parent heterosis or heterobeltiosis (the performance of a hybrid compared with that of the best parent in the cross) and useful, economic or standard heterosis (the performance of a hybrid compared with high yielding variety in the region). From a practical point of view, standard

heterosis is the most important of the two levels of heterosis because it is aimed at developing desirable hybrids superior to the existing high yielding commercial varieties (Chaudhary, 1984).

Bulk segregant analysis (BSA) is a marker assisted breeding strategy in which the process of genotyping aids in reducing the sample size to DNA sample by grouping plant according to their expression of particular trait. BSA measures variation in pools of segregants that have sorted according to phenotype and uses correlation to assign a likely map location of gene of interest. So through BSA technique gene linked to R*f* loci can be identified (Ahmadikhah *et al.*, 2007).

Keeping in view the above facts the present investigation is being undertaken with the following objectives:

1. Molecular characterization of *Rf* loci using SSR markers
2. Validation of the restoration of fertility and identification of restorers and maintainers.
3. Study of inheritance pattern of restorer gene for WA cytoplasm.
4. Bulk Segregant Analysis (BSA) of the sterile and fertile bulks with respective markers in F_2 generation of crosses between CMS line and restorer lines.
5. Assesing general and specific combining ability effects of restorers, maintainers and their hybrids respectively
6. Identification of heterotic hybrids for seed yield and yield attributes

Review of Literature

2. REVIEW OF LITERATURE

Plateauing trend in the yield of HYV (high yielding varieties), declining and degrading natural resources like land and water and acute shortage of labour make the task of increasing rice production quite challenging. Among several options besides crop management, the innovative genetic option of hybrid rice technology is practically feasible and readily adoptable (Viraktamath *et al.*, 2010). Hybrids play an important role in enhanced yield production by heterosis breeding. The applications of concept of diversity among parental lines, identification of restorers and maintainers, inheritance of restoring fertility (*Rf*) gene, heterosis, combining ability of identified restorers and maintainers are the important aspects in development of hybrid rice. The literature available on these aspects is reviewed under the following heads

2.1 Hybrid rice

2.2 Screening rice varieties for presence of *Rf* loci and identification of maintainers and restorers

2.3 Morphological diversity analysis

2.4 Heterosis in rice

2.5 Inheritance of *Rf* loci and bulk segregant analysis

2.6 Combining ability and gene action

2.1 HYBRID RICE

Ever since the first report on the phenomenon of heterosis (Jones, 1926) and role of cytoplasm in inducing male sterility in rice (Sampath and Mohanty, 1954), attempts have been made to explore the possibilities of exploiting hybrid vigour. Shinjyo and Omura (1966) were the first to develop cytoplasmic genetic male sterile lines in cultivated rice by substituting nuclear genes of Taichung 65, a japonica variety into the cytoplasm of Chinsura Boro II an indica variety. The IRRI, American and Indian scientists subsequently brought out still more precisely the prospects of exploiting hybrid rice (Athwal and Virmani, 1972) succeeded by substituting the nuclear genes of popular japonica varieties grown in the USA, such as Calrose into the cytoplasm of Birco (PJ 279120) and *O. glaberrima*,

accessions. Similarly Athwal and Virmani (1972) substituted Pankhari 203 in the background of TN-1 cytoplasm.

Although a lot of basic research has been done on various aspects of hybrid rice by different groups, the technology failed to develop commercial hybrid until Chinese scientists successfully developed a stable cytoplasmic genetic male sterility-fertility restorer system in 1974 using the accidentally found cytoplasm of *Oryza sativa* f. *spontanea* (WA) and produced world's first commercial F_1 hybrid (Lin and Yuan, 1980). Hybrid rice term was successfully used in China from 1976, when the Chinese scientists developed the very high yielding F_1's from recessive male sterile lines and dominant restorer genes. In rice, commercial exploitation of hybrid vigour by the use of cytoplasmic genetic male sterile system (CGMS), maintainer (B line) and restorer (R line) was started from 1976 in China and thereafter in other rice growing countries.

Recognizing the potential of this technology, the Indian Council of Agricultural Research (ICAR) launched a mission-mode project on hybrid rice during 1989. This project was further strengthened with the financial support from the UNDP, MAHYCO Research Foundation and NATP. Concentrated efforts coupled with wholehearted support from funding agencies have enabled the country to enter into the era of hybrid rice. Efforts to develop and use of hybrid rice technology in India was initiated during 1970. But the research works were systematized and intensified since 1989 with a mission mode project. With the concerted research work, the country developed half a dozen rice hybrids each from public and private sectors within a short span of five years. The first four rice hybrids were released in the country *viz.*, APHR-1, APHR-2, MGR-1 and KRH-1 during 1994. Subsequently, two more hybrids viz. CNRH-3 and DRRH-1 were also released. During last 15 years, more than 1000 experimental hybrids have been developed and tested at different network centers. As a result of systematic evaluation and involvement of private sector, 33 hybrids were recommended for large scale cultivation by either Central Variety Release Committee or State Variety Release Committees. Basmati rice hybrid is also developed and released by IARI, New Delhi (Anon, 2009).

The released hybrids have an average yield advantage of 15-20 per cent over the pureline check varieties. Totally 17.11 m. ha area was under hybrid rice in some leading Asian rice growing countries during the year 2005-06 (Khush, 2007). While in India more than 1.4 m ha area was under hybrid rice cultivation during 2007-08 (Anon., 2008), mostly cultivated in Eastern Uttar Pradesh, Chattisgarh, Madhya Pradesh, Orissa, Maharashtra, West Bengal, Andhra Pradesh, Karnataka, Tamil Nadu, Punjab, Haryana and Bihar. In India during the year 2011-12, hybrid rice had occupied an area of 1.3 m. ha and an additional rice production of 1.5 to 2.5 m. tonnes was added to our food basket through this technology.

2.2 SCREENING RICE VARIETIES FOR PRESENCE OF *Rf* LOCI AND IDENTIFICATION OF MAINTAINERS AND RESTORERS

In plants, cytoplasmic male sterility (CMS) is a maternally inherited trait that leads to the production of non-functional pollen. CMS is frequently caused by chimeric open reading frames in the mitochondrial genome and may be suppressed or counteracted by nuclear genes known as restorer-of-fertility (*Rf*) genes (Hanson and Bentolila, 2004). This phenomenon is widely used for hybrid seed production. In rice (*Oryza sativa* L.), three main CMS/R*f* systems which based on pollen degeneration and genetic evidence of restoration, Wild abortive (WA), Hongolian (HL) and Boro II (BT) CMS systems are effectively utilized in rice cultivation (Rao, 1988 and Yuan, 1994).

The availability of stable cytoplasmic male sterility and fertility restoring system is vital for commercial exploitation of heterosis in rice. Rice hybrid for unfavorable environment can be developed using elite parental lines adapted to these environments. The establishment of testcross nursery to identify restorers and maintainers is the first step in three-line heterosis breeding. Therefore, it is imperative to identify maintainers and restorers from local germplasm for development of component lines in a hybrid Programme (Ali *et al.*, 2014).

The WA-CMS line is derived from wild rice and belongs to the sporophytic CMS/Rf system, in which more than 90% of pollen sterility can be rescued by one of two major restorer genes, *Rf3 or Rf4*, in F_1 hybrid plants (Li *et al.*, 2007). BT

type CMS is restored by nuclear fertility restorer gene *Rf1*, which was mapped on chromosome 10 (Akagi *et al.*, 1996). HL type fertility restoration gene *Rf5* was also mapped on chromosome 10 (Huang *et al.*, 2003). Bazrkar *et al.*, (2008) tagged four Rf genes for WA –CMS system using SSR markers on chromosomes 1 (*Rf3*), 7 (*Rf4*), 10 (*Rf6*) and 12 (*Rf7*) by recessive class analysis. In rice, a variable number of fertility restorer genes can restore complete fertility of certain CMS line in various restorer lines. One or two dominant restorer alleles (*Rf3* and/or *Rf4*) are usually suggested to be responsible for the fertility of WA-CMS (Tan *et al.* 1998). *Rf* genes tagged with closely linked SSR markers would be facilitating marker assisted selection (MAS) in hybrid rice breeding program by reducing time and workload for identifying potential restorers.

To develop a PCR-based molecular marker closely linked to the restorer gene in the wild-abortive (WA) type of cytoplasmic male sterility (CMS) system, Runchun *et al.*, (2000) used F_2 population consisting of 210 rice plants from a cross between Zhenshan 97A and the strong restorer line IR24 for mapping. An ISSR marker, UBC-835, generated polymorphism between the two parents. Linkage analysis in the mapping population showed that UBC-835 was closely linked to the restorer gene of the CMS-WA system and the distance between UBC-835 and the restorer gene was 3.58 cM. The same result was obtained by linkage analysis with OSR33 located on chromosome 10 in the rice genome by screening more than 150 SSR loci, however, no SSR locus was found to link with the restorer gene in the polymorphic SSR loci on chromosomes 1 and 7.

Jing *et al.* (2001) mapped *Rf4* locus on long arm of chromosome 10 at a genetic distance of 3.7 cM and 3.4 cM from RM171 (OSR33) and RM228 respectively by SSLP markers analysis of F_2 population from a cross between Zhenshan 97A and a strong restorer line IR24. They also mapped RM244, another SSLP marker on the short arm of chromosome 10, linked with a fertility restorer locus in an F_2 population from a cross between Zhenshan 97A and a weak restorer line IR64.

Kumar *et al.* (2002) conducted an experiment during the wet season of 2001 at Pusa, Bihar, India. Three cytoplasmic male sterile lines of rice were

randomly crossed with 9 elite lines/genotypes, and the 27 hybrids obtained were screened to identify the maintainers and restorers based on spikelet fertility reaction. Gautam, Dhanlaxmi and Prabhat were identified as restorers and Pusa 1040 as maintainer for the CMS line IR 58025A. Dhanlaxmi, Saroj, Pusa 1107, PSRM-1-16-48-1 and RAU 1411-4 were identified as restorers and Gautam as maintainer for the CMS line IR 68897A. Prabhat, Pusa 1040, PSRM-1-16-48-1 and RAU 1411-10 were identified as restorers and RAU 1411-10 as maintainer for the CMS line IR 68886A

Komori *et al.* (2003) conducted genetic analysis of two near isogenic lines from Koshihikari (japonica variety) using nine RFLP markers (converted to PCR based markers) and mapped the *Rf1* locus between S12564 Tsp509I and C1361 MwoI with the genetic distance between the two marker loci estimated to be 0.3 cM.

Liu *et al.* (2004) carried out the high-resolution mapping of the *Rf* gene using RAPD and microsatellite markers in three BCF_1 populations. The genetic linkage analysis revealed that two *Rf* loci respond to cms-HL and these are located in different regions of chromosome 10. The loci, *Rf5* co-segregates with the SSR marker RM3150 and is flanked by RM1108 and RM5373 at a distance of 0.9 cM and 1.3 cM respectively. Another *Rf* locus *Rf6* (t), co-segregates with RM5373, and is flanked by RM6737 and SBD07 at genetic distances of 0.4 cM.

Fujii and Toriyama (2005) carried out the characterization of the ms-CW pollen grains and mapping of the restorer gene for ms-CW-type CMS. The genetic linkage analysis revealed that five SSR markers (RM6737, RM304, RM171, RM5841 and RM228) on the long arm of chromosome 10 were linked with the *Rf4* gene. *Rf4* was flanked by two SSR markers RM171 and RM6737 at distances of 3.2 and 1.6 cM, respectively. Also, within the region between *Rf4* gene and RM171, two sterile-specific markers AB443-400 and AB443-500, located at 0.5 and 1.03 cM from the gene (Ahmadikhah and Karlov, 2006)

Rosamma and Vijayakumar (2005) observed differential fertility reactions by most of the genotypes when seven cytoplasmic-genic male sterile (CMS) lines of rice having wild abortive (WA) cytoplasmic male sterility source and one

having *Oryza perennis* CMS source were crossed with 34 entries to assess their maintainer/restorer behavior. Among the genotypes tested, 'Annapoorna', 'Kanchana', IR 36, 'Mattatriveni' and 'Aiswarya' were recognized as effective restorers for WA cytoplasmic male sterile lines. 'Jyothy' produced completely sterile hybrids with all CMS lines. 'Aruna', 'Pavizham' and PTB 10 were maintainers for five CMS lines

Bisne and Motiramani (2005) developed thirty-two rice hybrids from the crosses between 4 cytoplasmic male sterile (CMS) lines with wild abortive (WA) cytoplasmic background (IR68885A, IR62829A, DRR2A and PMS10A) and 8 testers (BKP232, R827-287, Pusa Basmati, R1060-1674-1-1, R714-2-103, Culture 1001, Super rice 2 and R304-34) through line × tester design. The identification of maintainer and restorer lines was conducted by observing spikelet and pollen fertility. A very low magnitude of pollen and spikelet fertility was observed for the hybrids. The hybrids showed more than 70% spikelet fertility and 80% pollen fertility. R304-34 was an effective maintainer for IR68885A and was an effective restorer for IR62829A. R827-287 was an effective maintainer for IR62829A and was an effective restorer for IR68885A, DRR2A and PMS10A.

Pradhan *et al.* (2006) recorded fertility restorer namely RP-3644-36-15-8-4, RP-3644-41-9-5-5, 15392, TM 970267 and RP-3644-36-15-8-4 for CMS Pusa 3A line based on the F_1 fertility restorer study. The results indicated 11 maintainer lines for CMS Pusa 3A.

Durai and Nadarajan (2007) determined pollen and spikelet fertility of the hybrids and found seven male parents, *i.e.* BR 736-20-3-1, IR 31406, IR 50400-641-2-2-2, IR 8866-20-3-1-4-2, MDU 3, MDU 4 and Ponni, as effective restorers for all the five male sterile lines, *i.e.* PMS 9A, PMS 10A, V 20A, IR 58025A and IR 62829A. The other three pollen parents, *i.e.* BR 20370-B-2, IR 20 and IR 49457-33-1-2-2-2, were either partial restorers or partial maintainers of the CMS lines. No effective maintainer was identified for any of the CMS lines.

Ahmadikhaha *et al.* (2007) found that fertility in rice WA system is controlled by more than two loci, one on the short arm of chromosome 1, one on the short arm of chromosome 10, one on the long arm of chromosome 10 and an

unknown *Rf* gene in the rice genome. Results also showed that lines IR28, Amol1 and Amol2 carry *Rf4* gene linked with SSR marker RM171 on the long arm of chromosome 10, lines IR36 and IR60966 carry *Rf3* gene linked with SSR marker RM1 on the short arm of chromosome 1, line IR62030 carries *Rf5* gene on the short arm of chromosome 10, and finally line IR24 carries *Rf4* gene on the long arm of chromosome 10 and an unknown *Rf* gene, respectively.

Sattari *et al.* (2007) in their marker-aided selection system for two major *Rf* genes (*Rf3* and *Rf4*) governing fertility restoration in rice, identified a new STS marker (S10019/BstUI) for *Rf4* located on chromosome 10 and RG140/PvuII marker for screening fertility restoration in non-WA cytoplasm such as the Dissi and Gambica CMS systems. Further studies on association of the S10019 marker linked to the *Rf1* locus of BT-CMS (Komori *et al.,* 2003) and to *Rf5* locus of HL-CMS (Huang et al. 2003) with the *Rf4* locus of WA-CMS on chromosome 10 revealed that *Rf*4, in addition to WA-CMS, can also restore fertility in the Dissi and Gambiaca CMS systems.

Bazrkar *et al.* (2008) tagged four fertility restorer genes (*Rf*) to simple sequence repeats (SSR) markers on chromosomes 1, 7, 10, 12 by studying 222 individual plants from a F_2 population of a cross between IR58025A /IR42686R. A new *Rf* locus designated as *Rf7* on chromosome 12 was found to be linked to RM7003 at a genetic distance of 13.3 cM (LOD 6.12) and RM 6344 linked to *Rf4* locus on chromosome 7 . The SSR markers RM443 and RM315 were flanking the *Rf3* gene at a genetic distance of 4.4 (LOD 10.29) and 20.7 cM (LOD 3.98) on chromosome 1 and RM258 and RM591 were flanking *Rf6* at a genetic distance of 4.4 (LOD10.29) and 23.3 cM (LOD 3.39) located on chromosome 10, respectively.

Sattari *et al.* (2008) studied the genetic relationship among three WA, Dissi, and Gambiaca cytoplasmic male sterility (CMS) systems, revealed that for the WA-CMS system, *Rf3* was located at a distance of 2.8 cM from RM490 on chromosome 1 and *Rf4* was located at 1.6 cM from RM1108 on chromosome 10. For the Dissi-CMS system, *Rf*3 was located on chromosome 1 at 1.9 cM from RM7466 and *Rf4* on chromosome 10 was located at 2.3 cM from RM6100. The

effect of *Rf3* on pollen fertility appeared to be stronger than the effect of *Rf4*. In the Gambiaca-CMS system, only one major locus was mapped on chromosome 1 at 2.1 cM from RM576.

Tan *et al.* (2008) investigated the genetic mode and allelism of fertility restorer (*Rf*) genes and the relationship between *Rf* and CMS. They observed fertility of all test-cross F_1 plants shows that the restorer-maintainer relationship is similar for HL-CMS and BT-CMS, while it is variant for WA-CMS and HL-CMS (or BT-CMS), respectively. Genetic analysis of *Rf* genes indicates that HL- or BT-CMS are controlled by single dominant *Rf* gene and WA-CMS is controlled by one or two pairs of dominant *Rf* genes, which reflects the characters of the gametophytic and sporophytic restoration CMS type. It was concluded that there are at least three *Rf* loci in different accessions with *Rf* genes for each CMS type.

Leenakumari *et al.* (1998) reported new restorers and maintainers for WA CMS line in rice. They screened 43 genotypes for their restoration ability in CMS lines belonging to WA cytoplasmic source and found seven to be complete restorers, eighteen effective maintainers and sixteen partial restorers. The frequency of maintainers was higher among local genotypes.

Akhter *et al.* (2008) evaluated two hundred thirty nine (239) rice genotypes to know their status in hybrid rice gene pool during 2005 and 2006. From these 239 test crosses, twelve (12) restorers and 16 maintainers (8 Basmati and 8 non basmati lines) were identified for use in hybrid rice breeding programs. Four basmati and eight coarse lines were identified as restorers from the tested genotypes. Most of the genotypes were found partial restorer and partial maintainer.

Ingale *et al.* (2008) evaluated 220 hybrids, where 145 genotypes were crossed with 10 stable CMS lines. Total 40 effective restorer and 77 mantainers were identified from 145 genotypes for 10 CMS lines. Among the effective maintainer PMS-5A, COMS -9A, IR-62275 A, PMS 11A and IR 68892A were most effective. The average proportion of maintainers, partial mantainers/ partial restorer and effective restorer were 35:47:18.

Jaiswal and Sharma (2009) reported that super Basmati behaved as an effective maintainer for CMS line DRR 2A but was an effective restorer for CMS lines PMS 2A and UPRI 95-17A. Kasturi was an effective maintainer for CMS lines IR68890A and DRR 2A while it was an effective restorer for CMS line PMS 2A.

Khatibani *et al.* (2009) made genetic analysis of restorer fertility gene (s) for WA cytoplasm using F_2 population from a cross between IR42686R and IR58025A. They reported that out of 181 pairs of microsatellite markers, 30 markers (16.6%) showed polymorphism between IR42686R and IR58025A. Microsatellite markers RM443 and RM315 were flanking *Rf3* gene at a genetic distance of 4.4 and 20.7 cM on chromosome 1 respectively while RM6344 was closely linked at a distance of 6.6 cM to *Rf1* on chromosome 7. The third gene *Rf2* was flanked on either side with SSR markers RM258 and RM591 on chromosome 10 at a genetic distance of 4.4 and 22 cM respectively.

Kumar *et al.* (2010) evaluated forty rice hybrid derived from two CMS lines and 20 testers were evaluated for fertility restoration behaviour of testers. Out of 20 testers, eight were found as partial restorers for both CMS lines. Six pollen parents were found as partial restorer for one CMS *i.e.* NMS 4A whereas, same parents were found as restorer for another CMS line *i.e.* IR 58025A and six were found as complete restorer for both the CMS lines.

Babu *et al.* (2010) evaluated 300 F_2 plants of the cross KCMS 26A × IET 19886 were grown under aerobic situation and fifty randomly selected plants were analyzed for per cent spikelet fertility and the individual plants were grouped as effective restorer (>80% SF), partial restorer (21-79% SF), partial maintainers (1-20% SF) and maintainer (< 1% SF).

Umadevi *et al.* (2010) made cross between eight CMS lines with 31 genotypes as 'testers' to get 248 hybrids. The 248 hybrids were subjected to pollen and spikelet fertility analysis. Among the 248 hybrids 168 hybrids were identified as restorers, 52 as partial restorers, 28 as maintainers. Ten testers *viz.*, IR 62037, IR 72865, IR 68427, MDU5, TP1021, RR363-1, RR 347-1, RR 286-1, ACK 99017 and ASD06-08 were identified as restorers for all the eight CMS lines.

Ghara *et al.* (2012) identified IR9 cultivar as a restorer line based on the pollen and spikelets fertility and this line was confirmed by the molecular markers RM1 linked to the *Rf3* fertility restorer gene on chromosome 1 and RM171 markers and RM490 markers on chromosome number 10 and linked with *Rf4* gene.

Khan *et al.* (2012) investigated hundred rice hybrids derived from two CMS lines (IR 58025A and Pusa 6A) and fifty tester (male) following L x T mating design to identify potential restorers and maintainers. Genotypes were categorized as Restorers (> 80% spikelet fertility), Partial restorers (20 to 79% spikelet fertility), Weak maintainers (10 -20% spikelet fertility) and Maintainers (< 10 % spikelet fertility). Out of 50 male lines 19 lines behaved like restorer and 3 lines behaved like maintainer with both of CMS (IR58025A and Pusa 6A) lines.

Shah *et al.* (2012) used nearly 30 SSR markers have been, of which 9 markers were selected from the genomic regions of chromosome 1 and 10 on which *Rf3* and *Rf4* genes were located. Among 30 SSR markers used, 25 SSR loci generated polymorphic patterns and a total of 231 alleles were amplified. The number of alleles per locus ranged from 5 to 17 with a mean of 9.4 alleles per locus. The PIC values for 25 SSR markers varied from 0.74 (RM195, RM10318 and RM258) to 0.92 (RM302).

Soni *et al.* (2006) conducted a study to identify restorers and maintainers from a combination of 3 CMS lines having WA cytoplasm and 9 entries. Among the crosses made NPTR-2 were identified as restorer for IR79156A and IR58025A. Similarly germplasm lines ET-1-10, ET-1-12 and ET-1-1 showed potential restorer for APMS6A, SR-6-SW-8, IR79156A. Genotypes TOX981-11-2-3, SR-6-SW-8 and IRFAN-115 reported as partial maintainer for APMS6A and IR58025A.

Das *et al.* (2013) reported that the narrow genetic base and inadequate number of parental lines are the major constrains for the development of location specific hybrid varieties in rice. The commercial exploration of hybrid in rice has been made possible by identification of parental lines *i,e.*, maintainers and restorers. They conducted a study on ten cytoplasmic male sterile (CMS) lines

and twenty five elite rice genotypes of diverse source of origin to evaluate the genotypes in order to identify potential restorers and maintainers from test crosses. The F_1s (crossed between genotypes and CMS lines) expressed different fertility reactions. Among the tested genotypes, twelve genotypes expressed restorer (R) reaction and two exhibited maintainer (M) reaction. The identified maintainers and restorers were locally well adopted. The identified genotypes can play a pivotal role in hybrid rice development

Bedi and Sharma (2014) reported that the availability of stable cytoplasmic male sterility and fertility restoring system is vital for commercial exploitation of heterosis in rice. The biological material used by them comprised of three CMS lines *viz.,* CRMS 31A, IR 58025A and IR79156A and five testers *viz.,* NPT 453-2, NDR 8054 (IR 77768-25-NDR-B-108-14), CR 2330-3-3-2-1-1, NPT 76-8 and PR-115. Parents NPT 76-8 and CR 2330-3-3-2-1-1 were identified as potential restores, whereas NPT 453-2, NPT 76-8, PR-115 and NDR 8054 (IR 77768-25-NDR-B-108-14) were identified as potential maintainers.

Ali *et al.* (2014) conducted an experiment to identify stable maintainers and restorers for three CMS lines having wild abortive type sterility inducing genes in local rice germplasm. One hundred and twenty nine test crosses were made by using 43 aromatic rice genotypes and three CMS lines. Pollen sterility and spikelet fertility of the raised F_1s were assessed. Out of 43 F_1s with IR58025A, two were found complete sterile (IR58025A/Kaliijira-9 and IR58025A/ Sorukamini-2) and three as full fertile (IR58025A/Agali, IR58025A/Benaful, and IR58025A/Khasa). The local aromatic line, Kalijira-9 and Sorukamini were identified as maintainer for IR68885A which was common with IR58025A. The local aromatic line Benaful was identified as restorer for IR58025A and IR62829A.

Singh *et al.* (2014) conducted a study to screen one hundred breeding lines with SSR markers RM6100 and RM 10313 for the presence of *Rf4* and *Rf3* gene and identified 61 lines carried both *Rf3* and *Rf4* genes and these lines can be utilized in hybrid rice breeding as restorers.

Islam *et al.* (2015) screened 148 exotic rice germplasm to assess pollen sterility at flowering stage. Sixteen genotypes showed 100% pollen sterility status which were considered as completely male sterile lines (A-line). Sixteen genotypes were also identified as completely fertile due to 80% and above pollen and spikelet fertility. For identification of proper maintainer lines, the identified 16 CMS lines crossed with established known maintainer lines *viz.* IR 58025B, IR 62829B, GAN46B, IR 68888B and BRRI1B. Based on pollen male sterility status of the F_1 lines it was indicated that 10 out of 16 were maintained by IR 58025B line, 8 CMS lines were maintained by IR 62829B, three CMS were maintained by IR 68888B and one CMS line was maintained by GAN46B and BRRI1B. Restoration potentiality of identified 16 suspected restorer genotypes were assessed through judgement of pollen and spikelet fertility of F_1's developed through crossing with five standard CMS lines. Based on 80% and above pollen and spikelet fertility of F_1's, seven suspected restorers were identified as restorer against IR 58025A, two against GAN46A, five against IR 62829A and two against IR 68888A.

Kiani (2015) carried out a study with the objective of validating linked SSR markers to *Rf* genes and adopting Marker Assisted Selection (MAS) for restorer/non-restorer line detection in Wild Abortive (WA) type of Cytoplasmic Male Sterility (CMS). Twelve SSR markers reported to be linked to *Rf* genes were analyzed in the mapping population of NedaA/Pajouhesh. Among these, three markers, namely, RM258, RM171 and RM3148 proved to be associated with *Rf* genes.

El-Namaky *et al.* (2016) screened 300 rice cultivars and breeding lines using four simple sequence repeats (SSR or microsatellites) RM171, RM258, RM315 and RM443 to detect the allelic status with respect to the fertility restoring genes (*Rf3* and *Rf4*) . The results revealed that 90 lines had *Rf3*, 65 lines had *Rf4* and 45 lines had *Rf3* and *Rf4* alleles out of 300 lines screened. Offspring of all test lines except HHZ 8-SAL9DT1-Y1, HHZ 5-SAL9-Y3-1 and IDSA 77 exhibited high pollen and spikelet fertility (> 80%), thus confirming they bear R*f* alleles.

Waza and Jaiswal (2016) conducted a study to identify restorers and maintainers using twenty one premium grain quality genotypes of rice with three WA cytoplasmic male sterile lines. Six genotypes (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1 and HUR-JM-59221) were found to exhibit stable restorer behaviour for all the 3 CMS lines (IR-58025A, IR-68897A and Pusa 6A). Moreover, Pusa-44 was found to exhibit the stable fertility restoring ability for IR-58025A, whereas Pusa Basmati-1121 revealed the stable restoring potential for IR-68897A. Three pollen parents (Pusa Basmati-1, Pusa-1460 and HUR-LP-191123) exhibited stable maintainer behaviour for all the 3 CMS lines.

2.3 MORPHOLOGICAL DIVERSITY ANALYSIS

The nature and magnitude of genetic improvement generally depend on the amount of genetic variability present in a population. The major thrust area for such genetic improvement depends on selecting efficient breeding system and identifying desirable parents in hybridization programmes. Genetic diversity plays an important role in plant breeding since progeny originating from diverse parents exhibit greater heterosis and provide broad spectrum of variability in segregating generations (Khush, 1974). Therefore, a meaningful classification of genotypes will enable the breeder to identify the best parents with wide genetic divergence and to utilize some of the selected diverse parents in the hybridization programme.

Chakravorty and Ghosh (2012) assessed genetic divergence among 51 landraces of rice based on 18 traits following Mahalanobis's D^2 analysis with the grouping of 51 rice genotypes into 11 clusters. The maximum intra cluster D^2 value was shown by cluster X followed by cluster IX, III and I, while the inter-cluster value was maximum between cluster IX and X Among the genotypes, maximum contribution towards genetic divergence came from the characters *viz.*, culm lenth, culm diameter and grain length. Hybridization among the genotypes from the cluster I, III, IX and X which had maximum inter-cluster distances and desirable values for flag leaf angle, grain breadth, grain weight, kernel weight, number of primary branches per panicle and number of grains/panicle is likely to produce heterotic combinations and wide variability in segregating generations.

Gosh and Sharma (2012) estimated genetic variability, heritability and genetic advance on 10 diverse parents (3 CMS lines and 7 advance breeding lines) and 21 hybrids of rice. The magnitude of difference between PCV and GCV observed was relatively low for all the characters *viz.*, days to 50 % flowering, flag leaf length, flag leaf width, flag leaf area, plant height, pollen fertility, sterile spikelets per panicle, fertile spikelets per panicle, spikelets per panicle, spikelets fertility, panicle length, grain yield per plant, test weight and head rice recovery, indicating less environmental influence. The high heritability along with high genetic advance were registered as per cent of mean for grain yield per plant, pollen fertility (%), sterile spikelets per panicle, fertile spikelets per panicle, spikelets fertility (%), head rice recovery (%), 1000 seed weight, spikelets per panicle.

Shivaprasad *et al.* (2013) characterized 470 rice (*Oryza sativa* L.) accessions including five checks collected from different regions using 19 quantitative character. A principal components plot and distance between genotypes in different cluster groups were used to group the accessions. The rice genotypes grouped into divergent cluster XII and XVIII are expected to give promising and desirable recombinants in the segregating generations. Also, traits contributing maximum to genetic divergence *viz.*, seed vigour followed by fertile grains/ panicle, fertile grains/ panicle and panicle length may be utilized in selecting genetically diverse parents.

Lakshmi (2013) evaluated seventy genotypes of rice (*Oryza sativa* L.) to study the nature and extent of correlation among yield and yield attributing characters, days to 50 per cent flowering, days to maturity, number of effective tillers per plant, plant height, panicle length, number of grains per panicle, 1000-grain weight, grain yield per plant, kernel length, kernel breadth and L/B ratio. The results revealed that grain yield per plant to be positively and significantly associated with days to maturity, number of productive tillers per plant, plant height and kernel length indicating importance of these traits as selection criteria in yield improvement programmes.

Ketan and Sarkar, (2014) observed a wide range of variability for nineteen quantitative characters in 26 indigenous aman rice cultivars. Five genotypes *viz.*, Sabita, Kamini, Kumorogor, Sadakamisoru and Narkelchari were superior in grain yield per plant and grain L/B ratio simultaneously. The magnitude of PCV was higher than the corresponding GCV for all the characters. High heritability was observed days to 50 per cent flowering, plant height, 1000 grain weight, panicle length, florets number per panicle, kernel length and kernel L/B ratio. Number of grains per panicle recorded the highest genetic advance followed by floret number per panicle, plant height and number of secondary branches. High heritability in conjunction with high genetic advance was registered for plant height, days to 50 per cent flowering and number of secondary branches. High heritability with low genetic advance was observed for panicle length, panicle weight, kernel length and kernel L/B ratio. Grain yield per plant was significantly correlated with number of secondary branches per panicle at phenotypic level while panicle weight, florets number per panicle, number of grains per panicle and fertility percentage at genotypic and phenotypic level. The florets number per panicle imparted the highest positive direct effect on grain yield per plant.

Rai *et al.* (2014) conducted an experiment with 40 genotypes of rice during *Kharif* 2013. The data were recorded for 13 quantitative characters to study genetic variability, heritability, genetic advance, correlation and path coefficient. High GCV and PCV were observed for grain yield per plant and biological yield per plant. High heritability coupled with high genetic advance as percent mean for all the character except days to flowering, days to maturity, number of tillers per plant, number of panicle per plant and plant height. Correlation and path-coefficient analysis, concluded that, biological yield per plant and harvest index exhibited maximum positive direct effect on grain yield seems to be primary yield contributing characters and could be relied upon for selection of genotypes to improve genetic yield potential of rice.

Kumar *et al.* (2014) estimated nature and magnitude of divergence in 134 rice germplasm accessions during *kharif* 2013 using Mahalanobis D^2 statistics. The genotypes were grouped into five clusters. Cluster means indicated

considerable differences in the mean values of different traits. The genotypes of cluster II and cluster III exhibited high seed yield. 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. The hybridization involving genotypes belong to these clusters is expected to give desirable segregants in rice breeding programmes. Number of filled grains/panicle contributed highest towards divergence.

Rajput *et al.* (2014) examined the genetic diversity existing among 41 genotypes of rice. High estimates of GCV and PCV were observed for economic yield followed by harvest index and flag leaf length. High heritability coupled with high genetic advance was recorded for spikelets per panicle. The forty-one genotypes were grouped into seven heterogeneous clusters. The characters such as grain yield, harvest index, number of panicles per plant and biological yield per plant should be given top priority for effective selection. The present investigation revealed that Cluster V and VII are most diverse to each other and the genotypes constituted in these clusters may be used as parents for future hybridization.

Allam *et al.* (2014) assessed genetic diversity among 23 genotypes of basmati rice representing different regions of India on the basis of yield and quality characteristics utilizing Mahalanobis D^2 analysis. Based on the genetic distance (D^2 values), the rice genotypes were grouped into six clusters. The highest genetic divergence was observed between the clusters IV and I exhibiting wide diversity. The genotypes representing cluster VI were more yielding combined with excellent cooking quality. Among different traits, plant height, kernel length, elongation ratio and amylase content had maximum contribution towards total divergence may be used as selection parameters in segregating generations.

Lingaiah *et al.* (2014) carried out an experiment to estimate the variability parameters for quantitative traits in 33 rice cultivars. Analysis of variance revealed the existence of significant differences among genotypes for all traits studied. The genotypic and phenotypic coefficients of variations were moderate to high for No.

of grains/panicle, test weight and yield. High heritability coupled with high genetic advance as percent of mean was observed for number of grains/panicle, test weight and yield indicating the role of additive gene in controlling these characters.

Bharathi *et al.* (2016) studied genetic divergence among 32 rice (*Oryza sativa* L.) genotypes using D^2 technique for ten characters. The 32 genotypes were grouped into 9 clusters. In D^2 analysis, filled grains per panicle followed by ear bearing tillers per panicle and days to 50% flowering contributed maximum for the divergence. The inter-cluster distance was maximum between clusters V and IX and between clusters V and VII. Based on these studies, crosses may be made between genotypes of clusters IX (MTU 1010, BPT 2741, and MTU 1001) and V (BPT 1768) followed by clusters VII (RGL 2537) and cluster V (BPT 1768) to obtain new desirable recombinants in rice.

2.4 HETEROSIS IN RICE

Although rice is a self pollinating crop, strong heterosis is observed in their F_1 hybrids. Heterosis or hybrid vigour in the first generation (F_1) seeds, obtained by crossing genetically distant breeding lines, is well known in crop breeding (Shull, 1952). Heterosis is the superiority of F_1 hybrids over their parents. Heterosis may be positive or negative depending upon the breeding objectives. Both positive and negative heterosis is useful for crop improvement. The first scientist to report the phenomenon of heterosis in rice was Jones (1926). The idea of commercially exploiting hybrid rice was first mooted in India by Richharia (1962).

Hybrid seed production can only be economized if standardized cytosterile lines and proper restoration systems are available. Yuan and Fu (1995) detailed all the stages that should be followed to obtain male-sterile lines, restorers and maintainers. Hybrid rice would then be produced through a so-called three-line system, Cytoplasmic male sterility and the fertility restoration system have been primarily used to develop rice hybrids in and outside China (Virmani *et al.*, 2003). This technology has served to be an attractive and alternative to break yield barriers and boost rice production under fragile environmental conditions as

hybrid rice has a yield advantage of 15-20 per cent over the conventional high yielding varieties (Virmani *et al.*, 2003).

Joshi (2001) studied heterobeltiosis and standard heterosis in 14 crosses between rice (*Oryza sativa* L.) cultivars (improved and landraces) and three wild aborted male sterile parents. These crosses showed marked variations in the expression of heterobeltiosis and standard heterosis for yield and yield components. Grain yield manifested highly significant heterobeltiosis and standard heterosis in five crosses. Heterobeltiosis ranging from –55 to 139% and standard heterosis from –11 to 369% were observed. Highest heterotic effect among the yield components was for panicle number plant-1 followed by spikelet number and panicle length. With appropriate choice of parental lines, it is possible to develop F_1 rice hybrid possessing distinct yield superiority over the best-inbred lines.

Malarvizhi *et al.* (2003) identified thirteen hybrid combinations as promising based on standard heterosis for yield ranging from 18.5 per cent to 40.0 per cent. The hybrid IR 58025 A/IR 60821-34-1-2 R recorded the highest standard heterosis of 40.0 per cent followed by IR 58025 A/IR 8666-30-3-1-4 R (39.5%), IR 58025 MC 10 R (35.6 %), IR 69616 A/IRRI 3054 (29.6 %) and IR 68888 MR 4595- 54-1 R (28.4 %).

Vanaja and Babu (2004) reported that yield increase was largely due to significant and favourable heterosis in yield components *viz..,* number of spikelets panicle^{-1}, panicle length, leaf area/plant (at maximum tillering stage) and number of panicles m^{-2}. Five top heterotic crosses over their mid and better parents for each trait were identified.

Faiz *et al.* (2006) evaluated the F_1's developed from lines x two tester mating design along with parental genotypes to estimate heterosis for yield and yield influencing traits *i.e.,* plant height, number of productive tillers per plant, number of spikelets per panicle, number of filled grains per panicle, sterility % and grain yield. Both the CMS lines reduced the plant height of their respective F_1 hybrids. The highest positive heterosis over better parents was observed for grain

yield (41.83%), number of productive tillers per plant (11.04%) and number of filled grains per panicle (7.39 %) in the cross of IR69616A x Basmati 385.

Sarial *et al.* (2006) evaluated heterotic potential of basmati fertility restorer for grain yield and its component traits. Five of the basmati restorer having fertility restoration > 80% produces hybrids with heterobeltiosis ranging from 20.64 to 150.66% and superiority over check ranging from 15.17 to 284.55%. Hybrids were superior to their parents for grain yield per plant, biological yield per plant, days to 50% flowering, number of effective tillers per plant and number of primaries per plant indicated substantial heterosis. However, superiority of parents over hybrids for harvest index indicated negative heterosis for harvest index, 1000 grain weight and days to maturity.

Pandey and Tripathi (2006) evaluated hybrids developed using two WA CMS line and 15 restorers under 8 environments. All the hybrids exhibited significant and positive heterobeltiosis and standard heterosis for grain yield per plant ranging from 1.33-55.32 and 1.36-47.88 respectively. Heterosis for single plant yield was achieved due to posistive and significant heterosis for component characters like number of seeds/ panicle, panicles/plant, 1000 seedsweight, panicle length, biological yield/plant and harvest index.

Singh *et al.* (2006) developed thirty six hybrids utilizing two CMS lines and 18 testers. These were studied for extend of heterobeltiosis and standard heterosis in ten characters under irrigated condition. Standard heterosis for grain yield/plant was manifested through more number of ear bearing tillers/ plant, fertile grains/ panicle, days to 50% flowering, 100-grain weight, biological yield and harvest index.

Torres and Geraldi (2007) estimated some useful parameters which can be used to investigate the genetic control of agronomic characters in crosses combining cold tolerance and productivity. A partial diallel design was used in crosses between six tropical *indica* rice cold susceptible genotypes (group 1) and seven *japonica* or *indica/japonica* cold tolerant rice genotypes (group 2). Parents and crosses were evaluated for agronomic characters under field conditions in two different experiments. The results showed significant mid-parent heterosis for all

characters (plant height, tiller number, days to 50% flowering, panicle length, grains per panicle, sterility, and one-hundred grain weight).

Akhter *et al.* (2008) evaluated 65 test crosses and identified 7 restorers and 11 mantainers for use in hybrid research programme. In addition seven best heterotic combinations were also identified from the test crosses on the basis of filled grains per panicle and spikelets fertility percentage. All these seven heterotic hybrids have more than 75% spikelet fertility, acceptable maturity days and plant height.

Sharma and Malik (2008) conducted an experiment with 70 hybrids evolved from crossing 14 elite *indica* lines and 5 medium duration testers in a line × tester fashion to estimate extend and magnitude of heterosis for grain yield and its components. High heterosis in yield was accompanied by heterosis for one and more of the major yield components. The maximum heterosis for grain yield was 63.27% in case of UPRI 99-73-1 × Jaya.

Venkatesan *et al.* (2008) revealed that estimates of heterosis values were low for physical characters when compared to yield and yield components. Nine hybrids showed positive and significant heterosis over mid parent, better parent and standard check for grain yield per plant, of which AD95157/IR50, MDU5/IR50, AD95157/ADT143, MDU5/ADT36, AD95157/ADT36 and AD95137/ADT36 were top rankers.

Ratnakar *et al.* (2009) using 30 hybrids estimated the heterosis in CMS based hybrids of rice with respect to grain yield and its components. The result indicated among the hybrids, PMS 8A x NDRK 5028 recorded maximum grain yield with 105.80 and 40.56% heterosis over the better parent and standard cultivar, respectively

Amudha *et al.* (2010) undertook an experiment to identify heterotic rice hybrids for aerobic condition based on physiological and root characters associated with water stress tolerance in rice. Panicle harvest index, a substitute for spikelet fertility is used as a secondary trait in the selection of drought tolerant genotypes. Four hybrids viz., IR 68885A / IR 73718-3-1-3-3, IR 67684A / CT-6510-24-1- 2, IR 70369A / IR 73718-3-1-3-3 and IR 70372A/ PSBRC 80

exhibited heterotic vigour for yield and maximum number of yield components and showed better adaptability to aerobic conditions. These hybrids can be commercially exploited under aerobic condition.

Chandirakala and Thiyagarajan (2010) explored heterosis in 88 crosses of indica rice hybrids. The magnitude of relative heterosis, heterobeltiosis and standard heterosis with (CORH 2 and ADTRH 1) were estimated. Top yielding hybrids viz., GD 98049 × IR63875-196—2-2-1-3, GD 98014 × TKM 11, GD 98049 × TKM 11 and GD99017 × TKM 12 exhibited significant standard heterosis over CORH 2 and ADTRH 1. Most of the high yielding hybrids manifested significant positive heterosis for yield contributing traits viz., number of productive tillers per plant, panicle length, number of filled grains per panicle, spikelets fertility and 1000 grain weight.

Dar (2013) evaluated 27 F_1 hybrids raised in Line x Tester mating design by pollinating three CMS lines SKUA-7A, SKUA-11A and IR-68888A with nine testers namely Jhelum, Pusa Sugand-5 (PS-5), China-988, Shalimar rice-1, SKAU-382, SKAU-389, K-08-59, K-08-60 and K-08-61 in *Kharif* 2011. Significant and desirable heterotic effects were observed for most of the characters studied. Maximum standard heterosis for grain yield per plant was registered by SKAU-389 x K-08-60, Jhelum x K-08-60, SKAU-389 x PS- 5, Jhelum x SKAU-389, China-988 x SR-1, PS-5 x SKAU-7A, K-08-60 x SKAU-11A and K-08-61 x IR-68888A.

Rahimi *et al.* (2010) evaluated Fifteen F_1 hybrids and their parents generated by half diallel crosses of six diverse rice cultivars. Assessment of standard heterosis based on check variety Dorfak showed that there was significant heterosis for all the traits studied in the 15 hybrids. For grain yield, the Dorfak × Domsefid cross had the highest heterosis. This hybrid had good heterosis values for many traits such as growth period, reproductive period and 1000 grain weight and was recommended as the most promising combination for developing high yielding hybrid rice varieties.

Soni and Sharma (2011) in their studies on heterosis for grain yield among 27 hybrids from three CMS lines with nine testers showed highly significant

differences for all the traits.They observed the relative heterosis ranged from -81.7 to 78.89% and heterobeltiosis from -87.69 to 60.67% for seed yield per plant. They also obtained five hybrids *viz.,* IR58025A /IRFAN-115, IR58025A/SR-6-SW-8, IR58025A /ET 1-13, APMS 6A /ET 1-12, and APMS 6A /NPTR-2 showed significant heterosis for grain yield and seven hybrids indicated significant negative heterosis for earliness and three hybrids for plant height.

Tiwari *et al.* (2011) observed significantly superior manifestation of heterobeltiosis for grain yield for 43 hybrids ranging from 11.63 to 113.04% and 46 hybrids over standard check (Sarjoo 52) ranging from 10.48 to 71.56%. Most of crosses showed significant heterosis for number of fertile spikelets and number of spikelets per panicle. Heterobeltiosis and standard heterosis ranged from -15.12 to 9.49% for pollen fertility percent and 0.37 to -3.65% for panicle length.

Kumar *et al.* (2012) conducted 8 x 8 diallele analysis of 28 F_1's excluding reciprocals revealed that 23 crosses have significant positive standard heterosis for grain yield per plant. They showed that crosses *viz*; Pant sugandh dhan15 x UPR 2845-6-3-1, Pant sugandh dhan15 x UPR 3003-11-1-1 and Pant sugandh dhan17 x UPR 3003-11-1-1 exhibited high standard heterosis for grain yield (309.53, 244.45 and 255.56%), biological yield (158.47, 150 and 124.58%) and for harvest index (58.6, 37.8 and 58.4%).

Perera *et al.* (2013) had chosen two single crosses, Bg 379-2 x Mu 8-7 (Cross 1) and Bg 379-2 x Bw 400 (Cross 2) to study the heterosis and genetic effects in yield related agronomic characters of rice. F_1 hybrids along with their parents were evaluated in a Randomized Complete Block Design with four replicates. Cross 1 showed significant heterobeltiosis for leaf width (LW), panicle length (PL) and plant height (PH) while cross 2 showed significant heterobeltiosis for LW, culm length (CL), and PH. Additive genetic effect is higher than the dominance effect in days to 50% flowering (DF), CL and panicles per plant (PP) in Cross 1, while dominance effect is higher than the additive genetic effect in seedling height, LW, CL and PH in Cross 2. Bg 379-2 x Mu 8-7 is a more potential cross that agreed with breeding objectives, especially to extract short duration high yielding breeding lines.

Seesang *et al.* (2014) analysed 31 test crosses and identified 6 restorers and 9 maintainers based on pollen fertility. The estimation of heterosis to select superior genotypes was conducted on 12 hybrids resulting from crosses between 6 restorers and 2 male sterile lines. The results showed that two hybrids had a high hetero- beltiosis and standard heterosis, with yields of 7940 and 6810 kg/ha, respectively.

Bhatti *et al.* (2015) determined combining ability and heterosis for yield and its component traits in rice. The material consisted of F_1 hybrids of 30 crosses developed by crossing 10 lines with three testers.They have identified two crosses namely HPR 2639 X HPR 2143 and HPR 2529 X HPR 1156 having high heterosis over standard check for grain yield/plant biological yield/plant, grain fertility and plant height.

Sao and Motiramani (2015) analysed 24 F_1 hybrids derived from three female and eight male lines. The observations were recorded on 28 quantitative and qualitative traits. The highest heterotic effects observed for mid parents, better parent and standard heterosis were 233.33%, 97.50% and 60.14% for grain yield per plant noted for the crosses IR 58025A/R1679-1674-1-234-1.

2.5 INHERITANCE OF *Rf* LOCI AND BULK SEGREGANT ANALYSIS

Cytoplasmic male sterility (CMS) caused by lesions or rearrangements of mitochondrial genome is unable to produce functional pollens. But CMS can be restored by nuclear genes. The combination of cytoplasmic male sterility (CMS) in one parent and a restorer gene (*Rf*) to restore fertility in another are indispensable for the development of hybrid varieties. Therefore, the CMS systems are widely used for hybrid seed production (Yuan, 1992). However, searching for restorer genes is a good example where phenotyping is very time consuming and requires determination of spikelet sterility in testcross progeny (Komori *et al.*, 2003, Yao *et al.*, 1997 and Ahmadikhah and Karlov, 2006).

Khatibani *et al.* (2009) made genetic analysis of restorer fertility gene (s) for WA cytoplasm using F_2 population from a cross between IR42686R and IR58025A reported a 3 separate genes governing this trait through triplicate

dominant epistatic model giving a ratio of 63:1 (pollen fertile:sterile single plants), indicating three pairs of duplicate dominant alleles govern the trait

Genetics of fertility restoration in WA-CMS lines has already been investigated. However, conclusions regarding the number of nuclear genes controlling fertility restoration depend on the materials and methods used. The genetics of fertility restoration in WA-CMS lines has been shown to follow monogenic (Mishra, 2001) digenic (Bharaj *et al.,* 1991), digenic with different types of interaction (Sarker *et al.*, 2002), trigenic (Sarker *et al.,* 2002), and trigenic interactions (Huang *et al,* 1987). Nevertheless, most investigators tended to agree that restoration of wild abortive (WA) type CMS in rice is controlled by two nuclear genes (Zhang *et al.,* 1997, Yao *et al.,* 1997, Zhang *et al.,* 2002).

Teng and Shen (1994) reported that the fertility restoration of cytoplaslmic male sterility (CMS) in rice (BT-type sterility) controlled by a dominant restoring gene, *Rf1* located on chromosome 10 (Shinjyo, 1975). For the WA-type, however, either one or multiple genes were found to be involved (Gao, 1981). They crossed three Japonica restorer lines, C57 (an elite restorer for hybrid rice in North China), ZH157 (a "wide compatible" restorer) and T65R (established by Shinjyo 1975) with two Japonica CMS lines, Shuang-Bai (SBA, a BT type) and 02428A (a WA-type derived from Zhen-Shan 97A/024287) for genetic analysis.

Yao *et al.* (1997) identified *Rf* gene containing regions by surveying two bulks, made of 30 highly fertile and 46 highly sterile plants from a large F_2 population of the cross between Zhenshan 97A and Minghui 63, with RFLP markers covering the entire rice genome. The survey identified two likely R*f* genes, one nuclear fertility restorer gene (*Rf4*) for the WA type CMS was mapped on chromosome 10, 3.3 cM from G4003 and a distance of 22.4 cM interval between G4003 and C234 and another R*f* locus on chromosome 1 located closest to RG532 . They also identified fertility segregation of 15:1 ratio indicating that fertility restoration in this population is controlled by two major duplicate dominant genes.

Jing *et al.* (2001) conducted a study in F_2 population consisting of 210 excessive sterile individuals from a cross between Zhenshan 97A and a strong

restorer line IR24 was used for mapping of *Rf4*. The genetic distance from *Rf4* locus to RM171 (OSR33) and RM228 on long arm of chromosome 10 were 3.7 cM and 3.4 cM, respectively. These were the two closest SSLP markers flanking the *Rf4* locus. The two SSLP markers gave promise of application in molecular marker-assisted selection (MAS) for fertility restorer lines of the CMS-WA system.

The inheritance and molecular mapping of a fertility restorer gene in basmati rice quality restorer line PRR-78 was carried out by Mishra *et al.* (2003) using an F_2 mapping population from the cross IR58025A x PRR-78 employing microsatellite markers. Dominant monogyny control of fertility restoration was observed and further confirmed by test cross data. Out of the 44 sequence tagged microsatellite (STMS) markers used in the bulked segregant analysis, four differentiated the fertile bulk from the sterile bulk as well as the two parental lines from each other. One of these markers, RM258 located on chromosome 10, was found linked to the restorer gene at a distance of 9.5 cM.

Komori *et al.* (2003) conducted genetic analysis of rice in a segregating population consisting of 1042 plants using nine markers. The *Rf1* gene was mapped between S12564 *Tsp*509I and C1361 *Mwo*I. The genetic distance of the two marker loci was estimated as 0.3 cM. Based on the map information of the *Rf1* gene, it will be possible to introduce a limited chromosomal segment of the *Rf1* region derived from a donor into japonica rice varieties efficiently and effectively by marker-assisted selection (MAS).

Huang *et al.* (2003) carried out Bulked segregant analysis (BSA) in a BC_1 population derived from congguang 41A/MiYang 23//congguang 41B to map the nuclear fertility restorer gene *Rf5* for HongLian (HL) cytoplasmic male sterility. The parents and two bulks representing extremely fertile and sterile plants, respectively, were screened for polymorphism with 20 microsatellite primer pairs on chromosome 10, chosen on the basis of previous research. MRG4456 is linked to the fertility restorer gene *Rf5* at a distance of 1.57 cM, and another newly developed microsatellite primer HL01, was located at a distance of 0.63 cM to

Rf5. Closely linked DNA markers will facilitate not only breeding but also the purity management of hybrid seeds.

Liu *et al.* (2004) analyzed the inheritance patterns in the cms-HL/*Rf* system using the segregation of fertility in three BCF_1 populations, CG-41A × (CG-41B × MY23), YT-A × (YT-B × 93-11) and YT-A × (YT-B × MY23). Genetic analyses have made it clear that two major genes are generally involved in fertility restoration in the CMS-WA lines, whereas one gene is required to restore fertility to the CMS-BT lines.

Tao *et al.* (2004) studied the inheritance of male sterility restoration gene in testcross populations revealed that the complete restoring ability of WAB450-11,WAB450-13,WAB450-14, IRAT216, IRAT359, and the partial restoring ability of IRAT104 were controlled by dominant genes and the gene in WAB450-13, WAB450-14 and IRAT216was allelic or identical to *Rf1*.

Fujii and Toriyama (2005) carried out the characterization of the ms-CW pollen grains and mapping of the restorer gene for ms-CW-type CMS. Pollen grains of the male-sterile plants appeared to be normal and viable based on the fluorochromatic reaction test, but they did not germinate on normal stigmas. The 1:1 segregation of fertile and sterile plants in a BC_1/F_1 population from a cross between W1-R and a maintainer line demonstrated that fertility restoration is controlled by a single gene and fertility restoration functions gametophytically. They designated the fertility restorer gene *Rf*cw.

Sharma *et al.* (2005) observed the pollen and spikelet fertility in F_2 population of the cross involving PMS 2A and P 1292, a restorer line identified in basmati background, corresponded a trigenic segregation ratio of 27:30:7 for fully fertile, semi fertile plus semi sterile and completely sterile plants indicating three dominant genes imparted normal fertility in the segregating plants. Assuming two dominant genes one with strong effect (*Rf1*) and another with weak effect conferred the fertility of the plant and one dominant gene allowed the expression of weak restorer gene.

Rongbai *et al.* (2005) studied inheritance of fertility restorer of TGMS lines using UPRI 95-140TGMS, a spontaneous mutant with known fertility–

sterility transformation behavior under different temperatures Forty-four male fertile *indica* rice lines/ varieties were used as male parents to cross the TGMS line for inheritance study. Study has displayed mostly digenic nature of inheritance of TGMS and in some cases trigenic inheritance was evident. The monogenic pattern of segregation for fertility–sterility (3F:1S) in F_2 was indeed digenic in nature (12F:3PS:1CS), while some segregations (15F:1S) were trigenic (60F:3PS:1CS). The segregation patterns were strongly related to the genetic background. Thus, the results of the present study indicate the involvement of three pairs of independent and major recessive genes in the inheritance of TGMS in UPRI 95-140TGMS.

Ahmadikhah *et al.* (2007) carried out genotyping of rice line at the restoring fertility (*Rf*) loci, by crossing 38 lines with a sterile tester (*rfrf*) line. Pollen fertility test was performed to identify sterile and fertile plants in F_2 gneration. Bulked segregant analysis (BSA) in F_2 showed that fertility in rice WA system is controlled by more than two loci, one on the short arm of chromosome 1, one on the short arm of chromosome 10, one on the long arm of chromosome 10 and an unknown *Rf* gene in the rice genome. Results also showed that lines *IR28*, *Amol1* and *Amol2* carry *Rf4* gene linked with SSR marker RM171 on the long arm of chromosome 10, lines *IR36* and *IR60966* carry *Rf3* gene linked with SSR marker RM1 on the short arm of chromosome 1, line IR62030 carries *Rf5* gene on the short arm of chromosome 10, and finally line *IR24* carries *Rf4* gene on the long arm of chromosome 10 and an unknown *Rf* gene, respectively.

Sattari *et al.* (2008) analyzed the genetic relationship among three cytoplasmic male sterility (CMS) systems, consisting of WA, Dissi and Gambiaca, were studied. The results from an inheritance study showed that the pollen fertility restoration in all three CMS systems was governed by two independent and dominant genes with classical duplicate gene action.

Khatibani *et al.* (2009) did genetic analysis of restorer fertility gene (s) for WA (wild abortive) cytoplasm using F_2 population from a cross between IR42686R and IR58025A reported three separate genes governing this trait through triplicate dominant epistatic model giving a ratio of 63:1 (pollen fertile:

sterile single plants), indicating three pairs of duplicate dominant alleles govern the trait.

Alavi *et al.* (2009) mapped a fertility restorer gene using SSR and CAPS markers in rice line IR36 in a F_2 population developed from the cross Neda-A×IR36. The genetic linkage analysis indicated that three SSR markers (RM1, RM3233 and RM3873) and one CAPS marker (RG140/EcoRI) were linked to *Rf*3 on the short arm of chromosome 1 and fertility restoration governed by two dominant genes interacting in duplicate fashion. *Rf3* flanked by two SSR markers RM1 and RM3873 at distances of 5.6 and 14 cM, respectively.

Sheeba *et al.* (2009) analysed nine SSR and three CAPS markers reported to be linked to *Rf* genes along with two previously unreported SSR marker on two mapping population namely an F_2 population derived from the cross IR 62829A/ KMR 3 and a BC_1F_1 population from the cross IR 62829A/ IR 10198A/ IR 62829 A. In both the populations studied, the trait of fertility restoration was observed to be under digenic control. Result indicated RM6100 was observed to be closely segregating with fertility restoration in both the mapping populations and was located at a distance of 1.2 cM. The largest phenotypic variation was accounted for the region located between RM311 and RM6100.

Molecular tagging of fertility restorer genes for WA type of CMS system with SSR markers showed that four markers RM258, RM171, RM591 and RM3148 produced polymorphic bands between two parents. Linkage analysis of F_2 recessive class showed that RM258 and RM171 (on chromosome 10) flanked to restorer gene *Rf4* at the distances of 3.1 and 6.3 cM respectively. In this study polymorphism was not detected for *Rf3* gene using SSR markers RM1, RM443, RM315 and RM294 on chromosome 1 of rice but new SSR marker RM3148 was found that linked with *Rf* locus at a genetic distance of 19.7 cM (Nematzadeh and Kiani, 2010).

Hossain *et al.* (2010) conducted a study using three *indica/japonica* restorers (P1277-100, P1266-89, and P1266-8) and three 'WA'-type cytoplasmic male sterile lines (Pusa 3A, Pusa 5A and Pusa 6A) revealed that two or three major genes govern the fertility restoration, with epistatic interactions that differed

from cross to cross. Crosses Pusa 6A/P1277-100 and Pusa 3A/P1266-89 showed a segregation ratio of 12:3:1 and 2:1:1 in F_2 and BC_1 generations. The restorer P1266-89, when crossed with Pusa 5A, segregated in different digenic ratios of 9:3:4 and 1:1:2 in F_2 and BC_1 generations. The same restorer P1266-89 when crossed with Pusa 6A, segregated in ratios of 27:30:7 and 1:2:1 in F_2 and BC_1 generations, respectively, indicating three major genes governing fertility restoration. Restorer P1266-8 when crossed with Pusa 5A and Pusa 6A, gave the same segregation ratios of 27:30:7 in F_2 and 1:2:1 in BC_1 generation, indicating that fertility restoration is also governed by three major genes.

Nematzadeh and Kiani (2010) crossed between an Iranian CMS line Neda A with DN-33-18. The inheritance of fertility restoration in F_2 population of this cross was evaluated at flowering and grain filling stages. Pollen staining test showed segregation ratio of 15:1 (fertile: sterile), representing two nuclear independent dominant genes controlling the trait carried by fertile parent DN-33-18. Segregation for spikelet fertility in F_2 confirmed the results of pollen fertility test.

Waghmode and Mehta (2011) reported fertility restorer genes are governed by two independent genes and one is stronger than other. They observed dominant epistasis with a ratio of 12:3:1 and 2:1:1 in F_2 and back cross generations of five crosses, 9:6:1 and 1:2:1 in F_2 and back cross generations of four crosses and other 11 crosses exhibited 9:3:4 and 1:1:2 in F_2 and back cross generations.

2.6 COMBINING ABILITY AND GENE ACTION

Roy and Mandal (2001) in combining ability study in rice, revealed significant mean squares for GCA and SCA for yield and yield contributing traits. However, the estimates of SCA variances were found greater in magnitude than GCA variances for yield and most of the yield contributing traits. They also revealed correspondence between good general combiners and *per se* performance for majority of traits.

Reddy (2002) evaluated eight parents and its hybrids produced through diallel cross and reported none of the parents to be good combiner for all the characters. However, the best parents identified were Lunishree and Utkalprabha,

which showed significant positive GCA effects for grain yield and panicle weight, grain number and 100 grain weight. Among the 28 crosses only 11 significant positive SCA effects for grain yield under normal planting.

A 8 x 8 diallel crossing study for various agro-physiological characters in rice conducted by Mehmood *et al.*, (2002) revealed high additive effects for plant height, panicle length, panicle/plant and primary branches/panicle, however, panicle fertility, days to maturity and paddy yield expressed non-additive effects .

Swamy *et al.* (2003) evaluated twenty-four rice hybrids generated by crossing three new CMS lines with eight testers. Among the CMS lines IR 70370A was identified as good general combiner for seed yield plant-1, plant height, filled spikelets panicle-1, total spikelets panicle-1 and per cent spikelet fertility. Among the testers IRBPHN 89 was the best general combiner for seed yield plant-1, filled spikelets and per cent spikelet fertility, while GMR 17 was the best general combiner for earliness. Further, they reported that SCA variance was predominant for all the characters indicating operation of non-additive gene action, however presence of GCA variance was also evident in respect of plant height, filled spikelets panicle-1, total spikelets panicle-1, spikelet fertility per cent and seed yield plant-1 suggesting operation of both additive and non-additive gene action in the genetic control of these characters.

Vanaja *et al.* (2003) were studied twenty-eight hybrids, produced from diallel crossing excluding reciprocals among eight parents, along with the parents for combining ability for yield and 17 yield components. The study revealed importance of both additive and non-additive gene effects in governing yield and most of the yield components with preponderance of non-additive gene action for most of the yield components. Additive gene action was found important for 1000-grain weight, second uppermost internodal length and height of plant at harvest. The parent Vyttila 3 was found to be a good general combiner. The hybrids PK3355-5-1-4 x Hraswa, Vyttila 3 x IR60133- 184-3-2-2, Vyttila 3 x IR36, Vyttila 3 x Mattatriveni and IR36 x Mattatriveni have shown significant favourable *sca* effect for yield and different yield components.

Verma and Srivastava (2004) also found that GCA and SCA effects were highly significant for days to 50 per cent flowering, plant height, number of productive tillers, panicle length, number of spikelets/panicle and 100-grain weight, indicating the importance of both additive and non-additive effects in the inheritance of these traits as well as the greater importance of non-additive gene action. Most desirable specific combiners for hybridity involved high x low general combiners along with high x high with certain limitations of low x low general combiners (epistatic). The gene distribution estimates indicated the occurrence of asymmetry. The high to moderate estimate of narrow-sense heritability further supported the involvement of both additive and non-additive gene effects for different traits. At least one major group of genes controlled the inheritance of each trait. The photoperiod-sensitive plants with different critical day length were obtained from a particular intermediate sensitive × insensitive cross-combination.

In a line x taster analysis (Singh and Kumar, 2004), it was found that additive variance was more prevalent for days to 50 per cent flowering, plant height, effective tillers and grain yield, although line x tester contrast was also significant, indicating the importance of additive and non-additive effects.

Panwar (2005) reported that among the 30 F_1s produced through line × tester procedure in basmati parent's one line IET 13846 and 5 testers were having good GCA effects for grain yield per plant. Among the crosses 14 were exhibited positive significant SCA effects for grain yield per plant.

Combining ability analysis for grain yield and its component characters in rice were studied by Sharma *et al.* (2005).Variance due to GCA and SCA was significant for all traits. They further reported that additive and non-additive genes played significant role in controlling the expression of various traits. Preponderance of additive gene action for seedling height, panicles plant-1, grain length, grain length and breadth ratio, spikelets panicle-1 and non-additive gene action for the plant height, days to 50 per cent flowering, days to 80 per cent maturity, 1000-grain weight and grain yield plant-1 were reported.

Sunil (2006) studied combining ability in rice for earliness and yield potential which revealed non-additive gene action for these characters. Based on the per se performance and the gca effects, AD 95128 and AD 95134 were identified as superior parents for earliness. BTCE 23/99 and TKM 9 for yield potential and AD 95157 for both earliness and yield potential. The hybrid AD 95134 x IR 64 was the earliest for improving yield, AD 95157 x TKM 9 was identified as the best recombination for breeding.

Suresh and Anuselvam (2006) in 6 x 6 diallel study observed that estimates of GCA revealed a difficulty to study a good general combiner for all the traits. There was a close relationship between *per se* performance and GCA effects. The majority of crosses showed significant SCA effects, which involved atleast one parent having high GCA effects.

Faiz *et al.* (2006) evaluated two lines x two tester mating design along with four genotypes and their F_1's were studied to estimate combining ability for yield and yield influencing traits *i.e.,* plant height, number of productive tillers per plant, number of spikelets per panicle, number of filled grains per panicle, sterility percentage and grain yield. GCA effects were found higher for filled grains and number of spike-lets per panicle. Except plant height, mean performance of the parents was positively and strongly correlated with GCA effects. Within the CMS lines, IR 69616A found to be a good general combiner for most of the traits. Whereas within the testers, Basmati 385 was observed to be a good general combiner for most of the traits.

Hariprasana *et al.* (2006) in his line x tester analysis in rice revealed significant GCA and SCA effects for yield and/or its components indicating the role of both additive and non-additive gene action. Results further suggested that the good general combiners identified among the parents for grain yield also exhibited significant and positive GCA effects for one or more of its components. This was true for SCA effects also. However, in some of the crosses and parents same was not true. Rosama and Vijay Kumar (2005) observed a good correspondence of parents having different types of GCA effects (*viz.,* positive

significant, negative significant or non-significant) produced hybrids with high SCA effects

Kumar *et al.* (2006) reported that CMS line IR6888A for earliness and IR 58025A for yield and related traits like days to 50% flowering, plant height, productive tillers per plant, panicle length, number of fertile grains /panicle and 1000 grain weight. Four testers namely Pusa 1040, PSRm-1-16-48-11, RAU 1411-4 and RAU 1414-10 were rated as good general combiners for yield.

Rosamma and Vijayakumar in the year 2005 reported that parents with high, medium and low general combining ability produced hybrids with high *sca.* Hybrids with positive and significant SCA for grain yield were produced by almost all types of parental combinations. In their study, low x low combinations also produced hybrids with high SCA and this can be attributed to over dominance or epistasis. All these results revealed that there is no direct relation between GCA effects of parents and SCA effects of hybrid combinations. This can be also be explained from the point of gene action since *gea* is more due to additive gene action whereas SCA is due to dominance and epistasis.

Sarial *et al.* (2006) evaluated combining ability in basmati rice and reported restorer basmati 385 and KRH 241 as good general combiners for grain yield/ plant, biomass yield/plant, 1000 grain weight and effective tillers/ plant while kernel local for biomass yield/ plant and 1000 grain weight. High significant SCA effects were determined in 4 crosses for grain yield/ plant, biomass yield/plant and effective tillers/ plant.

Senguttuvel and Bapu (2007) carried out line × tester analysis using four lines and ten tester for seven biometrical traits i.e. days to flowering, plant height, number of productive tillers, panicle length, number of filled grains per panicles, 100 grin weight and single plant yield. Preponderance of additive gene action was predominant for days to flowering, plant height, number of productive tillers, panicle length, number of grain per panicle, 100 grain weight and single plant yield. Among the lines ASD 16 and ADT 38 and CB 98002, CB 98006 was the good general combiner for most of the traits.

Kumar *et al.* (2007) revealed that predominance of additive gene action for the traits like plant height, days to 50% flowering, days to maturity, dry matter harvest index, 100 grain weight and grain length. However, both additive and non additive gene effects was of equal importance for grain yield/plant, biological yield/plant, grain length and breadth ratio. Predominance of non-additive gene action was recorded for length of panicle and grain length. Both additive and non additive gene effects were played role in inheritance of yield and its component traits.

Rashid *et al.* (2007) evaluated 5 rice genotypes along with 6 F_1s were used of line produced by 2 line x 3 tester mating design, tester was higher than that of interaction of line x tester for all the characters. The highest significant heterosis (61.9) was observed in the cross Super Basmati/DM-107-4 for yield/plant. The female Super Basmati, male DM-25 and DM-107-4 were observed to be good general combiners for most of the characters studied. The crosses between Basmati-370/DM 25 and Super Basmati/DM-107-4 were observed good specific combiners for yield/plant. However, the cross combination Basmati-370/EL.-30-2-1 was identified as the promising specific combiner for further improvement in rice.

Torres and Geraldi (2007) estimated GCA and SCA using crosses between six tropical *indica* rice cold susceptible genotypes (group 1) and seven *japonica* or *indica/japonica* cold tolerant rice genotypes (group 2). The predominant direction of dominance effects was negative for days to 50% flowering, and positive for all the other characters. General combining ability (GCA) and specific combining ability (SCA) were significant for all characters, although the GCA effects of the two groups were more important than the SCA effects.

Shukla and Pandey (2008) estimated the presence of substaintial heterosis using 120 hybrids derived from 30 elite *indica* lines and four cultivars viz., Pant Dhan 4, Ajaya, Taichung 65 and IR 65598-112-2. Predominance of non-additive gene variance suggesting good prospects of hybrid breeding. Pooled analysis revealed high significant variances for lines, GCA, SCA and line × tester. Grain

yield recorded heterosis of 49.3%, 71.9% and 92.7% for *indica/indica*, *indica/ japonica* and *indica*/tropical *japonica* hybrid groups respectively.

Lakshmi *et al.* (2008) developed fifteen crosses from 5 lines and 3 testers along with parents were evaluated in line × tester design for analysis of grain yield and yield components. They have reported importance of both additive and no-additive gene action in the inheritance of the characters studied. Samba mashuri among the lines and lalnakanda 41 among the testers showed good general combining ability effects for yield and yield contributing characters.

Zhu *et al.* (2009) analyzed ten male sterile lines for the study of combining ability for yield and related traits. They found that the number of panicles, plant height, panicle length, grain number of panicle, full gain per panicle was mainly affected by the additive effects. The weight of 1000 grain weight and yield on single plant were affected by the additive effects and non-additive effects together and additive effects were greater than non- additive effect.

Akter *et al.* (2010) produced twenty hybrids using five CMS lines *viz.*, BRRI3A, BRRI9A, IR73328A, BRRI10A and IR78355A and four restorers *viz.*, BRRI12R, BRRI13R, BRRI14R and BRRI15R following line x tester mating design to find out the best combination (s) in respect of their combining ability effects among the parents and hybrids. The ratio of GCA and SCA variances were found less than unity for all the characters except plant height, days to maturity and panicles per square meter. The relative contribution of line, tester and combinations of line x tester interaction of ten characters were calculated and found that panicle weight contributed the highest followed by thousand grain weight, yield per plant and panicle per meter square in their hybrids. IR73328A was identified as good general combiner for shorter plant height and panicle per square meter. BRRI10A and IR78355A produced significant positive GCA for yield per plant which could be regarded as good general combiner for higher grain yield. Six hybrid combinations were found with significant and positive SCA effect of which the highest value was obtained from BRRI3A/BRRI14R followed by IR73328A/BRRI13R (2.23) for yield per plant.

Bagheri and Jelodar (2010) studied combining ability and heterosis on 12 F_1 hybrids along with seven rice genotypes to know the pattern of inheritance of some morphological traits for selecting superior genotypes. Variances of SCA were higher than the GCA variances for traits except for plant height which indicated predominance of non-additive gene action in the inheritance of the traits. The highest heterosis was observed in cross IR68899A x Poya followed by other eight crosses for yield and most of its related traits. The proportional contribution of testers was observed to be higher than that of the interactions of line x tester that revealed the higher estimates of GCA variance that is additive gene action among the testers used. Within CMS parents, IR62829A and among male parents, IR50 and Poya were observed to be good general combiners for most of the characters studied. The cross combinations IR62829A x Mosa-tarom, IR68899A x Poya, IR58025A x IR50 and IR58025A x Poya were observed to be good specific cross combinations for grain yield and most of its related traits due to highly significant SCA and heterotic effects.

Chaturvedi *et al.* (2010) reported presence of sufficient variation among the genotypes used in combining ability analysis. Prevalence of additive as well as non- additive gene action was indicated, for all the characters, by the analysis of variance for combining ability. Bahadur and Teke were found to be the best general combiners with respect to grain yield plant-1. These two parents were good general combiners for atleast four other desirable traits also. In the present study good x good combiners like Teke x Bahadur and Teke x Ranjit and good x poor combiners like Malong x Bahadur, Mehuru x Ranjit, Mehuru x Bahadur and Teke x Piolee were among the best specific combiners.

Kumar *et al.* (2010) reported line IR 58025A and eight tester parents was found to be a good general combiners. They had identified 13 crosses having good specific combining ability for grain yield and its component traits like seedlings height, number of leaves/ seedlings, days to 50% flowering, flag leaf length, plant height, panicle bearing tillers/plant, panicle length, spiekelts /panicle and grain yield/plant.

Rahimi *et al.* (2010) evaluated fifteen F_1 hybrids and their parents were in a randomized complete block design with three replications that generated by half diallele crosses of six diverse rice cultivars. The studied traits were growth period, reproductive period, flag leaf area, plant height, panicle length, number of panicles per plant, number of grains per panicle, 1000-grain weight, grain yield, brown grain length and brown grain width. The significance of specific combining ability (SCA) and general combining ability (GCA) for all studied traits revealed that both additive and non-additive gene effects contributed to the inheritance of the traits.

Saidaiah (2010) evaluated one hundred and fifteen crosses from five CMS lines and 23 restorers along with parents in line × tester design for grain yield and yield components. Predominance of non additive gene action was observed for all the characters, suggesting the development of hybrids in rice. The line APMS and PUSA 5A, IBL 57, SG27-77, SG 26-120 and KMR 3 were good general combiners for grain yield and contributing traits. IR 43, IR 55 and IR 60 were good general combiners for dwarf plant types.

Saleem *et al.* (2010) evaluated the performance of 27 F_1 hybrids along with 12 parents in Basmati rice in Line x Tester experiment. The estimates of variance of SCA effects, ratio of variance of GCA to SCA and degree of dominance indicated preponderance of non-additive gene effects for each trait. On over all basis, role of testers in the expression of most of the yield components were more than lines and line x tester interaction. Hybrids like Basmati Pak × Basmati-385, Super Basmati × Basmati-385, DM-107-4 × Basmati-385, Basmati 2000 × EL-30-2-1, Basmati 2000 × DM-25, DM-16-5-1 × Basmati-385 and Kashmir Basmati × DM-25 showed high mean performance, SCA effects and heterobeltiosis for grain yield and are proposed for heterosis breeding.

Thakare *et al.* (2010) while studying combining ability analysis for yield and grain quality traits found that the estimates of GCA effects indicate that among females, IR 68886A and IR 68897 A whileas, IR-44, IR-60, IR-9761, IR-4266-29-4- 2-2, IR-5638-139-2-2, IR-69701-9-3-1 and IR-71138-49-2-2 among males were good general combiners for grain yield plant-1. High SCA effects

were observed in the crosses, IR 68886 x IR-44, IR 68897A x IET-15554, IR 68897A x IR-56455-206-2, IR 68902A x IR-4266-29-4-2-2-2 and IR 68897A x IR-62161-184-3-1-3-2 and they found to be the best combinations for grain yield plant-1 and quality trait viz. Grain length, grain breadth, length: breadth ratio, hulling percentage, milling percentage and head rice recovery percentage.

Kumar in the year 2011 conducted a study in order to see the inheritance pattern of six rice genotypes for grain yield and its component characters in rice. Data from the F_1 generation and parents were analyzed using Hayman (1954) method of diallel analysis. The estimates of D (additive genetic variance) were significant for all the traits studied. The values of H1 and H2 as well as H2/4H1 indicated that there were unequal frequencies of the alleles at all the loci. An excess of dominant alleles was involved in five out of the ten traits of interest. The mean degree of dominance was more than unity for six out of the 10 traits studied, including grain yield/plant. The narrow sense heritability estimates were high for eight out of the ten traits studied.

Selvaraj *et al.* (2011) carried out a Line x tester analysis with 20 selected entries. General combining ability of parents and specific combining ability and heterosis among 64 hybrids for yield and its components were analyzed. Mean squares due to females were larger in magnitude than male parents for all the characters. The magnitude of SCA variance was higher than GCA variance for all the characters except grain yield where the GCA variance was higher. Among the lines, IR 50 was found to be a good general combiner for six traits. Among the testers, IR 64 recorded high *per see* performance along with high GCA effects for panicle length, filled grains/panicle, 1000 grain weight and grain yield plant^{-1}. Most of the hybrids recorded positive significant standard heterosis values for grain yield plant-1. IR 50/IR 64 recorded highest heterosis values for relative heterosis and TN 1/IR 64 recorded high heterobeltiosis and standard heterosis over the standard check ASD 16.

Suresh *et al.* (2012) reported that the analysis of variance for combining ability revealed significant differences among the genotypes tested for all the traits *viz.*, panicle weight, number of spikelets per panicle and seed yield per plant.

Among the CMS lines, CMS 2A, CMS 5A, CMS 6A and CMS 11A and among testers, Thanu, KMR 12, MSN 36, MSN 102, and MSN104 were identified as good general combiners as they recorded high (H) over all gca status. The hybrid CMS 11A × MSN 102 was a best specific combiner followed by CMS11A × Thanu, CMS11A ×KMR 12 and CMS 5A × MSN102. The SCA variance was predominant for all the characters indicating operation of non-additive gene action. The contribution of testers towards the total variance was found higher than lines and line x tester interaction suggesting predominant testers in influence for these traits. The hybrids CMS 11A × MSN102, CMS 11A × Thanu, CMS 11A × KMR 12 and CMS 5A × MSN 102 exhibited significant higher standard heterosis over the check KRH-4 with respect to seed yield.

Raju *et al.* (2014) evaluated 65 hybrids along with the parents and five checks *viz.,* KRH-2, DRRH-2, PA-6201 Jaya and IR- 64 for combining ability (Line x Tester design) at three locations. Among the lines APMS 6 A and among the testers JGL 8292, JGL 8605, JGL 17211, JGL 13515 and JGL 3844 were proved to be good combiners for majority of the characters including the yield, by exhibiting the high GCA effects. Out of 65 hybrids, the top five hybrids based on SCA effects were APMS 8 A x JGL 11110-2 (7.01), APMS 8 A x JGL 11110-1 (5.48), APMS 6 A x JGL 1111-2 (4.77), APMS 8 A x JGL 13515 (4.01) and APMS 8 A x JGL 8605 (3.93). The mean performance ranged from 27.64 g/plant to 31.22 g/plant. Among the 65 hybrids tested at three locations 21 hybrids, recorded significant positive GCA effects for single plant yield.

Bhati *et al.* (2015) carried out an investigation on combining ability for yield and its component traits in rice. The material consisted of F_1 hybrids of 30 crosses developed by crossing 10 lines with three testers. The cross HPR 2639 X HPR 2143 identified as good specific combination for grain yield/plant, panicle length, spikelets/panicle, grains/panicle, biological yield/plant, days to 50% flowering and plant height.

Materials and Methods

3. MATERIALS AND METHODS

The research pertaining to "Genotyping of *Rf* (Restoring fertility) loci of rice varieties of Kerala using molecular markers" was undertaken at the College of Agriculture, Vellayani, Thiruvananthapuram. The research involved utilization of both classical genetics as well as modern molecular tools. The material used in the course of experimentation and techniques followed for collection, analysis and interpretation of data are described in this chapter.

3.1 PLANT MATERIAL

The materials used in the research comprised of breeder seed of twenty one rice varieties collected from three different rice research centres representing two soil and climatically different rice growing tracts of Kerala namely Regional Agricultural Research station, Pattambi, Palakkad, Kerala Agricultural Universiy (KAU), Rice Research station, Moncompu, Alappuzha, Kerala and Rice research station Mannuthy, Thrissur, Kerala, along with four different CMS lines namely IR58025A, UPRI95-17A, CRMS31 and CRMS32A collected from IIRR, Hyderabad, GBPUAT, Pantnagar and NRRI, Cuttack respectively (Table 1).

3.2 ISOLATION OF GENOMIC DNA

Genomic DNA from these 21 rice varieties were isolated using the procedure of QIAGEN DNeasy plant mini kit. Samples were disrupted (≤100mg wet weight or ≥20mg lyophilized tissue) using the mortar and pestle with liquid nitrogen. 400µl of buffer AP_1 and 4µl of RNAse A were added, vortexed and incubated for 10min at 65°C. The tube was inverted 2-3 times during incubation. 130µl buffer P3 was added and mixed and incubated for 5 min on ice. The lysate was centrifuged for 5 min at 20,000 x g (14000rpm), the lysate was pipetted into a QIA shredder spin column placed in a 2ml collection tube and centrifuged for 2 min at 20,000 x g. The flow–through was transferred into a new tube without disturbing the pellet if present. 1.5 volumes of buffer AW1 was added by pipette and mixed well. Then 650 µl of the mixture was transferred into a DNeasy mini spin column placed in a 2 ml collection tube and centrifuged for 1 min at ≥ 6000 x g (≥8000rpm). The flow through was discarded and this step with the remaining

Table: 1: List of Rice varieties used in the experiment

Searial No	Name of Varieties	Place of collection
Male parents		
1	PTB-9	RARS, Pattambi
2	PTB-10	RARS , Pattambi
3	PTB-32	RARS , Pattambi
4	Aiswarya	RARS, Pattambi
5	Annapoorna (PTB-35)	RARS, Pattambi
6	Jyothi (PTB-39)	RARS, Pattambi
7	Bharathy (PTB-41)	RARS , Pattambi
8	Swarnaprabha (PTB-42)	RARS , Pattambi
9	Mattatriveni (PTB-45)	RARS, Pattambi
10	Jayathi (PTB-46)	RARS , Pattambi
11	Neeraja (PTB-47)	RARS , Pattambi
12	Kanchana (PTB-50)	RARS , Pattambi
13	Manupriya	RRS , Mannuthy
14	Varsha (PTB-56)	RARS , Pattambi
15	Kanakom (MO-11)	RRS , Moncombu
16	Karthika (MO-7)	RRS , Moncombu
17	Aruna (M0-8)	RRS , Moncombu
18	Remya (MO-10)	RRS , Moncombu
19	Aruna (MO-8)	RRS , Moncombu
20	Pavizham (MO-6)	RRS , Moncombu
21	Uma (M0-16)	RRS , Moncombu
Reported Restorers		
22	KMR-3R	DRR, Hyderabad
23	IR42266-29-3R	CRRI, Cuttack
Female parents		
24	IR58025A	IIRR, Hyderabad
25	UPRI95-17A	GBPUAT, Pantnagar
26	CRMS31A	NRRI, Cuttak
27	CRMS 32A	NRRI, Cuttak

sample were repeated. The spin column was placed into a new 2 ml collection tube, 500 µl Buffer AW2 were added and centrifuged for 1 min at ≥6000 x g. The flow through was discarded, another 500 µl Buffer AW_2 was added and centrifuged for 2 min at ≥20000 x g. The spin column was transferred to a new 1.5ml or 2ml micro centrifuge tube and 100 µl Buffer AE was added for elution. Then incubated for 5 min at room temperature (15-25° C) and centrifuged for 1 min at ≥6000 x g. These DNA samples were stored at -20°C.

3.3 MOLECULAR MARKERS

Thirteen DNA markers specific to the *Rf* genes were used to screen Kerala rice varieties for the presence of *Rf* loci based on the previous reports. The sequence and annealing temperature of each marker is given in Table 2.

3.4 AGAROSE GEL ELECTROPHORESIS

Stock solutions

50X TAE Buffer

Tris base	240g
Acetic acid	57.1ml
0.5M EDTA (pH-8.0)	186.12g
Final volume (Distilled H_2O)	1000ml

6X loading dye

Sucrose	4.0g
Bromophenol blue	0.025g
Volume (Distilled H_2O)	10ml

(Loading dye solution was stored at 4°C)

Agarose gel electrophoresis was carried out in a BIO-RAD, horizontal gel electrophoresis Unit. 0.8g of agarose was weighed and melted in 1x TAE buffer.

Table 02: Details of the markers used for screening of *Rf* loci

Sl. No	Marker	Linked gene	Chromosome No	Primer sequence	Annealing temp	Reference
1	RM 1	R*f3*	1	F:GCGAAAACACAATGCAAAAA R:GCGTTGGTTGGACCTGAC	53.8	Ahmadikhah *et al.*, 2007
2	RM 3233	R*f3*	1	F:GAAATTCGAAATGGAGGGAGAGC R:GGTGAGTAAACAGTGGTGGTGAGC	57	Alavi *et al.*, 2009
3	RM 3873	R*f3*	1	F:GCTATAGACGCCTCCTCCTTATCC R:AAAGCTAGCTAGGACCGACATGC	62.1	Alavi *et al.*, 2009
4	RM 315	R*f3*	1	F: CGGTCAAATCATCACCTGAC R: CAAGGCTTGCAAGGGAAG	56	Bazrkar *et al.*, 2008
5	RM 443	R*f4*	1	F: GGGAGTTAGGGTTTTGGAGC R: TCCAGTTTCACACTGCTTCG	50.7	Bazrkar *et al.*, 2008
6	RM 6100	R*f4*	10	F: TCCTCTACCAGTACCGCACC R: GCTGGATCACAGATCATTGC	55.3	Sigh *et al.*, 2005
7	RM 171	R*f4*	10	F: AACGCGAGGACACGTACTTAC R: ACGAGATACGTACGCCTTTG	58.7	Jing *et al.*, 2001
8	RM 258	R*f6*,R*f5*, R*F4*,	10	F: TGCTGTATGTAGCTCGCACC R: TGGCCTTTAAAGCTGTCGC	56.7	Huang *et al.*, 2003
9	RM 228	R*f4*	10	F: CTGGCCATTAGTCCTTGG	52.8	Mishre *et al.*, 2003

				R: GCTTGCGGCTCTGCTTAC		
10	RM 216	R*f4*	10	F: GCATGGCCGATGGTAAAG R: TGTATAAAACCACACGGCCA	56.5	Mishre *et al.*, 2003
11	RM 244	R*f6*	10	F: CCGACTGTTCGTCCTTATCA R: CTGCTCTCGGGTGAACGT	56.7	Mishre *et al.*, 2003
12	RM 591	R*f6*	10	F: CGGTTAATGTCATCTGATTGG R: TTCGAGATCCAAGACTGACC	55.3	Bazrkar *et al.*, 2008
13	RM 7003	R*f7*	12	F:GGCAGACATACAGCTTATAGC R:TGCAAATGAACCCCTCTAGC	56.5	Bazrkar *et al.*, 2008

After cooling the solution to 42-45°C, ethidium bromide was added at the rate of 3µl for 100ml. The solution was then poured on to a preset, sealed gel casting tray with a comb fixed in position, to a height of 3mm-5mm. The gel was allowed to solidify for 15-20 min. The comb and sealing tapes were then removed and tray was submerged in electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to height of 1mm. Required volume of DNA sample and loading dye [glycerol 30% + bromophenol blue] were mixed in the ratio 5:1 and loaded into the slots of gel using a micropipette near the negative terminal. The cathode and anode of the electrophoresis unit were attached to the power supply and a constant voltage of 60V was used for the run. The power supply was turned off when the loading dye moved about 3/4th of the gel. The gel was documented using SYNGENE gel documentation system.

3.5 QUANTIFICATION OF DNA

After ensuring the presence of DNA in samples by electrophoresis the quality and quantity of DNA was measured as follows

5µlof DNA dissolved in 0.1X TAE was added to 3ml of distilled water and read against distilled water used as blank at an absorbance of 260 nm and 280 nm, in an UV spectrophotometer. The concentration of DNA in sample was calculated using the formula;

$$\text{Amount of DNA (ng/ml)} = A260 \times \frac{\text{Volume of dist.water (µl)} \times 0.05 \times 1000}{\text{Amount of DNA}}$$

Where A260 = Absorbance at 260nm. The quality of DNA was judged from ratio of absorbance values at 260 nm and 280 nm. A ratio of 1.8-2.0 indicated best quality of DNA

3.6 VALIDATION OF REPORTED MARKERS LINKED TO FERTILITY RESTORATION (*Rf*) LOCUS.

Standardized the annealing temperature of each primer by using gradient PCR as given in Table 2. The PCR mixture contained 25 µl reaction volume containing

50 ng genomic DNA, 10 pmoles of each marker, 10 nM dNTP, 10x PCR buffer (10 nM Tris, pH 8.4, 50nM KCL, 1.8 nM $mgCl_2$ and 0.01mg/ml gelatin)and 1 U of Taq DNA polymerase. Amplifications were carried out in an Eppendorf master cycler nexus gradient PCR. Template DNA was initially denatured at 94°C for 4 min followed by 35 cycles of PCR amplification with the following parameters: a 30sec denaturation at 94°C, a 30sec annealing at required temperature identified for separate primers, 72°C for 1 min of primer extension and final extension 7 min. at 72°C. After completion of amplification, PCR products were stored at -20°C. The amplified product was resolved on a 1.7% agarose gel containing 0.5 µg/ml of ethidium bromide in 1xTBE buffer and visualized under SYNGENE G-Box F3gel documentation unit.

3.7 Genetic Divergence analysis

Data recorded for this experiment were subjected to the following statistical analysis.

3.7.1 COMPLETELY RANDOMIZED DESIGN (CRD)

CRD analysis was performed to analyse genetic divergence among 12 biometrical characters by using the following ANOVA table

TABLE 3: ANOVA for Completely Randomized design

Source of variation	Degrees of freedom (df)	Mean sum of squares(MSS)	Expected MSS
Replications	(r-1)	M_r	$g\sigma_r^2 + \sigma_e^2$
Treatments	(g-1)	M_g	$r\sigma_g^2 + \sigma_e^2$
Error	(g-1) (r-1)	M_e	σ_e^2
Total	(gr-1)		

Where,

r = number of replications

g = number of treatments (genotypes)

σ_r^2 = variance due to replications

σ_g^2 = variance due to treatments (genotypes)

σ_e^2 = variance due to error

3.7.2 Estimation of genetic variability parameters:

The variability among the genotypes for different traits was estimated as mentioned below.

i. Genotypic variance and phenotypic variance:

Phenotypic and genotypic components of variance were estimated by using the formula given by Burton and Devane (1953).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{\text{MSS due to genotypes - MSS due to error}}{r}$$

Phenotypic variance = Genotypic variance (σ^2_g) + Error variance (σ^2_e)

ii. Co-efficient of variability:

Both phenotypic and genotypic co-efficient of variability for all characters were estimated using the formula of Burton and Devane (1953).

$$\text{Phenotypic coefficient of variation (PCV \%)} = \frac{\sqrt{Phenotypic\ var\ iance}}{Grandmean} x100$$

$$\text{Genotypic coefficient of variation (GCV \%)} = \frac{\sqrt{Genotypic\ var\ aiance}}{Grandmean} x100$$

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

<10% : low

10-20% : moderate

>20% : high

iii. Heritability in broad sense (h^2):

The broad sense heritability (h^2_{bs}) was estimated for all characters as the ratio of genotypic variance to the total variance as suggested Lush (1949) and Allard (1960)

$$h^2 = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

According to Johnson *et al.* (1955) heritability estimates in cultivated plants can be placed in following categories.

5-10% - Low; 10.1-30% - Moderate; 30.1-60% - High

iv. Genetic advance (GA):

Genetic advance for each character was estimated by using the formula of Johnson *et al.* (1955).

$GA = h^2_{bs} \times \sigma_p \times K$

Where,

h^2_{bs} = Heritability estimate in broad sense

σ_p = Phenotypic standard deviation of the trait

K = Standard selection differential which is 2.06 at 5 per cent selection intensity

Further, the genetic advance as per cent of mean was computed by using the following formula

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

Genetic advance as per cent mean was categorized as given below as suggested by Johnson *et al.* (1955).

0- 10% - Low; 10.1-20% - Moderate; >20.1% - High

3.7.3 Genetic diversity analysis employing morphological traits

After testing the difference between genotypes for each of the character, a simultaneous test of significance of difference between the mean values of a number of correlated variables was done by using 'V' statistic, which in turn utilizes Wilk's criterion (Wilk, 1932). The sum of squares and sum of products of error and error + genotypes were used for this purpose. The estimate of ^ (Wilk's criterion) was done by using the following relationship:

$$\wedge = \frac{E}{E+V}$$

Where,

^ = Wilk's criterion

(E) = Determinant of error matrix and

(E + V) = Determinant of error + variety matrix

The genetic diversity in 45 genotypes for morphological characters was estimated using Mahalanobis's (1936) D^2 statistic technique. The D^2 value between i^{th} and j^{th} genotypes for p character was calculated as follows:

$$D^2 ij = \sum_{t=1}^{P} (Yit - Yjt)^2$$

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

Y it = Uncorrelated mean value of the i^{th} genotype for 't' character

Y jt = Uncorrelated mean value of the j^{th} genotype for 't' character

The various types involved in estimates of D^2 values are given below:

$$V(Stat) = -m \log_e \text{`^'} = - \left[ n - \frac{P+Q+1}{2} \right] \log_e \text{`^'}$$

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

n = Degrees of freedom for error + varieties

$\log_e$ '^'= 2.3026 log 10 '^'

P = Number of variables or characters (12)

Q = Number of genotypes – 1 (or d.f. for genotypes) 20

V (stat) is distributed as x^2 with PQ (240 = 12 x 20) degrees of freedom.

3.7.3.1Transformation of correlated variable

Computation of D^2 values was reduced to simple summation of differences in mean values of various character of the two genotypes *i.e.*, Di^2. Therefore, the transformation of correlated variable to uncorrelated ones was done before working out the D^2 values. Transformation was done by using pivotal condensation method.

3.7.4 Contribution of individual characters towards divergence

The character contribution towards genetic divergence was computed using the method given by Singh and Chaudhary (1977). In all the combinations, each character was ranked on the basis of $di = y_i^j - y_i^k$ values.

Where,

di = mean deviation

y_i^j = mean value of the j^{th} genotype for the i^{th} character and

y_i^k = mean value of the k^{th} genotype for the i^{th} character.

Rank 'I' is given to the highest mean difference and rank 'P' is given to the lowest mean difference

Where,

P is the total number of characters.

Finally, the number of times that each character appeared in the first rank is computed and per cent contribution of characters towards divergence was estimated using the formula

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

N = Number of genotype combinations where the character was ranked first.

M = No of all possible combinations of number of genotypes considered.

3.7.5 Clustering of genotypes into various clusters

Grouping of populations into different clusters was done using Tocher`s method as described by Rao (1952). The criterion used in clustering by this method is that any two varieties belonging to same cluster should at least on an average show a similar D^2 value than those belonging to different clusters. For this purpose, D^2 values of all combinations of each genotype were arranged in an increasing order of magnitude in a tabular form as described by Singh and Chaudhary (1977). To start with two populations having the smallest distance from each other were considered to which a third population

having the smallest D^2 value from the first two populations was added. Similarly, next nearest fourth population was considered and this procedure is continued. At certain stage where it was felt that after adding a particular population there was abrupt increase in the average D^2 value, that population was not considered for including in that cluster. The group of the first cluster was then omitted and the rest was treated in a similar way. This process was continued till all the genotypes were included in one or the other cluster.

A. Intra and inter-cluster distances

Based on D^2 values, average intra and inter-cluster distances were calculated based on the clusters formed.

B. Average intra-cluster distance

For the measurement of intra-cluster distances the formula used was $\sum D^2 i/n$ where, $\sum D^2 i$ was the sum of distance between all possible combinations and 'n' is the number of the genotype included in a cluster.

C. Average inter-cluster distance

Each cluster is taken one by one and their distances from other clusters were calculated. The distance between the two clusters to each of the members of other clusters divided by the product of number of genotypes in both clusters under consideration. The square root of the average D^2 value gave the genetic distance 'D' between the clusters.

3.8 HYBRIDIZATION PROGRAMME

Plant varieties were raised in the nursery by staggered sowing for synchronization of flowering. Seedlings of these varieties were then transplanted to main field as and when they attaind the maturity of 25 days. At boot leaf initiation stage, the male sterile female plants were uprooted in series from the main field and planted in pots. In the CMS lines individual plants with complete

pollen sterility was identified by observing the pollen grains under the microscope using one per cent Iodine potassium iodide (I_2KI) stain. Plant showing 100% pollen sterility were chosen for hybridization.

Plate 1: a. Germination of seed materials, b.Transfering germinated seedlings into cement pots and c. Transplanting parental lines into field

Emasculation was done in the female parents by the clipping method. One day before the pollination, spikelet's were clipped off one third from the top without damaging the stigma. Immediately after clipping, the panicles were covered with butter bag to prevent contamination from any foreign pollen.

Crossing between clipped male sterile female parent and 21 Kerala rice varieties was done on the next day morning as follows.

At 9.00AM the bloomed panicle from the male parent was collected and dipped in a glass containing water, for 1-2 minutes. Then the glass containing the panicle dipped in water was exposed to 200 watt bulbs inside the pollination

chamber. After half an hour these spikelets of the panicle started exerting their anthers. The pollen from exerted anthers was transferred to the emasculated spikelets of the recipient female parents by shaking them overhead. After conducting the pollination the appropriate information pertaining to pollination were labelled on butter paper cover. After three to four weeks the matured crossed seeds from female parents of the crossing programme were harvested and stored in refrigerator.

Plate 2: a. Pollination of female lines with pollen from male lines inside pollination chamber, b. Bagged panicles after pollination

3.8 IDENTIFICATION OF MAINTAINERS AND RESTORERS

The harvested F_1 seed of each cross were sown in nursery. Seedlings of these varieties were then transplanted to main field as and when they attain the

maturity of 25 days. At flowering stage Pollen fertility recorded as microscopic pollen grain count. Panicle from the main tiller of each plant was selected and several spikelets were randomly selected from different positions in the panicle. The anthers from each spikelet were squashed in a drop of 1% Iodine Potassium Iodide (I_2KI) solution on a glass slide separately and observed under a light microscope. The stained pollen grains were counted as fertile and unstained pollen grains were counted as unfertile. The total counts of fertile pollen grain were observed in relation to the total pollen grains in the five microscopic fields. The mean of five microscopic fields was then calculated. These mean values for fertile pollens and total pollens were used for calculating the pollen fertility percentage :

$$\text{Pollen fertility \%} = \frac{\text{No. of fertile pollen grains}}{\text{Total no. of Pollen grains}} x100$$

The spikelet fertility and sterility was calculated on the basis of five randomly selected panicles from each F_1 hybrid and parents at the time of maturity. The percentage of spikelet fertility was calculated using following formula.

$$\text{Spikelet fertility \%} = \frac{\text{Number of filled grains in a panicle}}{\text{Total no. of spikelet in a panicle}} x100$$

As per the pollen fertility and spikelet fertility percentage in the hybrids, the testers that could produce 0-1 per cent pollen fertility and 0-0.1 per cent spikelet fertility classified as maintainers, 1.1-50 per cent pollen fertility and 0.1-75 per cent spikelet fertility classified as partial maintainers, 50.1-80 per cent pollen fertility and 50.1-75 per cent spikelet fertility classified as partial restorers and >80 per cent pollen fertility and >75 per cent spikelet fertility classified as potential restorers as per the classification is given by (Virmani *et al.*, 1997).

3.9 Inheritance studies of restoring fertility gene

Goodness of fit for R*f* loci to expected segregation ratio was tested by means of chi-square analysis in F_2 population of cross between CMS lines and restorers identified.

3.10.1 CHI SQUARE TEST ANALYSIS

This method of evaluation would be Pearson's Chi square test (1900). The value of the test-statistic is

$$\chi 2 = \sum_{i=1}^{n} \frac{(Oi - Ei)^2}{Ei}$$

Where,

χ^2 = Pearson's cumulative test statistic, which asymptotically approaches a χ^2 distribution.

Oi = an observed frequency;

Ei = an expected (theoretical) frequency, asserted by the null hypothesis;

n = the number of cells in the table.

3.10 BULK SEGREGANT ANALYSIS

The detection for markers that were linked to the targeted *Rf* gene followed essentially the procedures described by Zhang *et al.*, (1994). Bulks were made by selecting individuals from the F_2 population for identifying chromosomal regions containing the *Rf* genes. Two bulk one sterile and one fertile were formed by mixing the DNA from 10 fully fertile and 10 fully sterile from corresponding F_2 plants was used to constitute fertile and sterile bulks, respectively, for BSA. Remaining all steps starting from DNA isolation, PCR and Gel documentation are same as above mentioned methods (3.2, 3.4, 3.5 and 3.6).

3.11 COMBINING ABILITY ANALYSIS

The mean value of 5 randomly selected plants recorded in each entry and replication were subjected to line x tester analysis as suggested by Kempthorne (1957) which is based on fixed effect model. Observation recorded on i x j^{th} cross grown in k^{th} replication can be linearly expressed as shown below for this design (Arunachalam, 1976).

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + rk + e_{ijk}$$

Where,

Y_{ijk} = any character measured on cross i x j in k^{th} replication

μ = population mean effect

g_i = *gca* effect of i^{th} parent

g_j = *gca* effect of parent j

s_{ij} = *sca* effect of cross i x j

rk = k^{th} replication effect

e_{ijk} = environmental effect particular to $(ijk)^{th}$ individual

Structure for the analysis of variance and expectation of mean sum of squares for line x tester design is presented in Table below

Table 4: ANOVA for L X T analysis

Source of variation	**Degrees of freedom (df)**	**Mean sum of squares**	**Expected mean square**
Replication	(r-1)	M	
Hybrids	(lt-1)		
Lines	(l-1)	M_1	σ_e^2 + r Cov(FS) - 2Cov(HS) + rt [Cov(HS)]
Tester	(t-1)	M_2	σ_e^2 + r Cov(FS) - 2Cov(HS) + rl [Cov(HS)]
Line x Tester	(l-1)(t-1)	M_3	σ_e^2 + r Cov(FS) - 2Cov(HS)]
Error	(r -1)(lt -1)	M_4	σ_e^2
total	(ltr-1)		

Where,

r = Number of replications

l = Number of lines (males)

t = Number of testers (females)

Cov (HS) = Covariance of half sibs

Cov (FS) = Covariance of full sibs

M_1 = Mean sum of squares due to females

M_2 = Mean sum of squares due to males

M_3 = Mean sum of squares due to female x male

M_4 = Mean sum of squares due to error

3.12.1 Estimation of variance

The GCA and SCA variances were expressed in terms of covariance full sibs (FS) and half sibs (HS) as indicated below.

GCA variance for lines = Cov (HS) lines = $\frac{M1-M3}{rt}$

GCA variance for testers = Cov (HS) testers = $\frac{M2-M3}{rl}$

SCA variance for hybrids = $\frac{M3-M4}{r}$

Cov (FS) = 1/3r [M_1+M_2+M_3 – 3M_4 + 6r Cov (HS) – r (l+t) Cov (HS)]

σ^2gca = Cov (HS)

σ^2sca = Cov (FS) – 2 Cov (HS)

3.12.2 Estimation of combining ability effects

A) General combining ability effects (gca)

i) Lines : $g_i = \left(\overline{Xi} - \overline{X}\right) = \frac{Xi..}{tr} - \frac{X..}{rlt}$

Where,

Xi.. = Total of i^{th} female parent over all parents and replications

Population mean $= \frac{X..}{rl}$

ii. Testers $= g_j = \frac{Xij.}{lr} - \frac{X..}{rlt}$

Where,

X.j. = Total of jth male parent over all female parents and replications

B) Specific combining ability effects (sca)

$$S_{ij} = \frac{X.j.}{r} - \frac{Xi.}{tr} - \frac{X.j}{rl} + \frac{X..}{rlt}$$

X_{ij} = Total of ij^{th} combinations over all replications

s_{ij} = SCA effect of the ij^{th} combination

The standard error (SE) and critical difference (CD) pertaining to the GCA effects of male and female parents and SCA effects of different combinations were calculated as follows.

$$\text{SE (GCA for line)} = \sqrt{\frac{M4}{rt}}$$

$$\text{SE (GCA for tester)} = \sqrt{\frac{M4}{rl}}$$

$$\text{SE (}sca\text{ effect)} = \sqrt{\frac{M4}{r}}$$

Where,

M_4 = Error variance (EMS)

r = Replication

l = Lines

t = Testers

CD = ($\sqrt{2}$) (SE) (table 't' for error d.f.) at 5% and 1%, respectively

3.12.3 Testing the significance of *gca* and *sca* effects

Significance of gca and sca effects was tested by 't' test as below:

t value for gca effects in lines = g_i / SEm g_i

t value for gca effects in testers = g_j / SEm g_j

t value for sca effects in crosses = S_{ij} / SEm S_{ij}

Where,

g_i = gca effect of i^{th} line

g_j = gca effect of j^{th} tester

S_{ij} = sca effect of the ij^{th} combination

SEm g_i = standard error of mean for lines

SEm g_j = standard error of mean for testers

SEm S_{ij} = standard error of mean for crosses

Calculated value of 't' was tested against table value of 't' at error degree of freedom at 5 per cent and 1 per cent levels of significance.

3.13 ESTIMATION OF HETEROSIS

Heterosis, expressed as per cent increase or decrease in the performance of F_1 hybrid over the mid-parent (average or relative heterosis), better parent (heterobeltiosis) and check parent (standard heterosis) was calculated as per the method of Hayes *et al.*, (1955).

$$\text{Per cent Heterosis over Mid Parent (MP)} = \frac{\overline{F1} - \overline{MP}}{\overline{MP}}$$

$$\text{Where, Mid Parent (MP)} = \frac{P1 + P2}{2}$$

$$\text{Per cent Heterosis over Better Parent (BP)} = \frac{\overline{F1} - \overline{BP}}{\overline{BP}}$$

$$\text{Per cent Heterosis over standard check hybrid (CH)} = \frac{\overline{F1} - \overline{CH}}{\overline{CH}}$$

$\overline{F1}$ =Mean performance of F1 hybrid

$\overline{P1}$ = Mean performance of parent one

$\overline{P2}$ =Mean performance of parent two

$\overline{BP}$ =Mean performance of better parent

$\overline{CH}$ = Mean performance of check parent

$\overline{MP}$ =Mean mid-parental value i.e. $(P1 + P2)/2$

For better parent value (BP) for each trait, superior value exhibited by any of the parent in a cross was taken for computation of heterosis.

3.13.1 Analysis of variance for hybrids

The analysis of Randomized Block Design was carried out based on the methods described by Panse and Sukhatme (1967). The model of ANOVA is furnished below.

Table 05: ANOVA table for Randomized block design

Source of variation	Degrees of freedom	Mean sum of squares	F-value
Replication	(r-1)	Vr	Vr/Ve
Treatment	(lt-1)	Vt	Vt/Ve
Error	(r-1)(t-1)	Ve	

The test of significance of calculated F value was done by referring the table F-values at 5 per cent and 1 per cent probability.

The standard error (SE), critical difference (CD), co efficient of variance (CV) and standard error of mean (SEm) for comparison of means was calculated as follows:

$$SE = \sqrt{\frac{2xVe}{r}}$$

CD (at 5%) = SE x 't' value at error degree of freedom at 5% level of significance

CD (at 1%) = SE x't' value at error degree of freedom at 1% level of significance

3.13.2 Standard Error of estimates and test for statistical significance of heterosis

To assess the standard error of estimates of heterosis, mean squares due to error (M_4) from RBD ANOVA was considered.

$$SE\ (MP) = \sqrt{\frac{3\ Ve}{2\ r}}$$ (For testing heterosis over mid parent)

$$SE\ (BP) = \sqrt{\frac{2 \times Ve}{r}}$$ (For testing heterosis over better parent)

$$SE\ (CH) = \sqrt{\frac{2 \times Ve}{r}}$$ (For testing heterosis over checks)

't' value for MP heterosis $= \frac{\overline{F1} - \overline{MP}}{SE(MP)}$

't' value for BP heterosis $= \frac{\overline{F1} - \overline{BP}}{SE(BP)}$

't' value for SH heterosis $= \frac{\overline{F1} - \overline{CH}}{SE(CH)}$

The calculated 't' value was compared to table 't' value at (r-1) (lt-1) degrees of freedom. Where,

M_4 = Error mean sum of squares from RBD ANOVA

$\overline{F1}$ = Mean of F_1

r = Number of replications in RCBD

$\overline{CH}$ = Mean of standard check

SH = Standard heterosis

SE = Standard error

$\overline{MP}$ = Mid parent mean

$\overline{BP}$ = Better parent mean

Results

4. RESULTS

To increase rice productivity and production, the scientists opted to develop and disseminate hybrid rice technology. For an efficient hybrid rice breeding programme, identification of restorers, maintainers and evaluation of parental lines and development of promising maintainer lines in to CMS lines forms an integral part of hybrid rice technology. Nuclear genes are required to restore pollen fertility to CMS lines. Restorer line carrying the restorer gene (*Rf*) to restore fertility is indispensable for the development of hybrid varieties. Different cytoplasm in rice has different mechanism for the cause of male sterility and they have different fertility restorer gene governed by nuclear genome. The use of molecular markers linked to *Rf* genes can enhance the selection efficiency, save time and avoid the complications associated with phenotype-based screening. To use any parental lines in heterosis breeding we need to know their ability to transfer superior performance to offspring. Since the combining ability of parents and hybrids does not always depend on the *per* se performance. The Line x Tester analysis gives reliable information about the nature and magnitude of gene action and combining ability effects present in the genetic materials. Till today there is not even a single rice hybrid developed in Kerala, main reson is non-availability of maintainers and CMS lines from Kerala rice varieties. So to solve this problem in Kerala present project "Genotyping of *Rf* (Restoring fertility) loci of rice varieties of Kerala using molecular markers" was undertaken and the result obtained are presented in following sections.

4.1 Molecular characterization of *Rf* loci using SSR markers.

4.2 Validation of the restoration of fertility and identification of restorers and maintainers.

4.3 Study of inheritance pattern of restorer gene for WA cytoplasm through Bulk Segregant Analysis (BSA).

4.4 Diversity analysis among 21 rice varieties

4.5 Identification of heterotic hybrids between CMS lines and identified restorers.

4.6 Locating heterotic combiners from the probable restorers and maintainers by assessing general and specific combining ability effects of restorers, maintainers in L x T analysis

4.7 Identification heterotic hybrids from cross between maintainers and restorers for seed yield and yield attributes

4.1 MOLECULAR CHARACTERIZATION OF *Rf* LOCI USING SSR MARKERS.

4.1.1 Isolation of genomic DNA and its quality and quantity

Breeder seeds of twenty one rice varieties collected from three different rice research centres of Kerala namely Regional Agricultural Research Station, Kerala Agricultural Universiy (KAU), Pattambi, Palakkad, Rice Research Station KAU, Moncompu, Alappuzha and Rice Research Station Mannuthy, Thrissur were grown in nursery of Department of Plant Breeding and Genetics, College of Agriculture, Vellayani. Genomic DNA was isolated from the 3-4 week old seedlings of the 21 rice varieties by using Quiagen Dneasy Minikit (Germany). The quality and quantity of DNA was checked both by agarose gel electrophoresis and UV spectrophotometer. The quantity of DNA was estimated by UV absorbance at 260nm along with blank. The amount of protein and RNA in the genomic DNA was also recorded at 280 nm using the same UV spectrophotometer. The ratio of absorbance at 260 nm and 280 nm ranges from 1.8-2 (Table 06), which indicated that the DNA was free from contaminants like RNA and protein etc. The quality of all the parental lines was also checked on agarose gel for its base pair size and RNA contamination. Resolution of genomic DNA on 0.8 per cent (w/v) submerged agarose gel showed that the genomic DNA of the parental genotypes was free from any mechanical or enzymatic degradation and was intact and good quality.

Table 06: Quality and Quantity of the 21 rice accession

Sl. No	Variety Name	A 260	A280	A260/A 280	DNA yield(ng/µl)
1	PTB 9	0.009	0.005	1.8	450
2	PTB 10	0.01	0.005	2	500
3	PTB 32	0.008	0.004	2	400
4	Aiswarya	0.011	0.006	1.83	550
5	Annapoorna (PTB 35)	0.008	0.004	2	400
6	Jyothi (PTB 39)	0.011	0.006	1.83	550
7	Bharathy (PTB 41)	0.01	0.005	2	500
8	Swarnaprabha (PTB 42)	0.009	0.005	1.8	450
9	Mattatriveni (PTB 45)	0.013	0.007	1.85	650
10	Jayathi (PTB 46)	0.012	0.006	2	600
11	Neeraja (PTB 47)	0.008	0.004	2	350
12	Kanchana (PTB 50)	0.013	0.007	1.85	650
13	Varsha (PTB 56)	0.008	0.004	2	400
14	Kanakom (MO 11)	0.013	0.007	1.85	650
15	Karthika (MO 7)	0.009	0.005	1.8	450
16	Aruna (M0 8)	0.01	0.005	2	500
17	Remya (MO 10)	0.008	0.004	2	400
18	Aruna (MO-8)	0.009	0.005	1.8	450
19	Pavizham (MO 6)	0.011	0.006	1.83	550
20	Uma (M0 16)	0.013	0.007	1.85	650
21	Manupriya	0.008	0.004	2	400

Plate 3: Testing the Quality of DNA

4.1.2 Screening for *Rf* loci

Thirteen microsatellite primers that were reported to be linked with fertility restoring genes (Table 2) in different chromosomal locations (chromosomes 1, 7, 10, and 12) (Bazrkar *et al.*, 2008, Nematzadeh A and Kiani G. 2010) were employed for polymorphism survey between 21 different rice varieties of Kerala (Table 1). The 21 are high yielding in Kerala condition and has other superior characteristics preferred by Kerala farmers including kernel colour (Table 07)

Analysis for *Rf3* gene was performed by SSR markers *viz.*, RM 1, RM 443, RM 3233, RM 3873 and RM 315 which were reported to be linked to this gene and analysis for *Rf4* gene was performed by SSR markers viz., RM 171, RM 216, RM 228, RM 258 and RM 6100 which were reported to be linked to *Rf4* gene. Rice varieties, PTB-9, Remya, Jayathi, Swarnaprabha, Manupriya, Bharthy, Uma, Jyothi, Mattatriveni, Neeraja, Aruna and Pavizham produced alleles with size ranging from 111bp-328 bp corresponding to SSR markers RM 1, RM 3233, RM 3873, RM 315, RM 443, RM 171, RM 6100, RM 328 and RM 216 confering the presence of *Rf3* gene and *Rf4* gene (Plate 5).

Rice varieties PTB 9, Swarnaprabha, Manupriya, Bharathy, Uma, Annapoorna, Jyothi, Karthika, Kanakom, Varsha, Aruna and Pavizham produced alleeles on amplification of DNA with SSR markers RM 244 and RM 591 with size ranging from 163-258 bp confering the presence of *Rf6* gene. Marker RM 7003 is linked to a restorer gene *Rf*7 produced an amplicon at 101 bp in varieties PTB 9, Remya, Jayathi and Jyothi (Plate 5).

Four lines viz., Remya, PTB9, Manupriya and Swarnaprabha had four (*Rf3*, *Rf4*, *Rf5* and *Rf6*) gene in common and rice variety Aruna, Uma and Bharathy had three gene viz., *Rf*, *Rf4* and *Rf6* (Table 08) in common. Variety Hraswa did not show the presence of any of the genes under study

Table 07: Important characteristics of high yielding varieties having *Rf* gene

SL No	Rice varieties	Characteristics
1.	PTB 9	High grain yield, White kernelled, short duration,
2.	Remya	High yielding, grains are red long and bold, moderately tolerant to Blast and Blight, suitable for all season, Medium duration
3.	Jayathi	Dwarf high yielding, grains are white, resistant to BPH, Green Leaf Hopper, Leaf folder and Blast.
4.	Swarnaprabha	White kernelled, short duration
5.	Manupriya	Red long and bold grain,
6.	Uma	High yielding, dwarf, non-lodging type. Resistant to BPH and gall midge. Medium bold red kernelled.
7.	Jyothi	Dwarf high yielding, grains are red medium and bold , short duration
8.	Bharathy	Grains are red long and bold, tolerant to BPH, moderately tolerant to blast, suitable to dry season
9.	Karthika	High yielding, Red kernelled, Dwarf
10.	Kanakom	High yielding, Medium red and bold seed,resistant to BPH, medium resistant to stem borer and resistant to rice tungro, suitable for 3 season.
11.	Varsha	Grains are red long and bold
12.	PTB-32	Red kernelled, long duration
13.	Kanchana	Grains are red long and bold, resistant to Blight, Balst, Stem Borer, short duration
14.	Hraswa	High yielding, Red kernelled, extra short duration
15.	Pavizham	High yielding, Red short and bold grains, Fairly resistant to Brown plant hopper. Moderately resistant to stack burn and sheath rot and fairly resistant to sheath blight
16.	Mattatriveni	Dwarf, High yielding. Red medium and bold seeds, Tolerant to Brown plant hopper
17.	PTB 10	Low yielding, Red kernelled, short duration

18.	Neeraja	Semi tall, High yielding and White grain, Moderately resistant to leaf folder, resistant to blast, suited to flood prone and waterlogged areas
19.	Annapoorna	Dwarf high yielding. Red short and bold seed,
20.	Aruna	High yielding, Grains are red long and bold, Tolerant to BPH, medium resistant to stem borer and Sheath root, short duration
21.	Aiswarya	Red long and bold grain, Resistant to blast and blight diseases. Resistant to Brown plant hopper.

Plate 04: Molecular assay using SSR markers for *Rf* genes in rice lines.

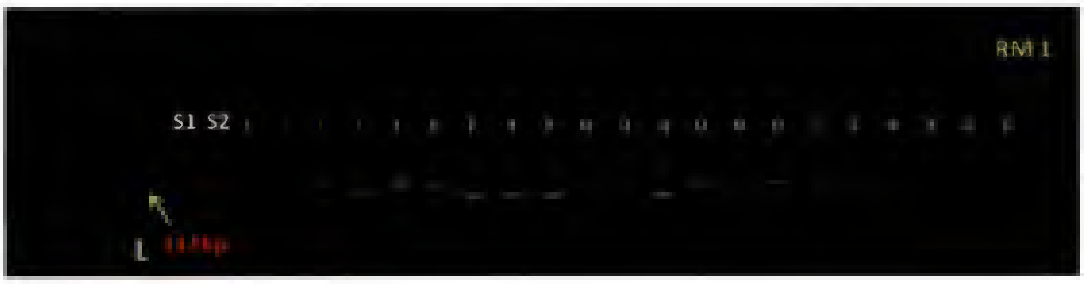


Plate 4(a)

Plate 4(b)

Plate 4(c)

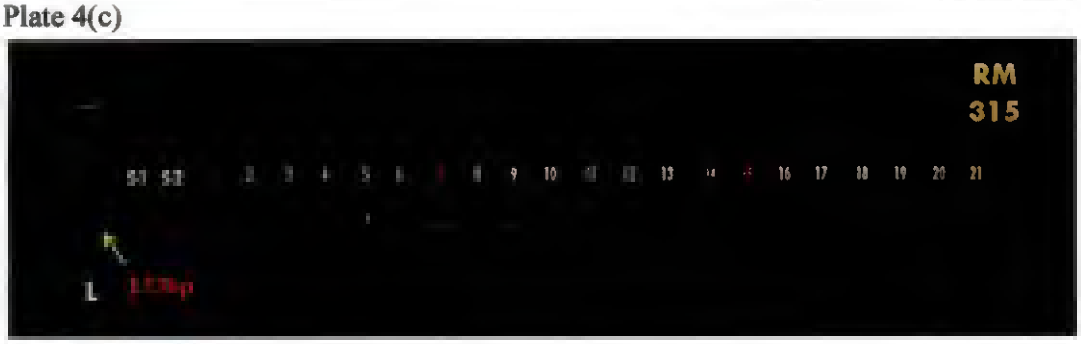


Plate 4(d)

Red marks indicate presence of gene

Plate 4(e)

Plate 4(f)

Plate 4(g)

Plate 4(h)

Red marks indicate presence of gene

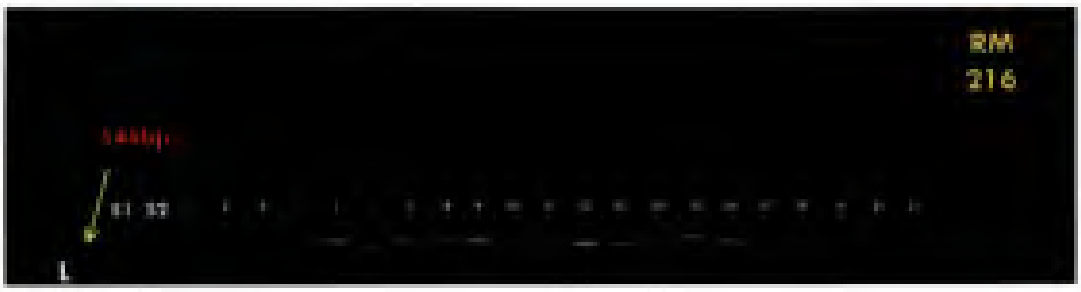


Plate 4(i)

Plate 4(j)

Plate 4(k)

Plate 4(l)

Red marks indicate presence of gene

L -100bp ladder , S1-KMR, S2-293R, 1) PTB-9, 2) Remya, 3) Jayanthi, 4) Swarnaprabha, 5) Manupriya, 6) Bharathy, 7) Uma, 8) Annapoorna, 9) Jyothi, 10) Karthika, 11) Kanakom, 12) Varsha, 13) PTB-32, 14) Kanchana, 15) Mattatriveni, 16) Neeraja, 17) Hraswa, 18) PTB-10, 19) Aruna, 20) Aiswarya, 21) Pavizham

Table 08: Scoring of rice varieties for the presence of *Rf* gene

	Rf3	*Rf4*	*Rf5*	*Rf6*	*Rf7*
PTB-9	—	—	—	—	—
PTB-10	—				
PTB-32		—			
Aiswarya	—				
Annapoorna (PTB-35)	—			—	
Jyothi (PTB-39)	—	—		—	—
Annapoorna (PTB-35)	—			—	
Jyothi (PTB-39)	—	—		—	—
Bharathy (PTB-41)	—	—		—	
Swarnaprabha (PTB-42)	—	—	—	—	
Mattatriveni (PTB-45)	—	—			
Jayathi (PTB-46)	—				—
Neeraja (PTB-47)	—	—			
Kanchana (PTB-50)	—				
Manupriya	—	—	—	—	
Varsha (PTB-56)		—		—	
Kanakom (MO-11)		—		—	
Karthika (MO-7)		—		—	
Uraswa					
Remya (MO-10)	—	—	—	—	
Aruna (MO-8)	—	—		—	
Pavizham (MO-6)	—	—		—	
Uma (MO-16)	—	—		—	

Table 09: Molecular Screening of rice varieties for Restoring fertility (*Rf*) gene

Primers	LINKED GENE	Allele size	Lines
RM 1	*Rf3*	113bp	Remya, Jayathi,Manupriya, Neeraja.
RM 3233		111bp	Remya, Jayathi, Swarnaprabha, Manupriya, Bharathy, Uma, Annapoorna, Jyothi, Mattatriveni, Neeraja.
RM 3873		215bp	PTB-9,Swarnaprabha, PTB -10, Aruna
RM 315		133bp	PTB 9, Uma, Kanchana, Mattatriveni
RM 443		124bp	PTB-9,Jayathi, Jyothi, Aiswarya, Pavizham
RM 171	*Rf4*	328bp	PTB-9,Remya,Mattatriveni, Neeraja, Aruna, Pavizham
RM 6100		144bp	PTB-9, Manupriya, Bharathy, Uma , Jyothi, Karthika, Varsha, PTB-32 , Aruna .
RM 228		154bp	Remya, Swarnaprabha, Bharathy
RM216		146bp	PTB-9 , Swarnaprabha, Bharathy , Kanakom.
RM244	*Rf6*	163bp	Swarnaprabha, Manupriya, Bharathy, Uma, Annapoorna, Jyothi, Karthika, Kanakom, Aruna, Pavizham
RM 591		258bp	PTB-9, Swarnaprabha, Manupriya, Bharathy, Varsha
RM 7003	*Rf7*	101bp	PTB-9, Remya, Jayathi, Jyothi
RM 258	*Rf4,Rf5,Rf6*	148bp	PTB-9, Remya, Swarnaprabha, Manupriya

4.2 VALIDATION OF THE RESTORATION OF FERTILITY AND IDENTIFICATION OF RESTORERS AND MAINTAINERS.

4.2.1 Raising of parents for hybridization programme

The twenty one rice varieties along with 4 CMS lines (Table 01) were raised in the nursery by staggered sowing for synchronization of flowering. Seedlings of these varieties were then transplanted to main field. At boot leaf initiation stage, the male sterile female plants were uprooted in series from the main field and planted in pots. In the CMS lines individual plants with complete pollen was chosen for hybridization. Emasculation was done in the female parents by the clipping method. One day before the pollination spikelets were clipped off one third from the top without damaging the stigma. Immediately after clipping, the panicles were covered with butter bag to prevent contamination from foreign pollen. Crossing between clipped male sterile female parent and 21 Kerala rice varieties was done on the next day morning.

4.2.2 Identification of restorers and maintainers

The harvested F_1 seed of each cross were sown in nursery and after 25 days they were transplanted in field following randomized block design with 3 replication. Pollen and spikelet fertility was counted at flowering and mature stage respectively.

4.2.3 Estimation of Pollen and Spikelet fertility:

The hybrids produced from 21 varieties and four CMS lines showed different level of pollen and spikelet fertility (Table 10). The pollen fertility per cent of hybrids was varying from 0.58 (CRMS31AXAruna) to 91 (UPRI95-17A x Jayathi). Rice varieties Remya, Manupriya, Varsha and Aiswarya showed pollen as well as spikelet fertility more than 80% with CMS line IR58025A. F_1 crosses of Remya, Jayathi, Annapoorna, Neeraja, Aiswarya and Pavizham with UPRI95-17A showed higher pollen as well as spikelet fertility. Crosses of Remya, Jayathi, Swarnaprabha,

Kanakom and Neeraja with CRMS31A recorded more than 80% pollen and spikelet fertility and crosses of Remya, Jayathi, Swarnaprabha, Annapoorna, Kanakom, Mattatriveni and Aiswarya with CRMS 32A also showed more than 80% pollen and spikelet fertility. Among all Kerala rice varieties Remya alone showed more than 80% pollen fertility as well as spikelet fertility when crossed with all four CMS lines IR58025A, UPRI95-17A, CRMS31A and CRMS 32A. Rice variety Jayathi were counted more than 80% pollen and spikelet fertility with UPRI95-17A, CRMS31A and CRMS 32A.Variety Aiswarya recorded more than 80% pollen and spikelet fertility with IR58025A, UPRI95-17A and CRMS 32A . Pollen and Spikelet fertility in crosses between Kanchana and four Male sterile lines (IR58025A, UPRI95-17A, CRMS31A and CRMS 32A) were found less than 1%. Other varieties such as Bharathy, Jyothi and Aruna also showed less than 1% pollen and spikelet fertility, but not with all four CMS lines, Bharathy with CRMS31A and CRMS32A showed 0.60%, 0% and 0.71%, 0% pollen and spikelet fertility respectively. Similarly other two varieties Jyothi and Aruna also found less than 1% pollen and spikelet fertility. So among all the varieties Remya is having more than 80% pollen and spikelet fertility and variety Kanchana is having pollen and spikelet fertility less than 1% with all the CMS lines under study (Table 10).

4.2.4 **Classification of Maintainer and Restorer:**

4.2.4.1 **Classification against IR58025A**

Pollen fertility of 21 crosses between CMS line IR58025A and 21 local rice varieties ranged from 0.61 % (IR58025A X Aruna) to 91% (IR58025A X Manupriya). Likewise, spikelet fertility ranged from 0% (IR58025A X Jyothi, IR58025A X Kanchana and IR58025A X Aruna) to 90% (IR58025A X Manupriya). Three pollen parents Jyothi, Kanchana and Aruna were identified as potential maintainer and pollen parent Remya, Manupriya, Varsha and Aiswarya) of fully fertile crosses were identified as restorers against IR58025A. Total ten varieties

Plate 5(a)

Plate 5(b)

Plate 5: CMS lines and rice varieties in nuresery bed

Plate 6: Field view of transplanted CMS lines and Kerala rice varieties

Plate 7: a. Clipped panicle, b. male panicle inside pollination chamber, c. pollination of clipped panicle

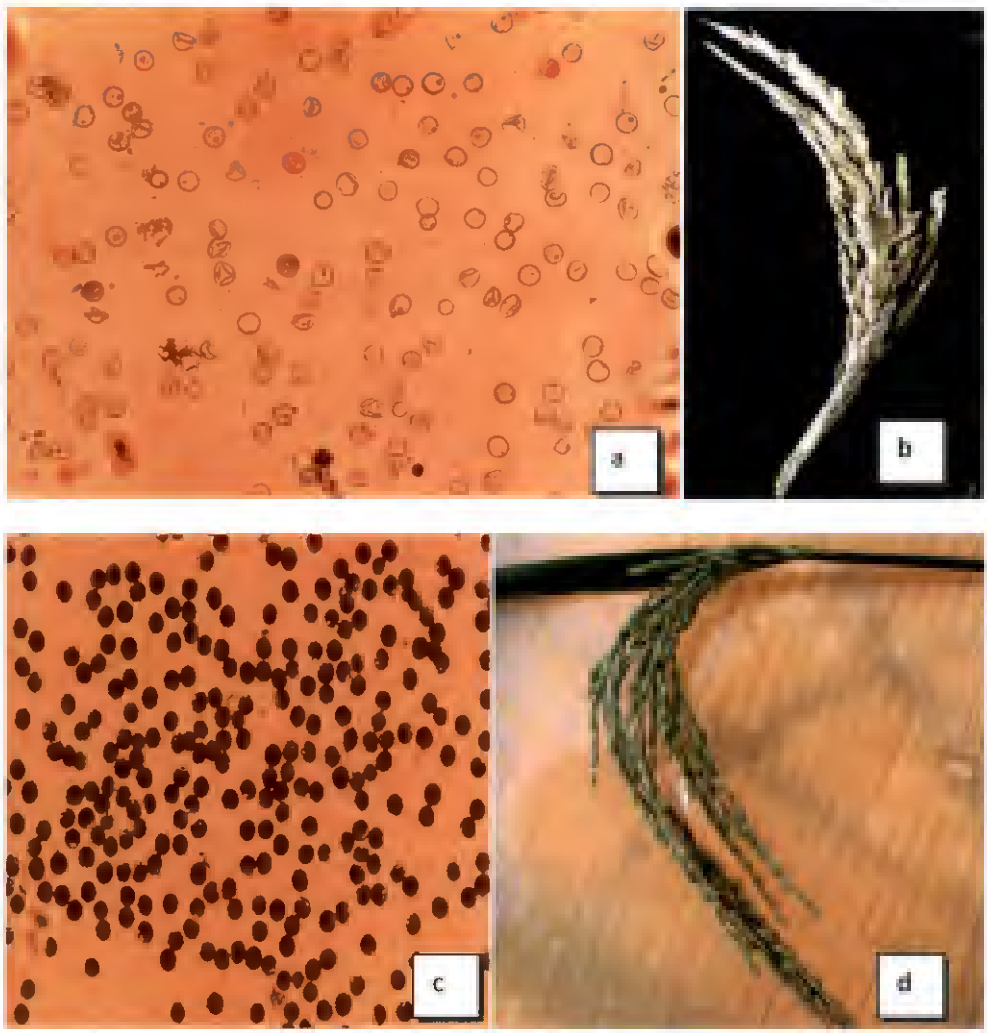


Plate 8: a. Sterile pollen, b. sterile panicle, c. fertile pollen, d. fertile panicle

Table 10: Pollen and Spikelet fertility of F_1 generation of crosses between Kerala Rice varieties and CMS lines

SL No	Cross combination	CMS LINES							
		IR58025A		UPRI95-17A		CRMS31A		CRMS 32A	
		PF%	SF%	PF%	SF%	PF%	SF%	PF%	SF%
Kerala Rice varieties									
1	PTB9	25	30	15	6	29	4	17	9
2	PTB10	42	25	33	21	49	13	51	23
3	PTB32	61	32	55	13	55	3	45	56
4	REMYA	85	83	90	88	89	81	83	80
5	JAYATHI	81	46	91	80	92	82	88	82
6	SWARNAPRABHA	85	84	80	77	90	87	82	81
7	MANUPRIYA	91	90	55	30	69	25	66	62
8	BHARATHY	44	12	66	25	**0.60**	**0**	**0.71**	**0**
9	UMA	69	42	80	62	73	52	75	42
10	ANNAPOORNA	81	65	86	82	52	82	89	86
11	JYOTHI	**0.82**	**0**	13	5	**0.86**	**0**	17	25
12	KARTHIKA	49	29	50	19	71	8	61	71
13	KANAKOM	62	16	75	66	90	83	87	81
14	VARSHA	87	86	65	15	50	9	77	58
15	KANCHANA	**0.65**	**0**	**0.71**	**0**	**0.74**	**0**	**0.81**	**0**
16	MATTATRIVENI	85	57	72	14	78	63	84	84
17	NEERAJA	86	74	90	88	89	85	79	76
18	HRASWA	78	41	70	74	68	59	40	15
19	ARUNA	**0.61**	**0**	33	40	**0.58**	**0**	41	35
20	AISWARYA	86	83	87	86	87	69	85	84
21	PAVIZHAM	74	28	88	85	78	50	78	61

Table 11: Classification of rice varieties into Restorers (R), Maintainers (M), Partial Restorers (PR) and Partial Maintainers (PM).

SL NO.	CROSS COMBINATION	CMS LINES			
		IR58025A	UPRI95-17A	CRMS31A	CRMS 32A
Kerala Rice Varieties					
3	PTB9	PM	PM	PM	PM
4	PTB10	PM	PM	PM	PM
5	PTB32	PM	PM	PM	PM
6	REMYA	**R**	**R**	**R**	**R**
7	JAYATHI	PM	**R**	**R**	**R**
8	SWARNAPRABHA	R	PR	**R**	**R**
9	MANUPRIYA	**R**	PM	PM	PR
10	BHARATHY	PM	PM	**M**	**M**
11	UMA	PM	PR	PR	PR
12	ANNAPOORNA	PR	**R**	PR	**R**
13	JYOTHI	**M**	PM	**M**	PM
14	KARTHIKA	PM	PM	PM	PR
15	KANAKOM	PM	PR	**R**	**R**
16	VARSHA	**R**	PM	PM	PR
17	KANCHANA	**M**	**M**	**M**	**M**
18	MATTATRIVENI	PR	PM	PR	**R**
19	NEERAJA	PR	**R**	**R**	PR
20	HRASWA	PM	PR	PR	PM
21	ARUNA	**M**	PM	**M**	PM
22	AISWARYA	**R**	**R**	PR	**R**
23	PAVIZHAM	PM	**R**	PR	PR

seemed to be partial maintainer (PTB9, PTB10, PTB32, Jayathi, Bharathy, Uma, Karthika, Kanakom, Hraswa and Pavizham) and four varieties partial restorer (Swarnaprabha, Annapoorna, Mattatriveni and Neeraja) (Table 11)

4.2.4.2 Classification against UPRI95-17A

Among crosses between UPRI95-17A and 21 Kerala rice varieties, the highest (0.71%) pollen sterility was observed in F_1 cross of UPRI95-17A x Kanchana and highest fertility (91%) was observed in F_1 cross of UPRI95-17A x Jayathi. The pollen parent Kanchana identified as maintainer in the cross UPRI95-17A x Kanchana and the pollen parents Remya, Jayathi, Annapoorna, Neeraja, Aiswarya and Pavizham found to be restorers in the crosses of UPRI95-17A x Remya, UPRI95-17A x Jayathi, UPRI95-17A x Annapoorna, UPRI95-17A x Neeraja, UPRI95-17A x Aiswarya and UPRI95-17A x Pavizham respectively. Ten varieties identified as partial maintainers (PTB9, PTB10, PTB32, Manupriya, Bharathy, Jyothi, Karthika, Varsha, Mattatriveni and Aruna) and four variety is partial restorers (Swarnaprabha, Uma, Kanakom and Hraswa) (Table 11).

4.2.4.3 Classification against CRMS31A

Pollen sterility and spikelet fertility of 21 F_1s between CRMS31A and 21 Kerala rice varieties were determined for identification of maintainer and restorer lines. Pollen fertility of 21 crosses ranged from 0.58% (CRMS31A x Aruna) to 92% (CRMS31A x Jayathi). Among 21 pollen parents, 4 were identified as maintainers (Bharathy, Jyothi, Kanchana and Aruna), five as restorers (Remya, Jayathi, Swarnaprabha, Kanakom and Neeraja), six as partial maintainer (PTB9, PTB10, PTB32, Manupriya, Karthika and Varsha) and six as partial restorers (Uma, Annapoorna, Mattatriveni, Hraswa, Aiswarya and Pavizham) (Table 11).

4.2.4.4 **Classification against CRMS32A**

Pollen fertility of 21 F_1 hybrids ranged from 0.71% (CRMS32A x Bharathy) to 86% (CRMS32A x Annapoorna). The F_1s of the crosses, CRMS32A X Bharathy and CRMS 32A X Kanchana showed less than 1% (0.71% and 0.81%) pollen fertility and 0% spikelet fertlity indicated that the pollen parents (Bharathy and Kanchana)

carry maintainer genes. So Bharathy and Kanchana might be designated as maintainer lines against CRMS32A. Likewise crosses CRMS32A x Remya, CRMS32A x Jayathi, CRMS32A x Swarnaprabha, CRMS32A x Annapoorna, CRMS32A x Kanakom, CRMS32A x Mattatriveni and CRMS32A x Aiswarya showed pollen fertility and spikelet fertility more than 80%, this designated that pollen parents Remya, Jayathi, Annapoorna, Kanakom, Mattatriveni and Aiswarya are effective restorers. Varieties PTB9, PTB10, PTB32, Jyothi, Hraswa and Aruna identified as partial maintainers and Manupriya, Uma, Karthika, Varsha, Neeraja and Pavizham as partial restorers

4.3 STUDY OF INHERITANCE PATTERN OF RESTORER GENE FOR WA CYTOPLASM

Harvested F_2 seed of crosses between UPRI9517A and identified restorers Remya, Jayathi, Annapoorna, Aiswarya and Pavizham were sown in nursery and after 25 days they were transplanted in field. Based on pollen and spikelet fertility, plants were classified into complete fertile, partial fertile, partial maintainer and complete sterile. Goodness of fit for *Rf* loci to expected segregation ratio was tested by means of chi-square analysis.

4.3.1 Pollen fertility study

The pollen fertility per cent is presented in the Table 12. Sixty plants of male sterile parent UPRI 95-17 A were selected and analysed for pollen fertility percentage which all comes under the category of complete sterile plants. All five restorer lines viz; Remya, Jayathi, Annapoorna, Aiswarya and Pavizham were recorded as complete fertile plants. F_1 hybrids of the cross between UPRI 95-17 A and five restores were recorded as complete fertile plants. A total of 150 F_2 individual plant populations each for UPRI95-17A x Remya and UPRI95-17A X Aiswarya were grown and analyzed for the pollen fertility percentage status of each individual plant. Likewise F_2 plant of UPRI95-17A x Jayathi (148), UPRI95-17A x Annapoorna (149) and UPRI95-17A x Pavizham (147) were also analysed for pollen fertility. Based on the pollen fertility results each of the plant were grouped into different categories.

Plate 09: F_2 population derived from crosses between CMS lines and Kerala rice varieties

4.3.1.1 UPRI95-17A X Remya

Out of 150 plants analyzed ninety nine plants were completely fertile, 8 plants were completely sterile, 25 plants were partially fertile and 18 plants were partially sterile, giving a good fit to digenic ratio 9:3:3:1 with a chiquare value of 6.72.

4.3.1.2 UPRI95-17A X Jayathi

Out of 148 plants analyzed 98 plants were completely fertile, 8 plants were completely sterile, 23 plants were partially fertile and 19 plants were partially sterile, giving a good fit to digenic ratio 9:3:3:1. Chisquare value calculated from F_2 population of this cross is 6.35.

4.3.1.3 UPRI95-17A X Annapoorna

Out of 149 plants analyzed 98 plants were completely fertile, 7 plants were completely sterile, 24 plants were partially fertile and 20 plants were partially sterile, which resulted to a good fit to digenic ratio 9:3:3:1 with a chiquare value of 5.78.

4.3.1.4 UPRI95-17A X Aiswarya

Out of 150 plants analysed for pollen fertility, 105 plants were completely fertile, 7 plants were completely sterile, 20 plants were partially fertile and 18 plants were partially sterile. In this case observed ration does not fit to the expected ratio 9:3:3:1 as the chisquare values are significant. In that case we converted two class viz; Partial sterile and Partial fertile into semi fertile class as suggested by Hossain et al., 2010 then the ratio fitted to 12:3:1 ratio with chisquare value 4.56.

4.3.1.5 UPRI95-17A X Pavizham

Out of 147 plants analyzed 85 plants were completely fertile, 9 plants were completely sterile, 29 plants were partially fertile and 24 plants were partially sterile, reveals an F2 segregation ratio of 9:3:3:1 with a chiquare value of 0.60.

The results revealed that fertility restoration is under dominant gene control and relatively few genes were involved in fertility restoration in the crosses studied.

4.3.2 Spikelets fertility study

The results of spikelet's fertility percentage is presented in the Table 13. Like

pollen fertility, spikelet fertility of parental lines, their F_1's and their F_2's were assessed. Sixty plants of the parent UPRI 95-17 A were selected and analysed for spikelet fertility percentage, all came under the category of complete sterile plants. All five restorer lines viz; Remya, Jayathi, Annapoorna, Aiswarya and Pavizham were recorded as complete fertile plants. F_1 hybrids of the cross between UPRI 95-17 A and five restores were also recorded as complete fertile plants. A total of 147 F_2 individual plant populations for UPRI95-17A x Remya, UPRI95-17A x Jayathi (148), UPRI95-17A x Annapoorna (146), UPRI95-17A x Aiswarya (150) and UPRI95-17A x Pavizham (145) were grown and analyzed for the spikelet fertility percentage status of each individual plant. Based on the spikelet fertility result each of the plant were grouped into different categories (Table 12).

4.3.2.1 UPRI95-17A X Remya

Out of 147 plants analyzed 97 plants were completely fertile, 7 plants were completely sterile, 24 plants were partially fertile and 19 plants were partially sterile, giving a good fit to digenic ratio 9:3:3:1 with a chiquare value 6.11.

4.3.2.2 UPRI95-17A X Jayathi

Out of 148 plants analyzed 98 plants were completely fertile, 7 plants were completely sterile, 23 plants were partially fertile and 20 plants were partially sterile, giving a good fit to digenic ratio 9:3:3:1 with a chiquare value 6.13.

4.3.2.3 UPRI95-17A X Annapoorna

Out of 146 plants analyzed 93 plants were completely fertile, 6 plants were completely sterile, 25 plants were partially fertile and 22 plants were partially sterile, which resulted to a good fit to digenic ratio 9:3:3:1 with a chiquare value 3.77.

Table 12: Chi-square analysis of inheritance pattern of restorer genes for pollen fertility

Parents/crosses	No. of plants CF (>85% fertility	No. of plants PF (85 -70% fertility)	No. of plants PS (70-10% fertility)	No. of plants CS (<10%)	Total no. of plants	Expected genetic ratio	Chisquare value
UPRI95-17A				60	60		
Remya	35				35		
Jayathi	40				40		
Annapoorna	42				42		
Aiswarya	36				36		
Pavizham	30				30		
F_1							
UPRI95-17A X Remya	30				30		
UPRI95-17A X Jayathi	30				30		
UPRI95-17A X Annapoorna	29				29		
UPRI95-17A X Aiswarya	30				30		
UPRI95-17A X Pavizham	29				29		
F_2							
UPRI95-17A X Remya	99	25	18	8	150	9:3:3:1	6.728 (NS)
UPRI95-17A X Jayathi	98	23	19	8	148	9:3:3:1	6.354 (NS)
UPRI95-17A X Annapoorna	98	24	20	7	149	9:3:3:1	5.785 (NS)
UPRI95-17A X Pavizham	85	29	24	9	147	9:3:3:1	0.603(NS)
UPRI95-17A X Aiswarya	105	20	18	7	150	9:3:3:1	11.63 (S)
	CF (>70% Fertility)	Semi fertile (PS+PF)		CS (<10%)	Total no. of plants	Expected ratio	Chisquare value
UPRI95-17A X Aiswarya	105	38		7	150	12:3:1	**4.568** (NS)

Table 13: Chi-square analysis of inheritance pattern of restorer genes for spikelet's fertility

Parents/crosses	No. of plants CF (>70% fertility	No. of plants Pf (30-70% fertility)	No. of plants Ps (1-30% fertility)	No. of plants CS (<1%)	Total no. of plants	Expected genetic ratio	Chisquare value
UPRI95-17A				60	60		
Remya	35				35		
Jayathi	40				40		
Annapoorna	42				42		
Aiswarya	36				36		
Pavizham	30				30		
F_1							
UPRI95-17A X Remya	30				30		
UPRI95-17A X Jayathi	30				30		
UPRI95-17A X Annapoorna	29				29		
UPRI95-17A X Aiswarya	30				30		
UPRI95-17A X Pavizham	29				29		
F_2							
UPRI95-17A X Remya	97	24	19	7	147	9:3:3:1	6.118 (NS)
UPRI95-17A X Jayathi	98	23	20	7	148	9:3:3:1	6.138 (NS)
UPRI95-17A X Annapoorna	93	25	22	6	146	9:3:3:1	3.771 (NS)
UPRI95-17A X Pavizham	90	25	23	7	145	9:3:3:1	2.163 (NS)
UPRI95-17A X Aiswarya	**99**	**20**	**17**	5	**141**	**9:3:3:1**	**11.47** (S)
	CF (>70% fertility	Semi fertile (PS+PF)		CS (<1%)	Total no. of plants	Expected genetic ratio	Chisquare value
UPRI95-17A X Aiswarya	99	37		6	141	12:3:1	**5.502** (NS)

4.3.2.4 UPRI95-17A X Aiswarya

Out of 141 plants analysed for pollen fertility, 99 plants were completely fertile, 5 plants were completely sterile, 20 plants were partially fertile and 17 plants were partially sterile. In this case observed ration did not fit to the expected ratio 9:3:3:1 as the chisquare values were significant. So we converted two classes *viz;* Partial sterile and Partial fertile into semi fertile class as suggested by Hossain et al., 2010 which fits to the expected 12:3:1 ratio with a chiquare value 5.50.

4.3.2.5 UPRI95-17A X Pavizham

Out of 145 plants analyzed 90 plants were completely fertile, 7 plants were completely sterile, 25 plants were partially fertile and 23 plants were partially sterile, reveals a F_2 segregation ratio of 9:3:3:1 with a chiquare value 2.16.

4.3.3 BULK SEGREGANT ANALYSIS

Bulks were made by selecting fertile and sterile individuals from the F_2 population of cross between UPRI95-17A and Remya, Jayathi, Neeraja and Pavizham. Two bulk one sterile and one fertile were formed by mixing the DNA from 10 fully fertile plants and identified fully sterile plants from corresponding F_2 plants was used to constitute fertile and sterile bulks, respectively. For genotyping the identified putative restorer lines, PCR amplification of four genotypes (Remya, Jayathi, Neeraja and Pavizham) were carried out, using 2 SSR primers having a tight linkage with *Rf3* and *Rf4* genes. It has been reported that SSR marker RM1 is linked with *Rf3* gene on the short arm of 1st chromosome (He *et al.*, 2002), and RM171 with *Rf*4 on the long arm of 10th chromosome (Jing *et. al*, 2001).

4.3.3.1 Polymorphism study between male sterile and restorer lines

Both primer pairs detected polymorphism between CMS line (UPRI95-17A) and 4 fertile lines (Plate 10). Although for the marker loci a polymorphism between sterile line and all restorer lines were observed, the existence of a polymorphism between sterile and fertile lines is not adequate to associate this polymorphism with the existence of a linkage between marker and the given gene. In such cases, the used markers are not specific for a gene, but are located in a tightly distance from the R*f*

Plate 10: Polymorphism between male sterile line and restorer lines used for BSA analysis

Plate 10(a)

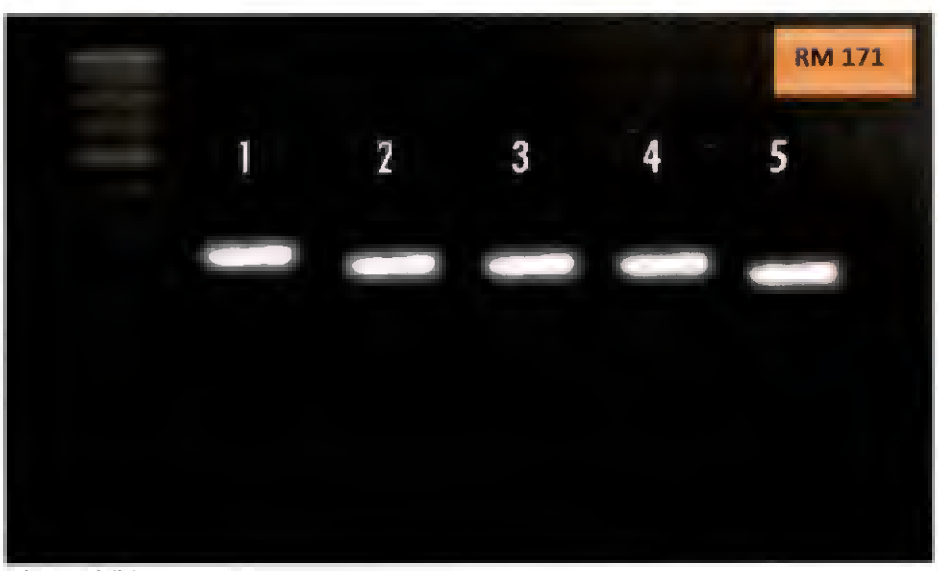


Plate 10(b)

1-UPRI95-17A,2- Remya, 3- Jayathi, 4- Neeraja and 4-Pavizham

Plate 11: Gel picture of 5 parents and their respective Fertile Bulk and Sterile Bulk in F_2 generation

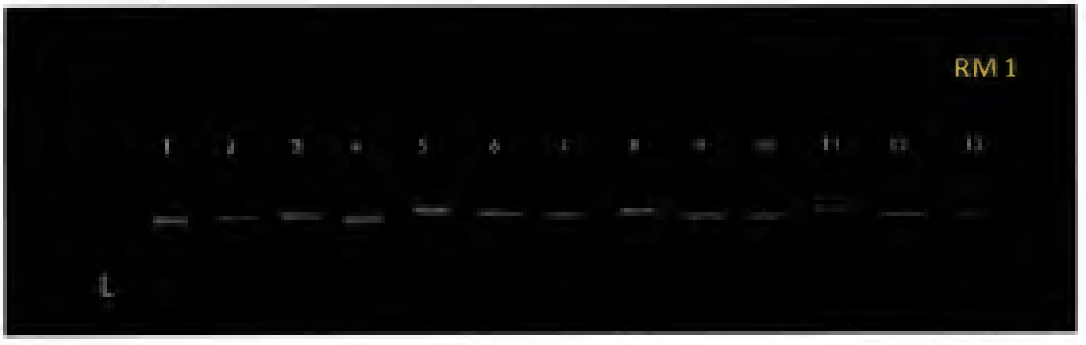


Plate 11(a)

1-UPRI95-17A, 2- Remya, 5- Jayathi, 8- Neeraja, 11- Pavizham 3, 6, 9, 12- Fertile bulk, 4, 7, 10, 13- Sterile

Plate 11(b)

1-UPRI95-17A, 2- Remya, 5- Jayathi, 8- Neeraja, 11- Pavizham 3, 6, 9, 12- Fertile bulk, 4, 7, 10, 13- Sterile

loci, it is better to follow the co-segregation of the marker and the given trait in a segregating generation using bulked segregant analysis (BSA).

4.3.3.2 Co-segregation study of *Rf* gene with marker locus

For carrying out the BSA analysis, individual F_2 plants were divided into two groups, sterile and fertile. Thus, groups of plants differed among themselves on alleles of the studied gene. When two primers (RM 1 and RM 2) were tested in PCR with DNA from four parents and respective bulks (BF-bulk fertile and BS-bulk sterile), it has been seen that all the fertile bulk and sterile bulk producing heterozygous and homozygous band respectibly. The amplicon size of fertile and sterile bulk is similar to their respective fertile and sterile parents, except for Pavizham with RM 1 and Jayathi with RM 171 where the F_2 bulks producing heterozygous band with amplicon size almost equal to the fertile parent.

No co-segregation was observed in the F_2 generation of crosses UPRI95-17A x Pavizham and UPRI95-17A x Jayathi between the *Rf* gene and the marker locus of RM1 and RM171 respectively. In F_2 generation of crosses UPRI95-17A x Remya, UPRI95-17A x Jayathi and UPRI95-17A x Neeraja co-segregation between marker RM 1and the trait was observed. While F_2 generation of crosses UPRI95-17A x Remya, UPRI95-17A x Neeraja and UPRI95-17A x Pavizham at the marker locus co-segregation between marker RM 171and the trait was observed.

4.4 DIVERSITY ANALYSIS AMONG 21 RICE VARIETIES

The observation on 12 metric traits from the 21 genotypes were subjected to genetic analysis to study

1. Variability parameters.
2. Correlation of different parameters with yield.
3. Path analysis
4. Genetic divergence study

4.4.1 Variability parameters

Analysis of variance showed significant differences for all the characters studied in the present investigation. The results of analysis of variance are presented in Table 14. The mean values for 12 characters for 21 rice genotypes are given in Table 15.

Table 14: ANOVA for 12 different characters

S.No.	Characters	Replication df:2	Genotypes df;20	Error df:40
1	Plant height (cm)	0.130	138.024**	1.104
2	Total no of tillers/plant	0.238	2.243**	0.579
3	Days to flowering	7.434**	45.768**	0.796
4	Number of Productive tillers/plant	0.537	1.8228	0.802
5	Panicle length (cm)	1.773*	7.408**	0.843
6	Pollen fertility %	0.760	192.526**	1.937
7	Number of Spikelets /panicle	2.171**	592.989**	0.513
8	Number of Filled grain /panicle	0.666	912.829**	1.057
9	Number of grain /panicle	0.595	118.445**	0.945
10	Grain length/breadth	0.016	0.301**	0.043
11	Number of grain /panicle	1.180	558.417**	3.060
12	Grain yield/plant (g)	0.003	13.699**	0.930

4.4.1.1 Plant height

Plant height ranged from 90.5cm to 117.5 cm with a general mean of 103.3cm. The shortest genotype was Jyothi (90.5 cm), while the tallest genotype was PTB-9 (117.5 cm).

4.4.1.2 Total number of tillers/plant

Total number of tillers per plant ranged from 9.5 to 13.08 with a general mean of 11. Rice variety Varsha (9.5) were recorded lowest no of tillers per plant and Swarnaprabha (13.08) recorded the highest.

4.4.1.3 Days to flowering

The number of days to flowering ranged from 78.5 days to 91 days with a general mean of 84.44. Among all the genotype, Mattatriveni was earliest (78.50 days) while (Karthika) was found to be late (91 days).

4.4.1.4 Number of Productive tillers/plant

The mean productive tillers per plant ranged from 7.97 tillers (varsha) to 11.5 tillers (Uma) with a general mean of 9.63tillers per plant.

4.4.1.5 Panicle length

The panicle length ranged from 20.17 cm to 26.65 cm with a general mean of 20.5 cm. The genotype Kanchana had shorter panicle length 20.17 cm and the genotype Hraswa exhibited the highest value of 26.65 cm. which was almost 6.5 cm longer than Kanchana.

4.4.1.6 Pollen fertility percentage

The mean values for pollen fertility percentage ranged from 54 (PTB-10) to 92.5 (Aiswarya) with a general mean of (78.54).

4.4.1.7 Number of Spikelets /panicle

The mean number of spikelet/panicle was 164.78 and the mean values ranged from 131.5 (PTB-32) to 181 (Pavizham).

4.4.1.8 Number of Filled grain /panicle

The mean values for number of filled grains per panicle ranged from 106.5 to 165. PTB 32 recorded the lowest value for filled grain where as Uma recorded highest filled grain/panicle which is almost 60 spiklets more than the variety PTB 32.

4.4.1.9 Days to maturity

The number of days to mature ranged from 98 days to 123 days with a general

mean of 111.83 days. Among all the genotype, PTB-9 was earliest (98 days) while (Remya) was found to be late (123 days).

4.4.1.10 Length and Breadth ratio

The range of variation observed for this character was from 1.9 to 3.3 with a mean value of 2.67. The genotype Pavizham (1.9) had minimum length/ breadth ratio while the genotype Neeraja (3.3) had maximum length/ breadth ratio.

4.4.1.11 Number of grain /panicle

The mean of number of grain /panicle was 157.23 and the mean values ranged from 125 (PTB-10) to 177 (Swarnaprabha). Among the genotypes tested Swarnaprabha recorded the highest no of grain per panicle.

4.4.1.12 Grain yield/plant

The mean for grain yield per plant was 23.78 g and the mean value ranged from 18 g (PTB-32) to 26.5 g (Uma). The genotype PTB-32 recorded the lowest grain yield where the genotype Uma exhibited highest grain weight.

4.4.2 GENETIC VARIABILITY, HERITABILITY AND GENETIC ADVANCE

Different variability parameters are given in Table 16 for 12 characters.

4.4.2.1 Plant height

The genotypic and phenotypic coefficients of variation estimates observed for this trait were low i.e., 8 and 8.06, respectively. The observed heritability estimates for this character was high (98.41) with moderate genetic advance as per cent of mean (16.35).

4.4.2.2 Total number of tillers

The genotypic coefficients of variation for this trait was low (8.28) but phenotypic coefficients of variation was moderate (10.79). The observed heritability

estimate was high (58.98) and with moderate genetic advance as per cent of mean (13.11).

4.4.2.3 Days to flowering

The genotypic and phenotypic coefficients of variation were low i.e., 5.60 and 5.70, respectively. The observed heritability estimate for this trait was high (96.58) while genetic advance as per cent of mean (11.35) was moderate.

4.4.2.4 Number of Productive tillers/plant

The genotypic coefficients of variation for this trait was low (7.41) where as phenotypic coefficients of variation for this trait were moderate i.e., 11.89. The observed heritability estimate was high (38.86) and with moderate genetic advance as per cent of mean (10.52).

4.4.2.5 Panicle length

The GCV and PCV for this trait was low *i.e.,* 7.55 and 8.46, respectively. The heritability observed for this trait was high (79.56). This character recorded moderate genetic advance as per cent of mean (13.87).

4.4.2.6 Pollen fertility

Moderate genotypic (12.42) and phenotypic (12.55) coefficients of variation were recorded with high heritability estimate of 98.008 and high genetic advance (25.34) as per cent of mean.

4.4.2.7 Number of Spikelets /panicle

Moderate genotypic (10.44) and phenotypic (10.45) coefficients of variation were recorded with high heritability estimate of 99.82 and high genetic advance (21.49) as per cent of mean..

4.4.2.8 Number of Filled grains /panicle

A moderate genotypic (14.94) and phenotypic (14.96) coefficients of variation were observed for this trait. The heritability estimate was very high (99.76) with a high genetic advance as per cent of mean (30.75).

4.4.2.9 Days to maturity

A low genotypic (6.81) and phenotypic (6.87) coefficients of variation were observed for this trait. The heritability estimate was very high (98.41) with a moderate genetic advance as per cent of mean (13.93).

4.4.2.10 Grain length and breadth ratio

A moderate genotypic (13.44) and phenotypic (15.52) coefficients of variation were observed for this trait. The heritability estimate was very high (75) with a high genetic advance as per cent of mean (23.97).

4.4.2.11 Number of grain per panicle

A moderate genotypic (10.59) and phenotypic (10.65) coefficients of variation were observed for this trait. The heritability estimate was very high (98.91) with a high genetic advance as per cent of mean (21.71).

4.4.2.12 **Grain yield per plant**

Moderate genotypic (16.54) and phenotypic (19.60) coefficients of variation were recorded with high heritability estimate of 71.20 and high genetic advance as per cent of mean (28.75).

4.4.3 CORRELATION OF DIFFERENT PARAMETERS WITH YIELD.

Result obtained in the correlation analysis to study the character association of traits studied is given below. Phenotypic (P) and Genotypic (G) correlations are given in Table 17.

Table 15: Mean performance of 21 rice varieties for 12 metric characters.

	Plant height (cm)	Total no of tillers/ plant	Days to flowering	Productive tillers /plant	Panicle length	Pollen fertility %	Spikelets /panicle	Filled grain /panicle	Days to maturity	L/B ration	No of grain /panicle	Grain yield/plant (g)
PTB-9	117.5	12.5	79.16	11.5	25.5	67	180.17	159.9	98	2.54	172.80	26.35
Remya	103.5	11.65	79.06	10	24.7	86	180.98	159.35	123	2.99	168.5	25.77
Jayathi	126.5	10	86.5	9	24.05	79	175.66	149.5	116.5	2.64	175.66	24.84
Swarnaprabha	111	13.08	80.5	11.5	24.05	88	179	162.5	109	3.1	177.22	26
Manupriya	104.5	11.5	82.5	10.37	26	79	179	154.65	109	2.9	166.5	25.57
Bharathy	100	10.69	87	9.5	25.16	78.5	171	153.5	116.5	3.28	163.16	24.65
Uma	97.5	12.75	79.5	11.5	25.1	82.5	179.75	165	118	1.9	179	26.5
Annapoorna	111	10.55	85.5	9.5	26.09	79.5	165	135	103.5	3.12	152.05	21
Jyothi	90.5	9.89	86.5	8.95	24.02	84	168	151.5	108.5	3.2	152	24.86
Karthika	96.65	10.25	91	9.5	22	80	177.83	158	119.5	2.8	165	25.67
Kanakom	107	11.35	81.77	9.74	24.33	78	175.75	156.35	116.5	2.1	155.5	25.5
Varsha	101	9.5	92.5	7.97	24.33	79.15	140.32	108.5	115.5	2.52	139.30	19.66
PTB-32	98	10.1	87.5	8.5	23	62	131.5	106.5	108	2.87	113.67	18
Kanchana	95.5	10.72	84.12	8.90	20.17	83.15	153.5	123.5	109.5	2.71	153	23.75
Mattatrivenai	97.5	10.25	78.5	9.22	23.5	77.5	152	112.93	100.5	2.7	166.5	22.5
Neeraja	101	10.66	90	8.5	24.1	87	149.5	127	126.5	3.3	152	20.57
Hraswa	96.79	9.5	89.5	8.61	26.65	75.5	136	112.62	116.5	2.9	142.3	20.92
PTB-10	106	11.55	93	10.5	23.5	54	134.5	109.5	102.5	2.89	125	20.25
Aruna	107.5	10.5	79.89	9	21	56	173	155	106.5	2.16	152.5	25.65
Aiswarya	96.5	11.94	79.86	10.75	25.5	92.5	178.5	159.5	119.5	2.7	166.5	25.83
Pavizham	105.5	12.5	79.5	11.22	20.5	79	181	160.71	118.5	1.9	163.77	25.5
MEAN	103.3	11	84.44	9.63	23.99	78.54	164.78	142.83	111.83	2.67	157.23	23.78
SED	1.051	0.761	0.892	0.89	0.91	1.39	0.716	1.02	0.97	0.20	1.74	0.96
CD	2.99	2.16	2.54	2.55	2.61	3.24	2.04	2.92	2.77	0.58	4.9	2.75

Table 16: Variability parameters of 12 characters

SL. No	Character	Variance		GCV (%)	PCV (%)	h2 (%) (Broad sense)	GA	GA as percent of mean (5%)
		Genotypic	Phenotypic					
1	Plant height (cm)	68.46	69.56	8	8.06	98.41	16.90	16.35
2	Total no of tillers	0.83	1.41	8.28	10.79	58.98	1.44	13.11
3	Days to flowering	22.48	23.28	5.60	5.70	96.58	9.60	11.35
4	Number of productive tillers/plant	0.51	1.31	7.41	11.89	38.86	0.91	10.52
5	Panicle length (cm)	3.28	4.12	7.55	8.46	79.56	3.32	13.87
6	Pollen fertility %	95.29	97.23	12.42	12.55	98	19.90	25.34
7	Number of spikelets /panicle	296.19	296.70	10.44	10.45	99.82	35.42	21.49
8	Number of filled grains /panicle	455.88	456.94	14.94	14.96	99.76	43.93	30.75
9	Days to maturity	58.75	59.69	6.81	6.87	98.41	15.66	13.93
10	LB ratio	0.12	0.17	13.44	15.52	75	0.64	23.97
11	Number of grains /panicle	277.67	280.73	10.59	10.656	98.91	34.14	21.71
12	Grain yield/plant	6.38	7.31	10.62	11.37	87.28	4.86	20.44

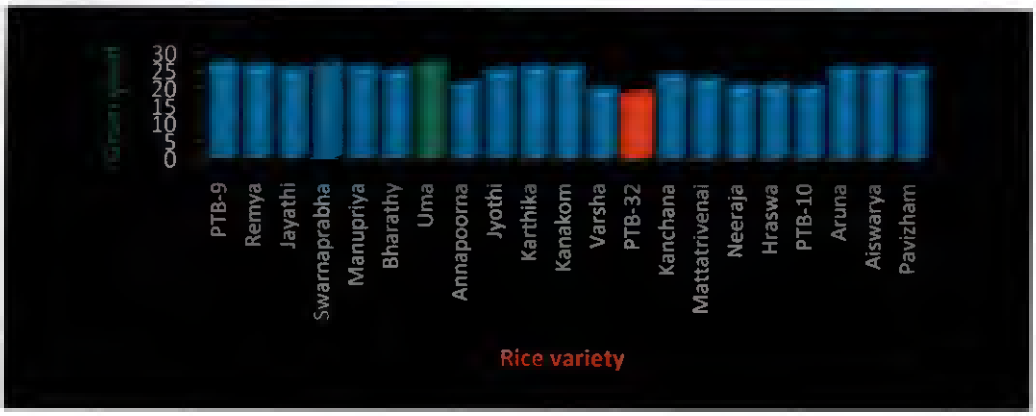


Fig 01: Variation in 21 rice varieties for grain yield/plant

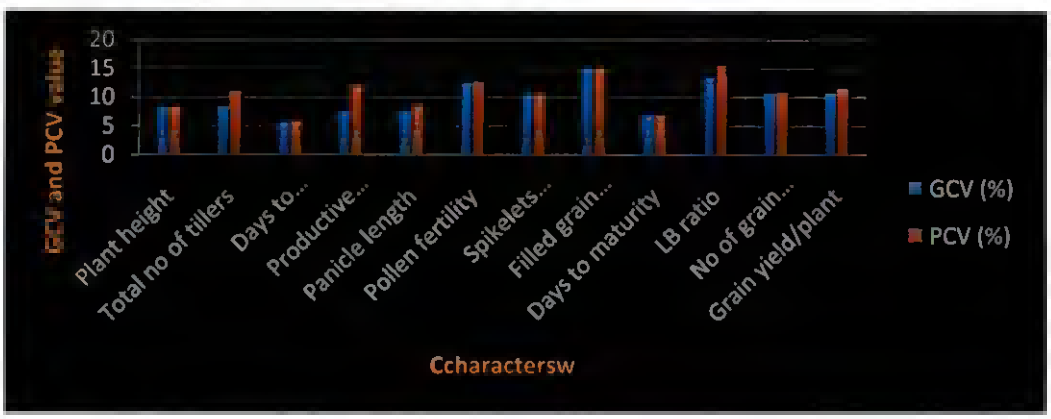


Fig 02: Comparison of PCV and GCV for 12 characters

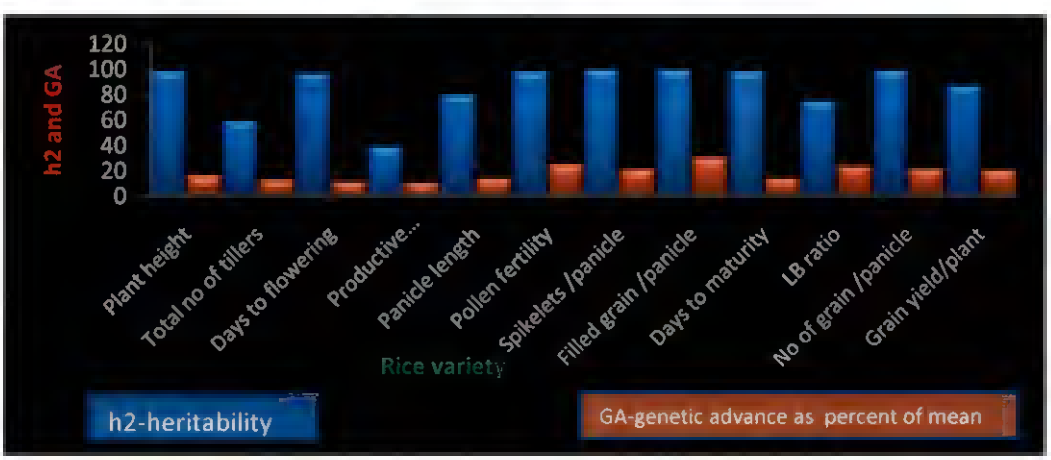


Fig 03: Heritability and genetic advance as per cent of mean for twelve characters

4.4.3.1 Plant height

Panicle length registered significant positive phenotypic (0.388) and genotypic (0.391) correlation with spikelet/panicle and positive correlation with number of grain per panicle (0.308).

4.4.3.2 Total number of tillers/plant

Total no of tillers exhibited significant negative correlation with days to flowering (-0.527P, -0.696G) and LB ratio (0.498P, -0.534G). Number of tillers has significant positive correlation with grain yield/plant (0.517P, 0.660G), productive tillers/plant (0.854P, 1.089G), panicle length (0.469P, 0.704G), spikelets/panicle (0.523P, 0.688G), filled grains/panicle (0.520P, 0.688G) and number of grains/panicle (0.457P, 0.580G) at both phenotypic and genotypic level.

4.4.3.3 Days to flowering

The days to flowering recorded a significant negative correlation with grain yield per plant (-0.624P, -0.674G), productive tillers (-0.446P, -0.751G), panicle length (-0.540P, -0.610G), spikelets/panicle (-0.645P, -0.659G), number of filled grains per panicle (-0.566P, -0.576G) and number of grains/panicle (-0.613P, -0.625G) at both phenotypic as well as genotypic level. The characters, L/B ratio (0.512P, 0.626G) showed positive significant association.

4.4.3.4 No. of Productive tillers/plant

Number of productive tillers per plant exhibited significant positive phenotypic correlation with grain yield per plant (0.500P, 0.889G), panicle length (0.365P, 0.730G), spikelets/panicle (0.526P, 0.843G), filled grains/panicle (0.517P, 0.857G) and number of grains/panicle (0.437P, 0.715G) at both phenotypic and genotypic level. It exhibited negative correlation with LB ratio (-0.366P, -0.441G).

4.4.3.5 Panicle length

Panicle length registered significant positive phenotypic correlation with grain yield per plant (0.610P, 0.745G), pollen fertility (0.300P, 0.306G), spikelets/panicle (0.756P, 0.843G), filled grains/panicle (0.699P, 0.777G), days to maturity (0.311P, 0.370G) and number of grains/panicle (0.771P, 0.859G). The character LB ratio showed negative significant correlation (-0.318) at genotypic level.

4.4.3.6 Pollen fertility

Pollen fertility registered positive non-significant correlation with grain yield per plant (0.090P, 0.099G). Character days to maturity (0.583P, 0.598G) and number of grains/panicle (0.327P, 0.333G) registered significant positive correlation with pollen fertility.

4.4.3.7 Number of spikelets /panicle

Spikelet/panicle registered significant positive phenotypic correlation with grain yield per plant (0.866P, 0.927G), filled grains/panicle (0.945P, 0.947G) and number of grains/panicle (0.854P, 0.859G). Character length-breadth ratio showed negative significant correlation (-0.417P, -0.483G).

4.4.3.8 Number of filled grain/panicle

Filled grain/panicle registered significant positive phenotypic correlation with grain yield per plant (0.847P, 0.914G) and number of grains/panicle (0.759P, 0.765G). Character length-breadth ratio showed negative significant correlation (-0.323P, -0.376G).

4.4.3.9 Days to maturity

Days to maturity registered positive non significant association with yield (0.140P, 0.132G). Character panicle length (0.311P, 0.370G) and pollen fertility (0.583P, 0.598G) showed significant positive association with days to maturity.

Table 17: Phenotypic and Genotypic correlation of 12 characters with Grain yield.

		PH(cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP
PH(cm)	P	1.000	0.194	-0.171	0.177	0.262	-0.293	0.388*	0.293	-0.212	-0.224	0.301	0.179
	G	1.000	-0.277	-0.162	0.302	0.280	0.299	0.391*	0.295	-0.213	-0.296	0.308*	0.205
TTPP	P		1.000	-0.527**	0.854**	0.469**	0.066	0.523**	0.520**	0.009	-0.498**	0.457**	0.517**
	G		1.000	-0.696	1.089**	0.704**	0.125	0.688**	0.688**	0.007	-0.534**	0.580**	0.660**
DF	P			1.000	-0.446**	-0.540**	-0.110	-0.645**	-0.566**	0.150	0.512**	-0.613**	-0.624**
	G			1.000	-0.751**	-0.610**	0.114	-0.659**	-0.576**	0.158	0.626**	-0.625**	-0.674**
NPTP	P				1.000	0.365*	-0.026	0.526**	0.517**	-0.059	-0.366**	0.437**	0.500**
	G				1.000	0.730**	-0.015	0.843**	0.857**	-0.089	-0.441**	0.715**	0.889**
PL(cm)	P					1.000	0.300	0.756**	0.699**	0.311*	-0.239	0.771**	0.610**
	G					1.000	0.306*	0.843**	0.777**	0.370*	-0.318*	0.859**	0.745**
PF%	P						1.000	0.169	0.193	0.583**	0.068	0.327*	0.090
	G						1.000	0.170	0.194	0.598**	0.083	0.333*	0.099
NSPP	P							1.000	0.945**	0.191	-0.417**	0.854**	0.866**
	G							1.000	0.947**	0.195	-0.483**	0.859**	0.927**
NFGP	P								1.000	0.198	-0.323*	0.759**	0.847**
	G								1.000	0.201	-0.376*	0.765**	0.914**
DM	P									1.000	-0.019	0.190	0.140
	G									1.000	-0.011	0.190	0.132
L:B	P										1.000	-0.384*	-0.452**
	G										1.000	-0.436**	-0.531**
NGP	P											1.000	0.825**
	G											1.000	0.860**
GYP	P												1.000
	G												1.000

PH- Plant height, TTPP- Tillers no of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant.

4.4.3.10 Length- breadth ratio

Length-breadth ratio registered significant negative correlation with grain yield per plant (-0.452P, -0.531G) and number of grains/panicle (-0.384P, -0.436G).

4.4.3.11 Number of grain/panicle

Number of filled grains per panicle exhibited a significant positive correlation (0.825P, 0.860G) with grain yield per plant whereas negative and significant correlation with days to flowering (-0.613P, -0.625G) and L/B ratio (-0.384P, -0.436G).

4.4.4 PATH COEFFICIENT ANALYSIS

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

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

As discussed in character association based on the importance of phenotypic effects the present results of phenotypic path coefficient of yield and yield contributing characters are presented in Table 18.

4.4.4.1 Plant height

Plant height had negative phenotypic direct effect on grain yield per plant (-0.214) while the correlation of plant height with grain yield was positive and but non-significant mainly due to positive indirect effect contribution through total no of tillers/plant (0.029), number of productive tillers per plant (0.021), pollen fertility

(0.057), number of spikelets/panicle (0.063), number of filled grains per panicle (0.102), L/B ratio (0.017) and number of grains/panicle (0.190).

4.4.4.2 Total number of tillers/plant

Total tillers/plant which exhibited a phenotypic positive direct effect on grain yield per plant (0.136) also had positive and significant correlation with grain yield per plant (0.517). The correlation was positive and significant due to positive indirect contribution through days to flowering (0.057), number of spikelet/panicle (0.105), number of filled grain per panicle (0.219) and number of grains/panicle (0.293).

4.4.4.3 Days to flowering

The days to flowering had direct phenotypic negative effect (-0.095) on grain yield and the correlation between days to flowering and grain yield per plant was negative and significant (-0.624). The correlation was negative and significant due to negative indirect effect through number of spikelets/panicle (-0.137), number of filled grains/panicle (-0.258) and number of grains/panicle (-0.364).

4.4.4.4 Number of productive tillers/plant

The number of productive tillers/plant had direct phenotypic negative effect (-0.172) on grain yield and the correlation between days to flowering and grain yield per plant was positive and significant (0.500). The correlation was positive and significant mainly due to positive indirect effect through total number of tillers/plant (0.123), days to flowering, number of spikelet/panicle (0.109), filled grains/panicle (0.225) and number of spikelets/panicle (0.109), filled grain/panicle (0.225) and number of grains/panicle (0.309)

Table 18: Direct and Indirect effects of components characters on grain yield

	PH(cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP (g)
PH(cm)	**-0.214**	0.0295	0.021	-0.038	-0.045	0.057	0.063	0.102	-0.008	0.017	0.190	0.179
TTPP	-0.046	**0.136**	0.057	-0.156	-0.078	-0.032	0.105	0.219	0.000	0.030	0.293	0.517**
DF	0.047	-0.081	**-0.095**	0.093	0.096	0.049	-0.137	-0.258	0.003	-0.034	-0.364	-0.624**
NPTP	-0.048	0.123	0.051	**-0.172**	-0.059	-0.017	0.109	0.225	-0.004	0.027	0.309	0.500**
PL(cm)	-0.048	0.052	0.045	-0.050	**-0.202**	-0.119	0.124	0.244	0.012	0.013	0.460	0.610**
PF	0.047	0.017	0.018	-0.011	-0.092	**-0.261**	0.080	0.143	0.021	-0.002	0.355	0.090
NSPP	-0.067	0.070	0.065	-0.093	-0.124	-0.104	**0.202**	0.391	0.007	0.024	0.537	0.866**
NFGP	-0.054	0.073	0.061	-0.095	-0.122	-0.092	0.196	**0.404**	0.009	0.022	0.490	0.847**
DM	0.045	-0.001	-0.007	0.018	-0.066	-0.145	0.037	0.100	**0.038**	0.001	0.120	0.140
L:B	0.055	-0.061	-0.048	0.070	0.039	-0.007	-0.072	-0.136	0.000	**-0.068**	-0.220	-0.452**
NGP	-0.064	0.062	0.054	-0.084	-0.146	-0.146	0.171	0.313	0.007	0.023	**0.633**	0.825**

PH- Plant height, TTPP- Tillers no of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant.

4.4.4.5 Panicle length (cm)

Panicle length had direct negative phenotypic effect (-0.202) on grain yield per plant. Where the correlation was positive and significant .The correlation was positive mainly due to positive indirect contribution through number of spikelet/panicle (0.124), number of filled grains/panicle (0.244) and number of grains per panicle (0.460).

4.4.4.6 Pollen fertility

Pollen fertility had direct negative phenotypic effect (-0.261) on grain yield per plant. Where the correlation was positive non-significant.The correlation was positive mainly due to positive indirect contribution through plant height, total number of tillers per plant, days to flowering, number of spikelet/panicle, number of filled grains/panicle, days to maturity, and number of grains per panicle

4.4.4.7 Spikelet/panicle

Number of spikelets per panicle had direct phenotypic positive effect (0.202) on grain yield per plant. Its correlation with grain yield per plant was also positive and significant (0.866). The correlation between number of spikelets per panicle and grain yield per plant was positive and significant mainly due to positive indirect effect through total number of tillers per plant (0.070), days to flowering, filled grains/panicle (0.065), days to maturity (0.007), L B ratio (0.024) and number of grains per panicle (0.537).

4.4.4.8 Number of filled grains per panicle

Number of filled grains per panicle had direct phenotypic positive effect (0.404) on grain yield per plant. Its correlation with grain yield per plant was also positive and significant (0.847). The correlation between number of filled grains per

panicle and grain yield per plant was positive and significant mainly due to positive indirect effect through total number of tillers per plant (0.073), number of productive tillers per plant (0.061), number of spikelets per panicle (0.196) and number of grains per panicle (0.490).

4.4.4.9 Days to maturity

Days to maturity had phenotypic positive direct effect on grain yield per plant (0.038) while the correlation of plant height with grain yield was positive and but non-significant mainly due to positive indirect effect through filled grain/panicle (0.100) and number of grain/panicle (0.120).

4.4.4.10 Length / breadth ratio

LB ratio had direct negative phenotypic effect on grain yield per plant (-0.068) and its correlation to grain yield per plant was negative and significant (-0.452). The correlation between L/B ratio and grain yield per plant was negative and significant, it is mainly due to negative indirect contribution through total number of tillers per plant (-0.061), number of spikelets/panicle (-0.072), filled grains/panicle (-0.136) and number of grains per panicle (-0.220).

4.4.4.11 Number of grains per panicle

Number of grains per panicle had direct phenotypic positive effect (0.633) on grain yield per plant. Its correlation with grain yield per plant was also positive and significant (0.825). The correlation between number of filled grains per panicle and grain yield per plant was positive and significant mainly due to positive indirect effect contribution through total no of tillers per plant (0.062), days to flowering (0.054), number of spikelets/panicle (0.171) and number of filled grains/panicle (0.313).

4.4.5 GENETIC DIVERGENCE

The quantitative assessment of genetic divergence was made by adopting Mahalanobis D^2 statistic for yield and its contributing characters. Genetic divergence was estimated for 21 rice varieties and the results obtained from the study are presented below.

4.4.5.1 Mahalanobis `D^2 values

The correlated unstandardized means of 12 characters studied were transformed to standardized uncorrelated set of variables by using pivotal condensation method. The statistical distance (Mahalanobis' D^2 value) between a pair of genotypes was obtained as sum of squares of differences between pairs of corresponding uncorrelated values of any two genotypes. These values were considered at a time and these were used for final grouping of genotypes.

4.4.5.2 Grouping of genotypes into various clusters

Twenty one genotypes were grouped into eight clusters based on D^2 values using Tocher's method (Rao 1952) such that the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. The distribution of genotypes into various clusters is shown in Table 19. Out of eight clusters, cluster I was the largest comprising 7 varieties followed by clusters VI with four varieties, cluster IV, V, VII and VIII each with 2 varieties, cluster II and III with one genotype each. The clusters II and III were represented by single variety indicating high degree of heterogeneity among the genotypes.

4.4.5.3 Average inter and intra cluster distances

The average intra and inter cluster D^2 values are presented in Table120. Intra cluster D^2 values ranged from zero (II and III) to 738.91 (VI). Maximum intra cluster distance was observed in cluster IV (738.91), followed by cluster VI (731.64), cluster

Table 19: Classification of genotypes into different clusters

Cluster	Number of genotypes	Germplasms
I	7	Remya, Manupriya, Bharathy, Uma, Jyothi, Karthika, Pavizham
II	1	Mattatriveni
III	1	Neeraja
IV	2	Varsha, Hraswa
V	2	PTB 32, PTB 10
VI	4	Jayathi, Swarnaprabha, Kanakom, Aiswarya
VII	2	Annapoorna, Kanchana
VIII	2	PTB 9, Aruna

VIII (594.18), cluster I (462.41), cluster V (334.71) and cluster IV (165.26), indicating that some genetic divergence still existed among these rice varieties. This could be made use of in the yield improvement through recombination breeding.

Table 20: Intra (diagonal) and inter clusters values and extant of diversity among the clusters

	I	II	III	IV	V	VI	VII	VIII
I	462.41	4823.88	3317.59	5320.96	7923.47	1676.6	2138.42	2354.69
II		0	4466.51	2586.62	5680.71	8478.55	2473.51	8125.9
III			0	1009.39	2953.68	3473.36	1216.17	5496.55
IV				165.26	1573.1	6421.33	1409.51	7458.16
V					334.71	7248.52	2678.36	6950.62
VI						738.91	2868.87	1324.52
VII							731.64	3224.1
VIII								594.18

From the inter cluster D^2 values of the eight clusters, it can be seen that the highest divergence occurred between cluster II and VI (8478.55) followed by cluster I and V (7923.47), cluster IV and VIII (7458.16), cluster V and VI (7248.52), cluster V and VIII (6950.62) and cluster IV and V (6421.33) suggesting that the crosses involving varieties from these clusters would give wider and desirable recombination.

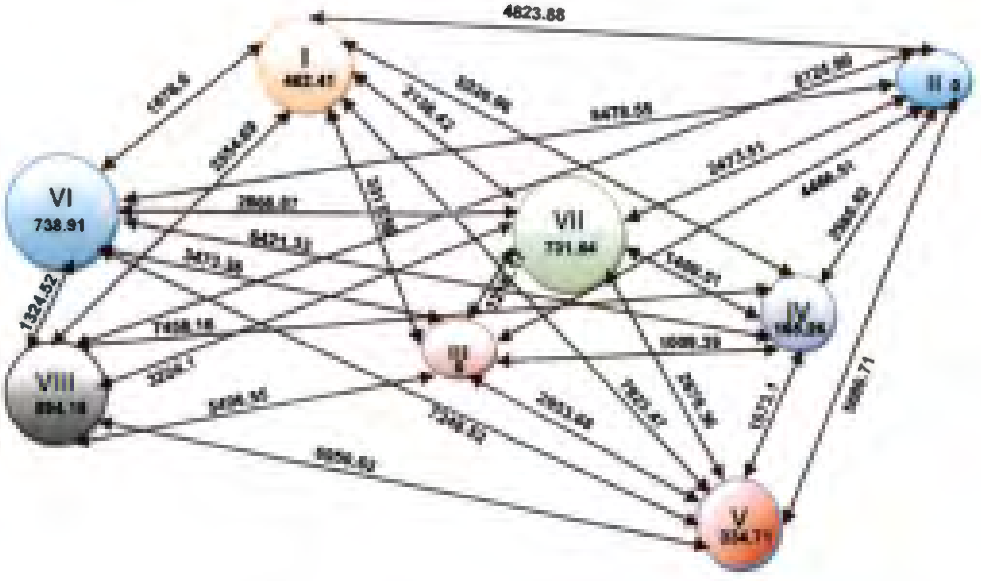


Plate 12: Cluster diagram with intra and inter cluster distance

While the lowest was noticed between cluster III and IV (1009.39), followed by cluster III and VII (1216.17), cluster VI and VIII (1324.52), cluster IV and VII (1409.51), cluster IV and V (1573.1) and cluster I and VI (1676.6).

4.4.5.4 Cluster means of the characters

The cluster means for each of eleven characters are presented in Table 21. From the data it can be seen that considerable differences existed for all the characters under study. The data indicated that the cluster mean for Plant height was highest in cluster VII (110.25 cm) and the lowest in cluster II (97.5 cm). Cluster VI recorded the highest number of total tillers per plant VI (11.6) and the lowest number of productive tillers per plant was in cluster IV (9.5). Cluster VIII recorded the highest number of days to flowering (62) and the lowest was recorded in cluster II (53.87). The number of productive tillers per panicle was the highest in cluster VIII (10.25) and the lowest in cluster IV (8.3). The data indicated that he the cluster means for panicle length was highest in cluster IV (25.5 cm) and the lowest in cluster V (23.25cm).

Pollen fertility percentage was recorded the ighest in cluster VIII (87) and the lowest in cluster V (58). Cluster VI recorded the highest grain yield per plant (15.43 g) while in cluster VIII it was low (6.64 g). Cluster VI recorded the highest (177.22) number of spikelets/panicle it was low in cluster V (133). Number of filled grains/panicle was recorded the highest in cluster I (157.53) and the lowest in cluster V (108). Cluster III (126.5) recorded the highest number of days to maturity and lowest mean recorded in cluster II (100.5). Number of grains/panicle was recorded the highest in cluster VI (168.72) and low in cluster in cluster V (119.33). Cluster VIII recorded

Table 21: Cluster mean for eleven characters.

	PH(cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	NGP	GYP
I	99.75	11.318	55.53	10.15	23.92	81.28	176.79	157.53	116.14	165.42	25.50
II	97.5	10.25	53.87	9.22	23.5	77.5	152	112.93	100.5	166.5	22.5
III	101	10.66	55.83	8.5	24.1	87	149.5	127	126.5	152	20.57
IV	98.89	9.5	54.19	8.29	25.49	77.32	138.16	110.56	116	140.80	20.29
V	102	10.82	56.41	9.5	23.25	58	133	108	105.25	119.33	19.12
VI	110.25	11.6	60.92	10.24	24.48	84.37	177.22	156.96	115.37	168.72	25.54
VII	103.25	10.63	56.94	9.20	23.13	81.32	159.25	129.25	106.5	152.52	22.37
VIII	112.5	11.5	62	10.25	23.25	61.5	176.58	157.45	102.25	162.65	26

PH- Plant height, TTPP- Tillers no of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant.

highest grain yield per plant (26g) while in cluster V it was low (19.12g).The result indicates that selection of genotypes having high values for particular trait could be made and used in the hybridization programme for improvement of that character.

4.4.5.5 Relative contribution of characters towards genetic divergence

The results showed that the contribution of filled grain /panicle was the highest towards genetic divergence (43%) followed by grain yield/plant (20%), number of grain /panicle (15%), spikelet's /panicle (11%), number of grain /panicle (7%), plant height (3%) and pollen fertility percentage (1%) (Table 22). No contribution towards genetic divergence was from the traits total number of tillers/plant, days to flowering, productive tillers/plant and Panicle length.

Table 22: Contribution of characters towards genetic divergence

Sl No	**Character**	**Contribution %**
1	Plant height(cm)	3
2	Total no of tillers/plant	0
3	Days to flowering	0
4	Number of productive tillers/plant	0
5	Panicle length	0
6	Pollen fertility %	1
7	Number of spikelets /panicle	11
8	Number of filled grains /panicle	43
9	Days to maturity	15
10	Number of grains /panicle	7
11	Grain yield/plant	20

4.5 IDENTIFICATION OF HETEROTIC HYBRIDS BETWEEN CMS LINES AND IDENTIFIED RESTORERS.

In the present study, magnitude of heterosis for each trait under study in the form of Mid-parent heterosis or relative heterosis, Better parent heterosis or heterobeltiosis and Standard heterosis or commercial heterosis was computed for all

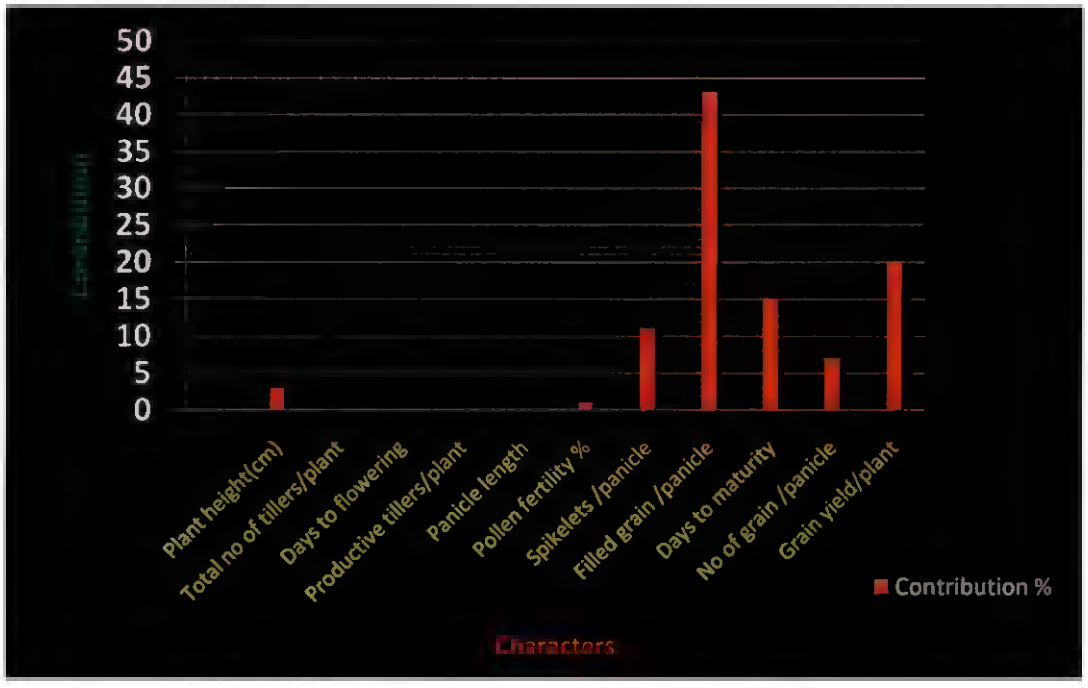


Fig 04: Contribution of 11 metric characters to total diversity

the eighty four (84) crosses. For traits in which an increasing expression of character is desirable, the crosses with significant and positive heterotic effects were considered superior and the trait where low scores are desirable, the crosses with significant and negative heterotic effects were considered promising.

Among 21 rice varieties three (PTB 9, PTB 10 and PTB 32) were found to be maintainers in the entire cross combinations with four CMS lines. While some varieties were restorer in one cross combination but partial maintainer or partial restorers in other crosses (Table 11). As the partial maintainers and partial restorers yield less in F_1 generation, they have not been included here. Only 22 cross combinations showing fertility restoration ability more than 80% (Table 10) were included in result and discussion part.

4.5.1 Plant height

Highest significant negative heterosis over mid parent was recorded in cross combination IR58025A x Manupriya (-15.65%) followed by IR58025A x Swarnaprabha (-13.48%), IR58025A x Varsha (-12.91%) and UPRI95-17A x Remya (-11.72), where as the highest significant negative heterosis over better parent was recorded in cross combination CRMS32A x Remya (-21.30%) followed by CRMS32A x Kanakom (-21.15%), CRMS31A x Kanakom (-20.83%) and IR58025A x Manupriya (-18.52%). While the highest significant heterosis in desirable direction over commercial check Uma was reported in cross combination CRMS32A x Kanakom (-11.48%) followed by IR58025A x Manupriya (-11.25%), CRMS31A x Kanakom (11.11%) and CRMS32A x Remya (-10.59%). Results are presented in Table 23.

4.5.2 Total number of tillers/plant

Almost all crosses revealed significant heterosis for this character (Table 23). Highest significant positive heterosis over mid parent was recorded in cross combination UPRI95-17A x Neeraja (29.2%) followed by UPRI95-17A x Remya (28.55%), UPRI95-17A x Aiswarya (24.29%), CRMS31A x Kanakom (22.48%) and

CRMS32A x Mattatriveni (21.38%). Hybrid UPRI95-17A x Remya (15.33%) registered highest significant positive heterosis over better parent followed by UPRI95-17A x Neeraja (13.07%), UPRI95-17A x Aiswarya (11.71%) and IR58025A x Manupriya (7.17%). Hybrid UPRI95-17A x Remya (40.40%) registered highest significant heterosis over commercial check followed by UPRI95-17A x Neeraja (37.65%), UPRi95-17a x aiswarya (36%), crms31a x swarnaprabha (32.28%) and crms31a x Kanakom (32.23%).

4.5.3 Number of productive tillers

Hybrid CRMS31A x Kanakom (24.83%) registered the highest significant positive heterosis over mid-parent followed by CRMS32A x Mattatriveni (20.92%), UPRI95-17A x Aiswarya (20.94%) and UPRI95-17A x Neeraja (17.74) (Table 22). The hybrid CRMS31A x Kanakom registered highest positive significant heterosis of 15.75 per cent over better parent followed by UPRI95-17A x Aiswarya (14.62%), UPRI95-17A x Neeraja (12.07%) and IR58025A x Manupriya (11.66). The hybrid UPRI95-17A x Aiswarya (25.12%) recorded significant positive heterosis over Uma followed by UPRI95-17A x Neeraja (22.33%), UPRI95-17A x Remya (21.55%) and IR58025A x Manupriya (19.60%).

4.5.4 Pollen fertility

Heterosis for this trait is presented in Table 24. Hybrid UPRI95-17A x Neeraja (13.68%) registered highest significant positive heterosis over mid-parent followed by CRMS31A x Jayathi (12.92%), CRMS31A x Swarnaprabha (12.85%) and CRMS31A x Neeraja (12.30). The hybrid IR58025A x Manupriya manifested highest significant positive heterosis of 10.92 per cent over midparent followed by CRMS31A x Jayathi (10.88%), CRMS31A x Kanakom (9.63%) and CRMS31A x Swarnaprabha (8.43%). The hybrid CRMS31A x Jayathi (9.56) recorded highest significant heterosis over standard check Uma followed by IR58025A x Manupriya (8.33), UPRI95-17A x Jayathi (8.33) and UPRI95-17A x Remya (7.24).

4.5.5 Number of spikelets per panicle

The hybrid CRMS31A x Kanakom (26.66%) showed highest significant heterosis over mid parent followed by CRMS32A x Kanakom (21.94%), UPRI95-17A x Neeraja (18.95%) and UPRI95-17A x Aiswarya (16.74%). The cross UPRI95-17A x Aiswarya (13.09%) recorded the highest better parent heterosis followed by UPRI95-17A x Remya (10.71%), UPRI95-17A x Neeraja (10.11) and UPRI95-17A x Pavizham (7.73%). The hybrid UPRI95-17A x Aiswarya manifested highest significant positive heterosis of 25 per cent over standard checks Uma followed by UPRI95-17A x Remya (22.37%), UPRI95-17A x Neeraja (21.71%) and UPRI95-17A x Pavizham (19.07%).

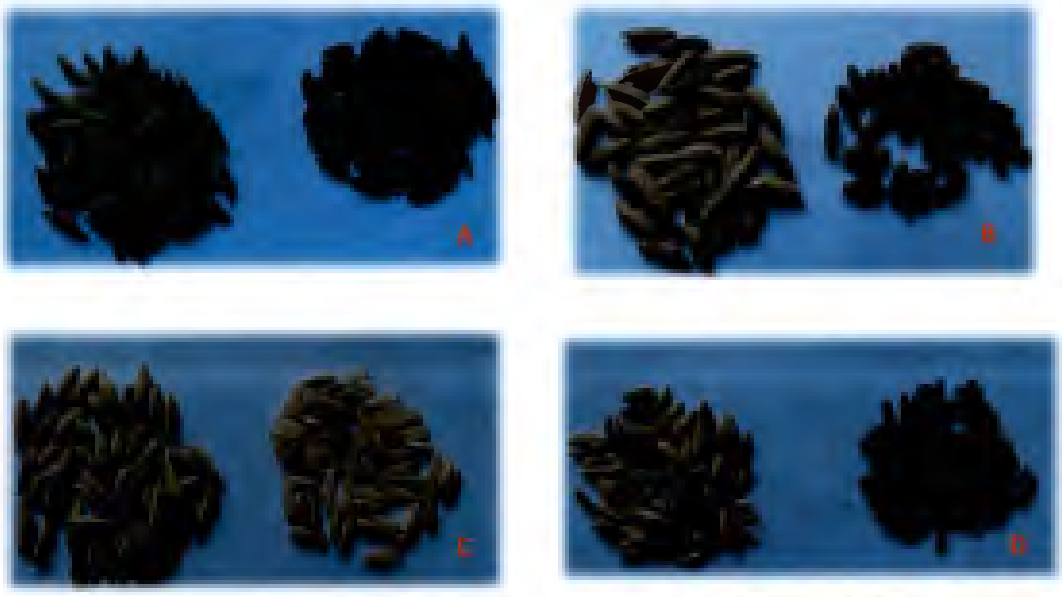
4.5.6 Number of filled grains per panicle

Most of the cross combinations revealed significant heterosis over mid parent, better parent and standard check (Table 24). The hybrid CRMS31A x Kanakom (29.40%) showed highest significant heterosis over mid parent followed by UPRI95-17A x Remya (25.44%), UPRI95-17A x Neeraja (23.65%) and CRMS32A x Kanakom (21.4). The hybrid UPRI95-17A x Remya manifested highest significant positive heterosis of 14.47 per cent over better parent followed by UPRI95-17A x Aiswarya (12.93%), UPRI95-17A x Neeraja (12.55%) and CRMS31A x Swarnaprabha (9.51%). The hybrid UPRI95-17A x Remya manifested highest significant positive heterosis of 25.92 per cent over better parent followed by UPRI95-17A x Aiswarya (24.23%), UPRI95-17A x Neeraja (23.80%) and CRMS31A x Swarnaprabha (17.09%).

4.5.7 Spikelet fertility

The hybrid UPRI95-17A x Remya manifested the highest significant positive heterosis of 20.24 per cent over midparent followed by UPRI95-17A x Neeraja (13.97%), CRMS31A x Kanakom (12.71%) and CRMS31A x Neeraja (12.51%). Only four hybrids registered positive heterosis over better parent but they were all non-significant (Table 24). Hybrid IR58025A x Manupriya (10.70%) showed the

Plate 13: Superior hybrids with commercial check Uma



A-UPRI95-17A x Remya, B-UPRI95-17A x Aiswarya, C- UPRI95-17A x Neeraja, D-CRMS31A x Kanakom

Plate 14: Kernel colour of Hybrids

highest significant heterosis over standard check Uma followed by UPRI95-17A x Remya (8.25%), UPRI95-17A x Neeraja (7.01%) and CRMS31A x Swarnaprabha (7.01%).

4.5.8 Yield per plant

The hybrid CRMS31A x Kanakom manifested the highest significant positive heterosis of 35.73 per cent over mid-parent followed by CRMS32A x Kanakom (22.72%), UPRI95-17A x Remya (22.57%) and UPRI95-17A x Neeraja (21.91%). Hybrid CRMS31A x Kanakom manifested the highest significant positive heterosis of 7.40 per cent over better parent followed by UPRI95-17A x Aiswarya (6.93%), CRMS31A x Swarnaprabha (6.41%) and CRMS31A x Jayathi (6.17%). Hybrid UPRI95-17A x Neeraja (24.31%) showed the highest significant heterosis over standard check Uma followed by UPRI95-17A x Remya (23.36%), CRMS31A x Kanakom (18.26) and UPRI95-17A x Pavizham (17.91%).

4.6 LOCATING HETEROTIC COMBINERS FROM THE PROBABLE RESTORERS AND MAINTAINERS

4.6.1 Raising parent for hybridization programme

Four identified maintainers (Kanchana, Jyothi, Aruna and Bharathy) and 11 restorers (Remya, Jayathi, Swarnaprabha, Manupriya Annapoorna, Kanakom, Mattatriveni, Varsha, Aiswarya, Neeraja and Pavizham) were raised in nursery. After 25 days they were transplanted in field. At boot leaf initiation stage, the female plants were uprooted in series from the main field and planted in pots. Emasculation was done in the female parents by the clipping method. The male parent was then crossed with female parent in L x T fashion. The F_1 seeds then collected from each cross and then planted in field in randomized block design with three replication.

Table 23: Estimated heterosis for the traits Plant height, Total no of tillers/plant and No. of Productive tillers/plant

Sl.No	Cross	PLANT HEIGHT			TOTAL NO OF TILLERS			PRODUCTIVE TILLERS		
		Heterosis over		Standard heterosis over	Heterosis over		Standard heterosis over	Heterosis over		Standard heterosis over
		MP	BP	UMA	MP	BP	UMA	MP	BP	UMA
1.	IR58025A XRemya	-12.43	-17.09**	-5.82**	19.24**	7**	30.26**	3.85*	2.69	12.49**
2.	IR58025A XSwarnaprabha	-13.48**	-17.84**	-7.26**	10.57**	-0.07	21.65*	-9.75**	-10.87**	-4.54
3.	IR58025A XManupriya	-15.65**	-18.52**	-11.25**	17.79**	7.19**	30.49**	16.29**	11.66**	19.60**
4.	IR58025A XVarsha	-12.91**	-15.4**	-8.92**	7.84**	-3.71*	17.21**	-1.76	-5.33**	1.39
5.	IR58025A XAiswarya	-0.93**	-2.35**	-0.88*	18.89**	6.85**	30.08**	4.24*	-0.30	6.78**
6.	UPRI95-17A XRemya	-11.72**	-16.42**	-5.05**	28.55**	15.33**	40.40**	11.16**	10.96**	21.55**
7.	UPRI95-17A XJayathi	4.01**	-0.29	10.34**	2.14	-8.59**	11.27**	-5.45**	-10.55**	-2.36
8.	UPRI95-17A XAnnapoorna	-6.48**	-11.60**	0.75**	9.51**	-0.59	21.01**	-5.97**	-10.67**	-2.49
9.	UPRI95-17A XNeeraja	-9.50**	-12.49**	-4.89**	29.2**	13.07**	37.65**	17.74**	12.07**	22.33**
10	UPRI95-17A XAiswarya	-0.24	-4.87**	-0.19	24.29**	11.71**	36**	20.94**	14.62**	25.12**
11	UPRI95-17A XPavizham	-3.21**	-6.37**	1.66**	16.44**	5.52**	28.46**	9.91**	4.787**	14.3**
12	CRMS31A XRemya	-6.40**	-17.30**	-6.04**	14.37**	1.58*	26.57**	-3.07**	0.63	2.40**
13	CRMS31A XJayathi	7.46**	-3.95**	6.28**	19.28**	5.65**	31.65**	13.80**	11.35**	13.30**
14	CRMS31A XSwarnaprabha	-7.05**	-17.64**	-7.04**	18.69**	6.16**	32.28**	11.16**	9.79**	14.60**
15	CRMS31A XKanakom	-10.86**	-20.83**	-11.11**	22.48**	6.11**	32.23**	24.83**	15.75**	17.78**
16	CRMS31A XNeeraja	-4.31**	-13.78**	-6.30**	18.54**	2.69**	27.97**	11.27**	9.56**	11.48**
17	CRMS32A XRemya	-11.38**	-21.30**	-10.59**	12.73**	0.68	23.88*	-4.95**	-2.04	1.10**
18	CRMS32A XJayathi	10.00**	-1.17**	9.36**	-17.29**	-26.33**	-9.36	-19.70**	-21.98**	-19.47**
19	CRMS32A XSwarnaprabha	-8.86**	-18.83**	-8.38**	8.03**	-2.82**	19.56**	-10.15**	-10.67**	-6.71**
20	CRMS32A XAnnapoorna	-4.23**	-15.07**	-3.20**	17.94**	6.54**	31.10**	14.64**	11.88**	15.48**
21	CRMS32A XMattatriveni	-2.57**	-10.15**	-6.17**	21.38**	3.58**	27.44**	20.92**	3.23**	6.55**
22	CRMS32A XAiswarya	4.30**	-1.19**	-2.59**	11.26**	-0.47	22.46**	6.52**	3.71**	7.04**

* Significant at 0.05 level, ** Significant at 0.01 level

Table 24: Estimated heterosis for the traits Pollen fertility, Spikelet/panicle and filled grain/panicle

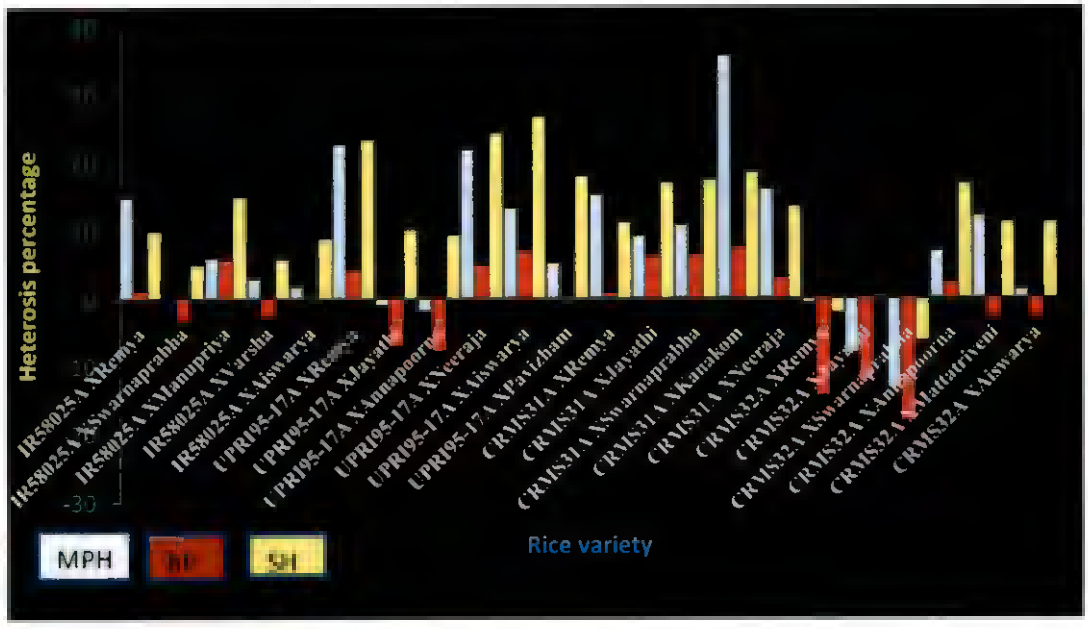
Sl No	Cross	POLLEN FERTILITY %			SPIKELETS/PANICLE			FILLED GRAINS/PANICLE		
		Heterosis over		Standard heterosis over	Heterosis over		Heterosis over	Standard heterosis over		Heterosis over
		MP	BP	UMA	MP	BP	UMA	MP	BP	UMA
1.	IR58025A XRemya	4.61**	3.65**	1.19*	2.41**	1.43**	4.76**	5.74**	-2.08**	1.67*
2.	IR58025A XSwarnaprabha	7.28**	3.69**	1.22*	-14.64**	-13.37**	-10.52**	-10.04	-15.37**	-12.11**
3.	IR58025A XManupriya	11.31**	10.92**	8.33**	0.22	2.93**	6.32**	11.88**	7.74	11.88**
4.	IR58025A XVarsha	6.09**	6.09**	3.57**	4.73**	0.53	3.83**	10.07*	1.71	5.62**
5.	IR58025A XAiswarya	-1.50**	-7.09**	2.30**	0.82	0.98	4.31**	1.87	-2.65*	1.09*
6.	UPRI95-17A XRemya	9.52**	7.24**	7.24**	15.53**	10.71**	22.37**	25.44**	14.47**	25.92**
7.	UPRI95-17A XJayathi	10.97**	8.33**	8.33**	14.71**	2.97**	13.81**	3.58**	-3.17**	6.51**
8.	UPRI95-17A XAnnapoorna	4.96**	2.77**	2.77**	13.72**	1.79*8	12.50**	7.05**	-1.93*	7.874**
9.	UPRI95-17A XNeeraja	13.68**	7.93**	7.93**	18.95**	10.11**	21.71**	23.65**	12.55**	23.80**
10.	UPRI95-17A XAiswarya	-1.03	3.96**	3.96**	16.74**	13.09**	25**	20.07**	12.93**	24.23**
11.	UPRI95-17A XPavizham	8.97**	4.76**	4.76**	10.19**	7.73**	19.07**	14.15**	7.59**	18.35**
12.	CRMS31A XRemya	8.89**	7.25**	5.98**	2.62**	-0.79**	7.68**	3.18**	-4.61**	1.99**
13.	CRMS31A XJayathi	12.92**	10.88**	9.56**	14.86**	3.93**	12.82**	6.83**	1.19**	8.1**
14.	CRMS31A XSwarnaprabha	12.85**	8.43**	7.14**	7.12**	6.04**	15.10**	16.64**	9.51**	17.09**
15.	CRMS31A XKanakom	11.65**	9.63**	8.3**	26.66**	6.19**	15.27**	29.40**	5.19**	12.48**
16.	CRMS31A XNeeraja	12.30**	7.23**	5.95**	10.63**	3.27**	12.10**	13.64**	4.78**	12.04**
17.	CRMS32A XRemya	0.30	-2.35**	-1.19	-16.71**	-19.01**	-13.15**	-17.12**	-22.87**	-18.72**
18.	CRMS32A XJayathi	6.71**	3.57**	4.80**	-20.43**	-27.60**	-22.36**	-25.94**	-29.37**	-25.56**
19.	CRMS32A XSwarnaprabha	2.78**	-2.35**	-1.19	-31.62**	-31.90**	-26.97**	-30.56**	-34.36**	-30.83**
20.	CRMS32A XAnnapoorna	6.34**	3.52**	4.76**	14.73**	4.08**	11.62**	14.30**	6.79**	12.54**
21.	CRMS32A XMattatriveni	-3.68**	-7.10**	1.19	2.20**	3.74**	11.25**	11.41**	3.68*	9.27**
22.	CRMS32A XAiswarya	-4.22**	-8.10**	1.19	5.56**	3.78**	11.29**	9.07**	4.69**	10.33**

* Significant at 0.05 level, ** Significant at 0.01 level

Table 25: Estimated heterosis for the traits Spikelet fertility and Grain yield/plant

Sl. No.	Cross	Spikelet fertility %			Grain yield/plant		
		Heterosis over		Standard heterosis over	Heterosis over		Standard heterosis over
		MP	BP	UMA	MP	BP	UMA
1.	IR58025A XRemya	12.63**	-6.73**	2.09**	14.77**	1.12	9.88**
2.	IR58025A XSwarnaprabha	0.82	-5.87**	3.32**	-0.04	-3.30*	5.06**
3.	IR58025A XManupriya	5.68**	1.12	10.70**	5.98**	5.76**	14.92**
4.	IR58025A XVarsha	1.51*	-2.24**	7.01**	2.93**	-2.63*	5.80**
5.	IR58025A XAiswarya	-7.33**	-7.80**	1.95	1.73	0.12	8.79**
6.	UPRI95-17A XRemya	20.24	0.01	8.25**	22.57**	4.13*	23.36**
7.	UPRI95-17A XJayathi	-9.68**	-10.31**	-1.55*	-1.03	-7.01**	10.15**
8.	UPRI95-17A XAnnapoorna	-7.67**	-6.81**	0.86	-1.83	-7.70**	9.34**
9.	UPRI95-17A XNeeraja	13.97*	-1.13	7.01**	21.91**	4.94**	24.31**
10.	UPRI95-17A XAiswarya	-4.44**	-5.45**	4.55**	13.25**	6.93**	26.67**
11.	UPRI95-17A XPavizham	1.58**	-3.40**	4.55**	5.25**	-0.45	17.91**
12.	CRMS31A XRemya	11.42**	-6.89**	-0.36	15.21**	0.86	11.24**
13.	CRMS31A XJayathi	-6.92**	-8.09**	0.88	9.20**	6.17**	17.10**
14.	CRMS31A XSwarnaprabha	5.69**	0.003	7.01**	10.80**	6.41**	17.37**
15.	CRMS31A XKanakom	12.51**	-4.07**	2.64*	35.73**	7.40**	18.46**
16.	CRMS31A XNeeraja	12.71**	-1.75*	5.13**	16.01**	2.96*	13.56**
17.	CRMS32A XRemya	8.98**	-9.55**	-1.54*	-0.70	-14.40**	-2.09
18.	CRMS32A XJayathi	-7.73**	-8.11**	0.86	-8.03**	-12.14**	0.49
19.	CRMS32A XSwarnaprabha	-2.48**	-8.47**	-0.36	-13.23**	-18.09**	-6.31**
20.	CRMS32A XAnnapoorna	-3.18**	-2.56**	6.06**	6.97**	2.26	16.96**
21.	CRMS32A XMattatriveni	2.35**	-5.08**	3.32**	12.12**	-2.73*	11.24**
22.	CRMS32A XAiswarya	-4.95**	-5.68**	4.28**	1.314	-2.73*	11.24**

* Significant at 0.05 level, ** Significant at 0.01 level



MPH mean relative heterosis . SH mean Standard heterosis

Fig 05: Different types of heterosis for grain yield

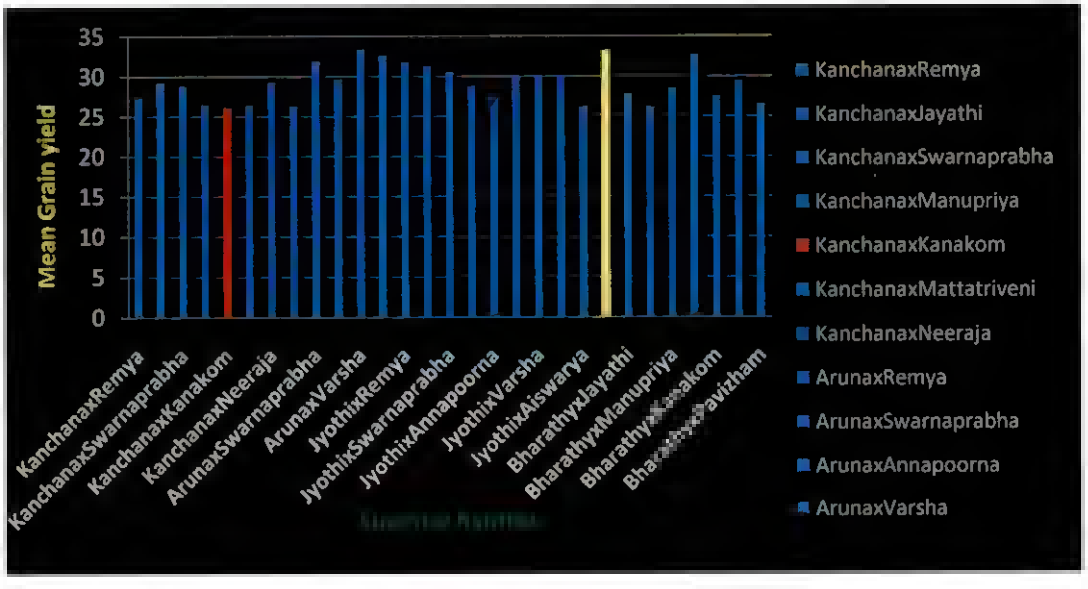


Fig 06: Mean performance of superior hybrids between maintainers and restorers

4.6.2 Analysis of variance (ANOVA)

The analysis of variance due to different sources for twelve characters studied *viz.*, plant height, days to flowering, total number of tillers, number of productive tillers, pollen fertility, days to maturity, number of spikelets/panicle, number of filled grains/panicle, number of grains/panicle, panicle length, length and breadth ratio and yield/plant are summarized in Table 26.

The ANOVA indicated that, there is no significance difference among replications except for two characters viz; days to flowering and length and breadth ratio. The differences among the genotypes were highly significant for all the characters based on their mean sum of square values and the difference among the parents was highly significant for all the traits. Highly significant difference were found between Crosses, Tester and Line x Tester for all characters and significant difference was found between Lines except for Plant height. Highly significant difference also found among Parent vs Crosses for all characters.

4.6.3 General combining ability effects (*gca*)

gca effect was analyzed for all the 12 traits related to the yield among all the four lines and eleven testers revealed that most of the genotypes showed significant GCA effect for most of the traits. The results are presented in the Table 27.

4.6.3.1 Plant height

Increased plant height is considered as having negative correlation with the high yielding trait in rice. Highest significant negative *gca* effect was recorded for Pavizham (-13.290) followed by the lines Neeraja (-8.249), jyothi (-4.372). However, high significant positive *gca* effect showed by the line Jayathi (14.932).

4.6.3.2 Days to flowering

Among the four lines evaluated, the line Kanchana (-3.515) exhibited highly significant *gca* effect in negative direction followed by Aruna (-0.484). While Bharathy (4.212) exhibited positive *gca* effect. In the testers, three testers exhibited highly significant negative GCA effects. Low *gca* effect for this trait is recorded by

Plate 15(a)

Plate 15(b)

Plate 15:a- Maintainers and Restorers in Field ,b. F_1 plant of cross between maintainers and restorers

the testers Manupriya (-4.022) and Kanakom (-1.772). Four testers showed significant positive *gca* effect with Neeraja being highest with GCA effect (6.810) followed by Aiswarya (4.227), Pavizham (0.810) and Remya (0.5606).

4.6.3.3 Total number of tillers

For this trait, only two lines Jyothi (0.678) and Kanchana (0.137) expressed highly significant positive *gca* effect and remaining two lines *viz;* Bharathy (-0.557) and Aruna (-0.258) showed significant negative *gca*. Among testers, Varsha (1.179), Swarnaprabha (1.047), Jayathi (0.926) and Neeraja (0.714) showed highly significant positive *gca* effect. Testers Mattatriveni (-1.431), Aiswarya (-1.366), Manupriya (-0.941), Annapoorna (-0.416) showed highly significant negative *gca* effect.

4.6.3.4 Number of productive tillers per plant

Highly significant positive *gca* effect for this character was expressed only by line Jyothi (1.664). While Bharathy (-1.145) and Kanchana (-0.389) showed negative *gca* effect. Among ten testers, only Neeraja (1.775), Swarnaprabha (1.6708), Jayathi (0.682), Pavizham (0.338) and Varsha (0.244) showed significant positive *gca* effect for this trait and remaining testers Aiswarya (-2.296), Manupriya (-1.296) and Mattatriveni (-0.980) showed significant negative *gca* effect.

4.6.3.5 Pollen fertility

Significant positive *gca* effect were observed for the line Kanchana (0.689) and Bharathy (0.416) where as significant negative effect for Aruna (-0.583) and Jyothi (-0.522). In the testers, five testers exhibited highly significant positive *gca* effect with Pavizham (2.113) being the highest followed by Annapoorna (1.863), Jayathi (0.947),

Table 26: Analysis of variance for combining ability studies in Rice

Source of Variation	Df	PH (cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP
Replication	2	42.41	4.87**	0.06	0.30	1.20	1.56	0.42	0.08	0.24	0.04	0.008**	0.03
Treatments	58	356.42**	113.99**	8.02**	12.37**	37.34**	116.10**	1394.25**	994.79**	1768.52**	13.04**	0.38**	26.83**
Parents	14	155.40*	203.64**	11.32**	11.78**	75**	300.04**	568.67**	345.13**	568.67**	11.62**	0.52**	8.42**
Crosses	43	314.90**	74.09**	7.05**	12.62**	24.52**	45.57**	1692.83**	1116.38**	2072.84**	12.78**	0.33**	24.79**
Parent v crosses	1	4956.21**	574.25**	3.28**	9.98**	61.19**	573.52 **	113.25**	4861.59**	5480.47**	44.07**	0.24**	372.45**
Lines	3	336.63	334.16**	9.39**	46.74**	13.88**	205.91**	1397.07**	757.91**	12243.17**	23.71**	0.54**	61.42**
Tester	10	708.41*	117.95*	11.27**	17.46**	34.53**	70.13**	2465.33**	1504.20**	1470.27**	15.48**	1.06**	31.42**
Line xTester	30	181.56*	33.47**	5.41**	7.60**	22.25**	21.35**	1464.91**	1022.95**	1256.67**	10.78**	0.07**	18.92**
Error	116	46.10	0.40	0.06	0.17	1.07	0.28	0.30	0.26	0.25	0.06	0.001	0.050
GCA	14	2.01**	0.61	0.21	0.07	0.03	5.18**	3.44**	1.41	12.32**	0.39	0.004	0.08
SCA	43	45.15**	11.02**	1.79	2.47*	7.05**	7**	488.20**	340.89**	418.80**	3.58**	0.02	6.29**
GCA/SCA Variance		0.04	0.05	0.12	0.03	0.004	0.74	0.007	0.004	0.029	0.109	0.16	0.01

PH- Plant height, TTPP- Total number of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant. * Significant at 0.05 level, ** Significant at 0.01 level

Table 27: General combining ability (*gca*) effect of parental lines for 12 characters

PARENTS	PH (cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP
LINES												
Kanchana	2.240	-3.515**	0.137**	-0.38**	0.689**	-2.891**	-5.908**	-6.298**	-19.412**	-1.13**	-0.0870**	-1.276**
Aruna	2.513*	-0.484**	-0.25**	-0.128	-0.583**	0.835**	-5.307**	-0.573**	-13.687**	0.407**	0.1745 **	-0.086*
Jyothi	-4.372**	-0.212	0.678**	1.664**	-0.522**	-0.917**	6.328**	5.061**	17.270 **	0.83**	-0.104 **	1.910 **
Bharathy	-0.382	4.212**	-0.55**	-1.14**	0.416**	2.973**	4.886**	1.811**	15.828**	-0.10**	0.0172**	-0.547**
SEm	1.185	0.105	0.16	0.072	0.180	1.131	0.095	0.089	0.088	0.032	0.006	0.039
TESTERS												
Remya	0.868	0.5606**	-0.051	0.110	0.863**	3.017**	2.966 **	3.656**	-0.117	0.147*	0.104**	-0.148*
Jayathi	14.932**	-1.606**	0.926**	0.682**	0.947**	2.019**	11.309**	8.373**	1.301**	-0.117*	-0.142**	0.817**
Swarnaprabha	8.225 **	-0.939**	1.047**	1.670**	0.280	- 0.730**	13.742**	11.926**	-0.978**	1.301**	0.092**	1.685**
Manupriya	-0.739	-4.022**	-0.94**	-1.296**	0.113	- 3.742**	-2.388**	0.229	-1.773**	-0.97**	0.031**	-0.227**
Annapoorna	4.400**	-2.856**	-0.41**	-0.073	1.863**	- 2.977**	3.600**	3.098**	-0.102	-1.77**	0.255**	1.142**
Kanakom	2.658	-1.772**	-0.33**	-0.174	-1.63**	- 0.780**	-11.94**	-11.09**	0.147	-0.102	-0.247**	-0.440 **
Varsha	-2.103	-1.439**	1.179**	0.244*	0.113	1.231**	4.759**	4.639**	0.159	0.147**	-0.137**	0.372 **
Mattatriveni	-3.789	0.227	-1.43**	-0.980**	-3.219**	- 3.250**	-30.812**	-18.836**	1.897**	0.159**	0.059**	-2.562**
Neeraja	-8.249**	6.810**	0.714**	1.775**	0.863**	1.979**	19.367**	14.045**	-1.603**	1.897**	0.553**	2.668**
Aiswarya	-2.913	4.227**	-1.36**	-2.296**	-2.303**	1.419**	-14.364**	-17.834**	0.922**	-1.60**	0.052**	-2.607**
Pavizham	-13.290**	0.810**	0.674**	0.338**	2.113**	1.814**	3.760**	1.800**	1.477**	0.922**	-0.622**	-0.698**
SEm	3.92	0.36	0.536	0.240	0.598	4.17	0.316	0.298	0.293	0.102	0.020	0.129

PH- Plant height, TTPP- Total number of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant. * Significant at 0.05 level, ** Significant at 0.01 level.

Remya (0.863) and Neeraja (0.863). Three testers Mttatriveni (-3.219), Aiswarya (-2.303) and Kanakom (-1.63) showed significant negative gca effect. Remaining 3 testers showed non significant *gca* effect for pollen fertility.

4.6.3.6 Days to Maturity

Highly significant negative *gca* effect was expressed by lines Kanchana (-2.891) and Jyothi (-0.917) Remaining three lines showed significant positive effect. Among eleven, five testers viz; Manupriya (-3.742), Mattatriveni (-3.250), Annapoorrna (-2.977), Kanakom (0.780) and Swarnaprabha (-0.730) showed significant negative *gca* effect reamaing all testers showed significant positive effect.

4.6.3.7 Number of spikelets per Panicle

The line Jyothi (6.328) exhibited highest significant positive *gca* effect followed by Bharathy (4.886), whereas significant negative GCA effect was observed in Kanchana (-5.908) and Aruna (-5.307). Among the testers the highest significant positive *gca* effect was observed for Neeraja (19.367) followed by Swarnaprabha (13.742), Jayathi (11.309), Varsha (4.759), Pavizham (3.760), Annapoorna (3.600) and Remya (2.966), where as significant negative *gca* effect were observed for Mattatriveni (-30.812) followed by Aiswarya (-14.364), Kanakom (-11.941) and Manupriya (-2.388).

4.6.3.8 Number of filled grains per panicle

Two lines Jyothi (5.061) and Bharathy (1.811) showed significant positive *gca* effect, whereas lines Kanchana (-6.298) and Aruna (-0.573) exhibited significant negative *gca* effect. In the testers, highest significant positive *gca* was reported for Neeraja (14.045) followed by Swarnaprabha (11.926), Jayathi (8.373), Varsha (4.639), Remya (3.656), Annapoorna (3.098) and Pavizham (1.800).While highest significant negative *gca* effect exhibited by Mattatriveni (-18.836) followed by Aiswarya (-17.834) and Varsha (-11.099).Tester Manupriya did not show any significant effect.

4.6.3.9 Number of grain per Panicle

The line that produced highest positive *gca* effect for number of grain per panicle was Jyothi (17.270) followed by Bharathy (15.828). Line Kanchana (-19.412) produced the highest negative *gca* followed by Aruna (-13.687). Tester Mattatriveni (1.897) exhibit the highest positive *gca* effect followed by Pavizham (1.477), Jayathi (1.301) and Aiswarya (0.922). While tester Manupriya (-1.773) produced the highest significant negative *gca* effect followed by Neeraja (-1.603) and Swarnaprabha (-0.978). Tester Remya, Annapoorna, Kanakom and Varsha showed non significant effect for this trait.

4.6.3.10 Panicle length

Lines Jyothi (0.832) and Aruna (0.407) exhibited significant positive *gca* effect whereas Kanchana (-1.135) and Bharathy (-0.105) produced significant negative effect for this trait. Among the eleven testers, Neeraja (1.897), Swarnaprabha (1.301), Pavizham (0.922), Mattatriveni (0.159), Remya (0.147) and Varsha (0.147) manifested highly significant positive *gca* effect. While testers, Annapoorna (-1.773), Aiswarya (-1.603), Manupriya (-0.978), Varsha (0.147) and Jayathi (-0.117) manifested highly significant negative *gca* effect. One tester Kanakom showed non significant effect for this trait.

4.6.3.11 Grain length and breadth ratio

General combining ability effects for this trait was highly significant in all the lines and testers. Lines Aruna (0.1745) and Bharathy (0.0172) produced positive significant effect whereas Kanchana (-0.0870) and Jyothi (-0.1046) produced negative significant effect. Among eleven testers, Neeraja (0.553), Annapoorna (0.255), Remya (0.104), Swarnaprabha (0.092), Mattatrivei (0.059), Aiswarya (0.052) and Manupriya (0.031) manifested positive significant effect. While Pavizham (-0.622),

Kanakom (-0.247), Jayathi (-0.142) and Varsha (-0.137) manifested significant negative effect.

4.6.3.12 Grain yield per plant

Among four lines only one line, Jyothi (1.910) showed highly significant positive *gca* effect, whereas significant negative *gca* effect was observed in Kanchana (-1.276) followed by Bharathy (-0.547) and Aruna (-0.086). Among the testers, Neeraja (2.668) manifested the highest positive significant gca effect followed by swarnaprabha (1.685), Annapoorna (1.142), Jayathi (0.817) and Varsha (0.372). While testers Aiswarya (-2.607), Mattatriveni (-2.562), Kanakom (-0.440), Manupriya and Remya (-0.148) showed significant negative *gca* effect.

4.6.4 Overall GCA status of parents

Yield is considered as a complex character dependent on interaction effect of a number of component traits. So, in order to achieve improvement in yield levels it is first necessary to bring improvement in the yield influencing traits. Therefore, it becomes very important to develop a system of working out pooled scores of GCA by utilizing the actual GCA values and also ensuring quantification of differences in GCA effects among parental genotypes.

In the Simple pooled GCA method, parents with significant *gca* effect in desired direction is given score of +1 and –1 score to parents with significant GCA in undesirable direction (Arunachalam and Bandyopadhyay, 1979).These values are added over different yield attributing traits to arrive at pooled score of *gca* effects.

The estimates of overall GCA status of parents (Table 28) revealed that, the testers Remya, Jayathi, Swarnaprabha, Annapoorna, Varsha, Neeraja and Pavizham had high overall GCA status. This result indicates that in general Remya, Jayathi, Swarnaprabha, Annapoorna, Varsha, Neeraja and Pavizham were good general

combiners. Similarly among lines Jyothi was identified as good combiner. The results implies that four lines and two testers studied were high combiners across all the traits, indicating their ability in transmitting additive genes in the desirable direction to their progenies. Further, these lines and testers may be further evaluated for the confirmation of their superiority as good parents for hybridization.

4.6.5 Specific Combining Ability Effect

Specific combining ability effect of a cross is the estimation and the understanding of the effect of non additive gene action for a trait. Non-additive gene action of a trait is an indicator for the selection of a hybrid combination. Therefore, a highly significant *sca* effect is desirable for a successful hybrid breeding program. The results of *sca* effect of 44 hybrids of the present study are given in the Table 29.

4.6.5.1 Plant height

The crosses Jyothi x Kanakom (-16.103), Kanchana x Pavizham (-12.947), BharathyxNeeraja (-12.168) exhibited highly significant negative sca effects.While the hybrid combination Jyothi x Pavizham (12.415) and Jyothi x Varsha (10.571) exhibited highest significant positive sca effect.

4.6.5.2 Days to Flowering

Among the 44 hybrids, the hybrid Kanchana1xMattatriveni (-4.651) had registered highly significant negative *sca* effect followed by Jyothi x Remya (-3.954), Jyothi x Pavizham (-3.871), Aruna x aiswarya (-3.681), Jyothi x Annapoorna (-3.204) and Aruna x Mattatriveni (-3.015). While, the hybrid Jyothi x Swarnaprabha (5.878) registered the higher significant positive *sca* effects followed by Kanchana x Annapoorna (5.431), Aruna x Pavizham (5.401) and Jyothi x Mattatriveni (4.378).

4.6.5.3 Total number of tillers per plant

The crosses Aruna x Varsha (2.860), Kanchana x Mattatriveni (2.742), Bharathy x Jayathi (1.812) and Jyothi x Pavizham (1.606) showed high significant positive *sca*

effects. Highest negative *sca* effect for this trait was observed in crosses Kanchana x Pavizham (-2.473), Arunam x Mattatriveni (-2.420) and Bharathy x Varsha (-2.172).

4.6.5.4 Number of productive tillers per plant

Productive tillers showed highest specific combining ability effect in the cross combination Aruna x Varsha (3.546) followed by the crosses Kanchana x Annapoorna (2.791) and Jyothi x Mattatriveni (2.311). However, highest significant negative *sca* effect was observed for the cross combination viz; Kanchana x Pavizham (-2.400), Kanchana x Varsha (-1.972) and Bharathy x Kanakom (-1.811).

4.6.5.5 Pollen Fertility

The cross combination Bharathy x Mattatriveni (4.583) and Aruna x Varsha (4.583) exhibited high significant positive *sca* effect for this trait followed by Bharathy x Jayathi (3.416), Kanchana x Annapoorna (2.893), Jyothi x Kanakom (2.272), Bharathy x Pavizham (2.416) and Jyothi x Swarnaprabha (2.022). Whereas, the crosses Aruna x Mattatriveni (-7.083), Kanchana x Varsha (-4.356), Kanchana x Aiswarya (-3.272), Jyothi x Jayathi (-3.643) and Bharathy x Kanakom (-3.000) expressed highly significant negative *sca* effect.

4.6.5.6 Days to Maturity

The *sca* effect for days to maturity was found to have negatively significant value for 16 cross combinations. Hybrid Bharathy x Kanakom (-3.555) had highest significant negative *sca* effect followed by Bharathy x Jayathi (-3.261), Jyothi x Varsha (-2.852), Jothi x Manupriya (-2.741), Aruna x Aiswarya (-2.675) and Bharathy x Manupriya (-2.636). Significant positive *sca* effect for this trait was observed in 19 hybrids.

4.6.5.7 Number of spikelets per panicle

Twenty five out of 44 hybrids showed positive significant *sca* effects for this trait. High *sca* effect was recorded for the cross combination Kanchana x Mattatriveni (37.530), Aruna x Varsha (25.691), Bharathy x Mattatriveni (24.969), Jyothi x Kanakom (23.313), Bharathy x Aiswarya (20.400), Aruna x Annapoorna (18.740) and

Bharathy x Annapoorna (15.003). However, high amount of significant negative value for *sca* effect was reported by the crosses Aruna x Kanakom (-50.661) followed by Kanchana x Aiswarya (-38.137), Aruna x Mattatriveni (-22.957), Jyothi x Mattatriveni (-39542), Bharathy x Varsha (-24.182) and Kanchana x Annapoorna (-17.768).

4.6.5.8 Number of filled grains per panicle

The cross combination Kanchana x Mattatriveni (27.834) exhibited high significant positive *sca* effect for no of filled garin followed by Aruna x Varsha (18.287), Jyothi x Kanakom (16.980), Bharathy x Kanakom (16.520) and Bharathy x Annapoorna (15.642). Highest significant negative *sca* effect was observed in cross combination Aruna x Kanakom (-47.461) followed by Jyothi x Mattatriveni (-43.245). Kanchana x Aiswarya (-30.221) and Bharathy x Varsha (-17.621).

4.6.5.9 Number of grains per panicle

The cross combination Kanchana x Mattatriveni (28.715) showed highest positive significant *sca* effect Jyothi x Kanakom (21.989) followed by Bharathy x Aiswarya (20.345), Aruna x Varsha (19.236), Bharathy x Annapoorna (18.381) and Jyothi x Pavizham (16.560). Highest significant negatrive *sca* effect was observed in cross combination Jyothi x Mattatriveni (-50.637) followed by Aruna x Kanakom (-47.942), Kanchana x Aiswarya (-33.746), Bharathy x Varsha (-23.113 and Bharathy x Remya (-21.594).

4.6.5.10 Panicle length

Almost all the cross combination showed significant *sca* effect for this trait. The crosses Aruna x Varsha (4.162), Aruna x Swarnaprabha (2.798), Bharathy x Mattatriveni (2.538), Jyothi x Pavizham (2.442), Jyothi x Remya (2.344) and Bharathy x Manupriya (2.169) showed high positive significant sca effect. Highest significant negative effect was observed for cross Bharathy x Varsha (-2.883) followed by Kanchana x Pavizham (-2.695), Jyothi x Mattatriveni (-2.565), Aruna x Manupriya (-2.287) and Bharathy x Swarnaprabha (-2.037).

Table 28: Pooled GCA score for yield and yield attributing traits in rice.

Parents	PH (cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP	Total	GCA status
LINES														
Kanchana	0	1	1	-1	1	1	-1	-1	-1	-1	-1	-1	-3	L
Aruna	-1	1	-1	0	-1	-1	-1	-1	-1	1	1	-1	-5	L
Jyothi	1	0	1	1	-1	1	1	1	1	1	-1	1	7	H
Bharathy	0	-1	-1	-1	1	-1	1	1	1	-1	1	-1	-2	L
TESTERS														
Remya	0	-1	0	0	1	-1	1	1	0	1	1	0	3	H
Jayathi	-1	1	1	1	1	-1	1	1	1	-1	-1	1	4	H
Swarnaprabha	-1	1	1	1	0	1	1	1	-1	1	1	1	7	H
Manupriya	0	1	-1	-1	0	1	-1	0	-1	-1	1	-1	-3	L
Annapoorna	-1	1	-1	0	1	1	1	1	0	-1	1	1	4	H
Kanakom	0	1	-1	0	-1	1	-1	-1	0	0	-1	-1	-4	L
Varsha	0	1	1	1	0	-1	1	1	0	1	-1	1	5	H
Mattatriveni	0	0	-1	-1	-1	1	-1	-1	1	1	1	-1	-2	L
Neeraja	1	-1	1	1	1	-1	1	1	-1	1	1	1	6	H
Aiswarya	0	-1	-1	-1	-1	-1	-1	-1	1	-1	1	-1	-7	L
Pavizham	1	-1	1	1	1	-1	1	1	1	1	-1	-1	4	H

PH- Plant height, TTPP- Total number of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant. H- High, L- Low

4.6.5.11 Length and breadth ratio

The hybrid JyothixRemya (0.356) exhibited high significant positive *sca* effect for this trait followed by Kanchana x Neeraja (0.263), Jyothi x Manupriya (0.203) and Jyothi x Aiswarya (0.202). Whereas significant negative *sca* effect was observed in Bharathy x Neeraja (-0.207), Jyothi x Jayathi (-0.196) and Aruna x Mattatriveni (-0.167).

4.6.5.12 Grain yield per plant

Highly Significant *sca* effects for this important trait were recorded by nineten hybrids out of 44 hybrids. The hybrid Aruna x Varsha (5.420) exhibited highly significant positive *sca* effect followed by Bharathy x Annapoorna (4.411), Jyothi x Pavizham (4.395), Kanchana x Mattatriveni (2.591) and Aruna x Swarnaprabha (2.570). However, significant negative *sca* effect was found for crosses Bharathy x Varsha (-3.295), Jyothi x Annapoorna (-3.132), Aruna x Jayathi (-2.861) and Aruna x Pavizham (-2.388).

4.6.6 Selection of best hybrids having high SCA effect for each character

Out of forty four crosses the top three crosses exhibiting high *sca* effects had been selected for each character (Table 30) and *gca* status of parents of such hybrids are presented as either low or high in Table 30.

4.6.7 Overall SCA status of parents

In the Simple pooled SCA method, hybrids with significant *sca* effect in desired direction is given score of +1 and –1 score to hybrids with significant *SCA* in undesirable direction (Arunachalam and Bandyopadhyay, 1979).These values are added over different yield attributing traits to arrive at pooled score of *sca* effects. Out of 44 hybrids 26 had high (H) overall SCA status across the twelve traits and the remaining 18 had low (L) overall SCA (table 31).

Table 29: Specific combining ability (*sca*) effects in crosses for yield and yield contributing characters

Crosses	PH (cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP
KanchanaxRemya	4.966	-0.318	0.096	0.581*	-0.439	1.219**	7.975**	7.974**	9.446**	0.367**	0.069**	1.308**
KanchanaIxJayathi	-0.897	1.181**	-0.568**	0.522*	-0.522	-2.146**	6.632**	8.301**	7.048**	1.597**	-0.017	2.072**
KanchanaIxSwarnaprabha	-3.357	-1.484**	0.384**	-0.509*	-1.856**	-2.064**	2.865**	4.271**	4.377 **	0.103	0.137**	0.793**
KanchanaIxManupriya	3.337	1.931**	0.475**	1.458**	1.977**	1.092**	9.483 **	7.768**	8.697**	1.315**	0.019	0.297*
KanchanaIxAnnapoorna	3.158	5.431**	0.183	2.791**	2.893**	0.183	-17.768**	-19.767**	-16.921**	0.360**	-0.028	-2.219**
KanchanaIxKanakom	6.190	2.348**	0.067	-0.201	0.393	0.986**	13.550**	13.960**	13.478**	0.329**	-0.115**	0.169
KanchanaIxVarsha	-5.411	0.348	0.298**	-1.972**	-4.356**	-2.138**	-10.640**	-7.274**	-6.325**	-1.600**	-0.108**	-2.192**
KanchanaIxMattatriveni	-7.585	**-4.651****	2.742**	0.699**	1.977**	-0.034	**37.530****	**27.834****	**28.715****	1.391**	-0.012	2.591**
KanchanaIxNeeraja	8.035*	-2.234**	-0.280**	-0.427**	1.893**	3.504**	2.350**	3.008**	0.761**	0.350**	0.263**	0.254*
KanchanaIxAiswarya	4.511	-2.318**	-0.923**	-0.541**	-3.272**	0.231	-38.137**	-30.221**	-33.746**	-0.817**	-0.042*	-0.962**
KanchanaIxPavizham	-12.947**	-0.234	-2.473**	-2.400**	1.310*	-0.832**	-13.841**	-15.856**	-15.532**	-2.695**	-0.167**	-2.112**
ArunaxRemya	-0.989	2.984**	-0.086	-0.319	1.500*	-1.536**	2.557**	-1.950**	-0.478	-1.328**	0.151**	-1.118**
ArunaxJayathi	0.006	1.484**	-1.217**	-1.628**	0.750	-1.365**	-9.925**	-12.45**	-13.709**	-1.835**	0.014	-2.861**
ArunaxSwarnaprabha	0.279	-1.848**	0.882**	1.383**	0.083	1.877**	13.484**	8.023**	8.129 **	2.798**	-0.010	2.570**
ArunaxManupriya	-7.382	-0.765*	-0.620**	-1.082**	-1.750**	-0.334	-8.874**	-5.640**	-4.710**	-2.287**	-0.088***	-1.566**
ArunaxAnnapoorna	0.508	-0.598	-0.145	-1.249**	-2.166**	-1.876**	18.740**	8.351**	11.197**	-0.542**	-0.039	0.940**
ArunaxKanakom	5.250	-0.015	-1.602**	-1.368**	0.333	1.073**	**-50.661****	**-47.461****	-47.942**	-0.460**	0.123 **	-1.753**
ArunaxVarsha	1.228	2.984**	**2.860****	**3.546****	**4.583****	5.583**	25.691**	18.287**	19.236**	**4.162****	0.146**	**5.420****
ArunaxMattatriveni	3.697	-3.015**	-2.420**	-1.561**	**-7.083****	0.730**	-22.957**	7.166**	8.047**	-1.364**	-0.167**	-2.135**
ArunaxNeeraja	-4.084	-2.931**	0.478**	1.411**	0.166	1.002**	8.749**	6.437**	4.189**	1.341**	-0.164**	2.301**
ArunaxAiswarya	3.692	-3.681**	0.339**	0.750**	1.666**	-2.675**	11.448**	10.693**	7.168**	0.244*	-0.093**	0.590**
ArunaxPavizham	-2.207	5.401**	1.532**	0.118	1.916**	-0.334	11.747**	8.548**	8.872**	-0.731**	0.128**	-2.388**
JyothixRemya	0.569	-3.954**	0.711**	0.930**	0.439	0.967**	11.844**	9.534**	12.626**	2.344**	**0.356** **	2.361**
JyothixJayathi	-2.317	0.212	-0.026	0.315	-3.643**	3.448**	3.238**	3.321**	4.922**	0.774**	-0.196**	0.945**
JyothixSwarnaprabha	4.455	5.878**	-0.657**	-0.229	2.022**	-1.843**	0.225	-0.455	2.147**	-0.864**	-0.101**	-0.789**
JyothixManupriya	-4.546	-1.704**	-0.243*	-1.309**	0.189	-2.791**	-6.897**	-9.062**	-8.585**	-1.198**	0.203**	-0.452**
JyothixAnnapoorna	-8.718*	-3.204**	-0.291**	-1.422**	-0.560	-1.322**	-16.005**	-4.227**	-12.657**	0.211*	0.089**	-3.132**
JyothixKanakom	**-16.103****	-1.954**	0.262**	-0.241	2.272**	0.111	23.313**	16.980 **	21.989**	0.036	-0.007	0.716**

JyothixVarsha	10.571**	-0.621	-0.986**	-0.136	0.522	-2.852**	9.132**	6.608 **	10.201**	0.321**	-0.024	0.067
JyothixMattatriveni	-5.039	4.378**	-0.023	2.311**	0.522	-1.870**	-39.542**	-43.245**	**-50.637****	-2.565**	0.095**	-1.228**
JyothixNeeraja	8.218*	1.462**	-0.678**	-1.778**	-1.227**	1.743**	-5.876**	-4.521**	-2.801**	-1.430**	0.108**	-2.181**
JyothixAiswarya	0.494	3.378**	0.323**	0.024	0.272	4.084**	6.288 **	10.342**	6.233**	-0.070	0.202**	-0.702**
JyothixPavizham	12.415**	-3.871**	1.606**	1.535**	-0.810	-0.008	14.278**	14.723**	16.560**	2.442**	-0.012	4.395**
BharathyxRemya	-4.546	1.287**	-0.721**	-1.193**	-1.500**	2.544**	-22.376**	-15.559**	-21.594**	-1.383**	0.135**	-2.551**
BharathyxJayathi	3.208	-2.878**	1.812**	0.791**	3.416**	**-3.261****	0.054	0.834**	1.737**	-0.536**	0.198**	-0.156
BharathyxSwarnaprabha	-1.377	-2.545**	-0.609**	-0.643**	-0.250	1.085**	-16.576**	-11.839**	-14.653**	-2.037**	-0.026	-2.575**
BharathyxManupriya	8.590*	0.537	0.388**	0.933**	-0.416	4.483**	6.288 **	6.934**	4.599**	2.169**	-0.134**	1.721**
BharathyxAnnapoorna	5.051	-1.628**	0.253**	-0.119	-0.166	1.409**	15.033**	15.642**	18.381**	0.030	-0.022	4.411**
BharathyxKanakom	4.663	-0.378	1.273**	1.811**	-3.000**	**-3.555****	13.798**	16.520**	12.474**	0.095	-0.066**	0.867**
BharathyxVarsha	-6.388	-2.712**	-2.172**	-1.437**	-0.750	2.156**	-24.182**	-17.621**	-23.113**	**-2.883****	-0.013	-3.295**
BharathyxMattatriveni	8.927*	3.287**	-0.298**	-1.448**	**4.583****	-2.636**	24.969**	8.244**	13.874**	2.538**	0.083**	0.772**
BharathyxNeeraja	-12.168**	3.704**	0.480**	0.794**	-0.833	0.701*	-5.224**	-4.924**	-2.149**	0.440**	**-0.207****	-0.374*
BharathyxAiswarya	-8.698*	2.621**	0.261**	-0.233	1.333*	2.918**	20.400**	9.185**	20.345**	0.643**	-0.066**	1.074**
BharathyxPavizham	2.738	1.295**	-0.666**	0.745**	2.416**	-0.008	-12.183**	-7.416**	-9.900**	0.985**	0.052**	0.105
SEm	**3.920**	**0.367**	**0.098**	**0.240**	**0.598**	**0.284**	**0.316**	**0.298**	**0.293**	**0.102**	**0.020**	**0.129**

PH- Plant height, TTPP- Total number of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant. * Significant at 0.05 level, ** Significant at 0.01 level

Table 30: *sca* effects and *gca* status for the 12 characters in the best crosses.

Characters	Desirable crosses	*Per se* performance	*sca* effects	*gca* status of parents	
				Female	Male
1. Plant height (cm)	JyothixKanakom	76.43	-16.103**	H	L
	KanchanaxPavizham	70.25	-12.947**	L	H
	BharathyxNeeraja	73.44	-12.168**	L	H
2.Days to flowering	KanchanaxMattatriveni	77.66	-4.651**	L	L
	JyothixRemya	82	-3.954**	H	H
	JyothixPavizham	82.33	-3.871**	H	H
3.Total number of tillers/plant	ArunaxVarsha	16.33	2.860**	L	H
	KanchanaxMattatriveni	13.99	2.742**	L	L
	BharathyxJayathi	14.73	1.812**	L	H
4. Number of productive tillers/plant	ArunaxVarsha	13	3.546**	L	H
	KanchanaxAnnapoorna	11.66	2.791**	L	H
	JyothixMattatriveni	12.33	2.311**	H	H
5. Pollen fertility (%)	ArunaxVarsha	84.33	4.583**	L	H
	BharathyxMattatriveni	82	4.583**	L	L
	BharathyxJayathi	85	3.416**	L	H
6.Days to maturity	BharathyxKanakom	114.86	-3.555**	L	L
	BharathyxJayathi	118	-3.261**	H	H
	JyothixVarsha	112.13	-2.851**	H	H
7.Spikelet/panicle	Kanchana1xMattatriveni	158.22	37.530**	L	L
	ArunaxVarsha	182.55	25.691**	L	L
	BharathyxMattatriveni	156.45	24.969**	H	L
8. No of filled grain/panicle	KanchanaxMattatriveni	136.05	27.834**	L	L
	ArunaxVarsha	155.70	18.287**	L	L
	JyothixKanakom	144.29	16.980 **	H	L
9. Number of grain/plant	Kanchana1xMattatriveni	158.22	28.715**	L	L
	JyothixKanakom	175.11	21.989**	H	L
	BharathyxAiswarya	167.66	20.345**	L	L
10. Panicle length (cm)	ArunaxVarsha	26.66	4.162**	L	L
	BharathyxMattatriveni	24.54	2.538**	L	L
	JyothixPavizham	26.14	2.442**	H	H
11.L:B	JyothixRemya	2.51	0.356 **	H	H
	KanchanaxNeeraja	3.596	0.263**	L	H
	JyothixManupriya	2.99	0.203**	H	L
12. Grain yield/plant (g)	ArunaxVarsha	33.32	5.420**	L	L
	JyothixPavizham	33.22	4.395**	H	H
	KanchanaxMattatriveni	26.36	2.591**	L	L

* Significant at 0.05 level, ** Significant at 0.01 level

Plate 16: Rice hynrids having high SCA effect for grain yield

4.6.8 Variance due to general and specific combining ability effects

The variance due to GCA and SCA for 12 different traits is presented in Table 32. The results revealed that, an estimate of SCA variance was predominant for all the characters studied. Non additive gene action was predominant for all the characters studied as the estimate of SCA variance was predominant over GCA variance. SCA variance was maximum for number of spikelets per panicle (488.204) followed by number of grains per panicle (418.805), number of filled grains (340.896), plant height (45.152), and days to flowering (11.022). GCA variance were recorded maximum for number of grain per panicle (12.32) followed by days to maturity (5.18), spikelet per panicle (3.44), plant height (2.01) and number of Filled grain (1.41) .

4.6.9 Proportional contribution of lines, testers and line × tester interaction on the performance of hybrids

The proportional contribution of lines, testers and line × tester interaction for twelve traits is presented in Table 33. The results revealed that the per cent contribution of lines was higher for number of grain per Panicle (41.207), days to maturity (35.78), days to flowering (31.463), number of productive tillers (25.827) and panicle length (12.942) and yield per plant (11.274). Whereas the per cent contribution of tester was high for most of the traits viz; yield per plant (73.722), plant height (52.136), total number of tillers (37.149), days to flowering (37.019), spikelet per panicle (33.868), pollen fertility (32.748), productive tillers (32.158), days to maturity (31.57) and number of filled grain (31.334). However the per cent contribution of lines x testers was more for grain length and breadth ratio (69.440), number of filled grain (63.928), pollen fertility (63.301), spikelet per panicle (60.374), panicle length (58.882) and total no of tillers (53.562).

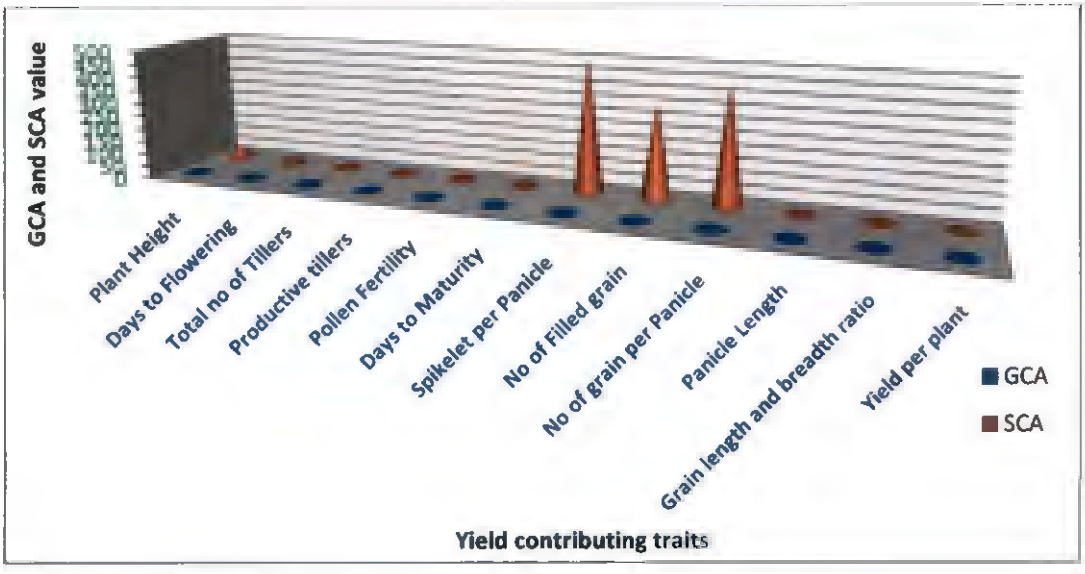


Fig 07: Comparison of GCA and SCA variances

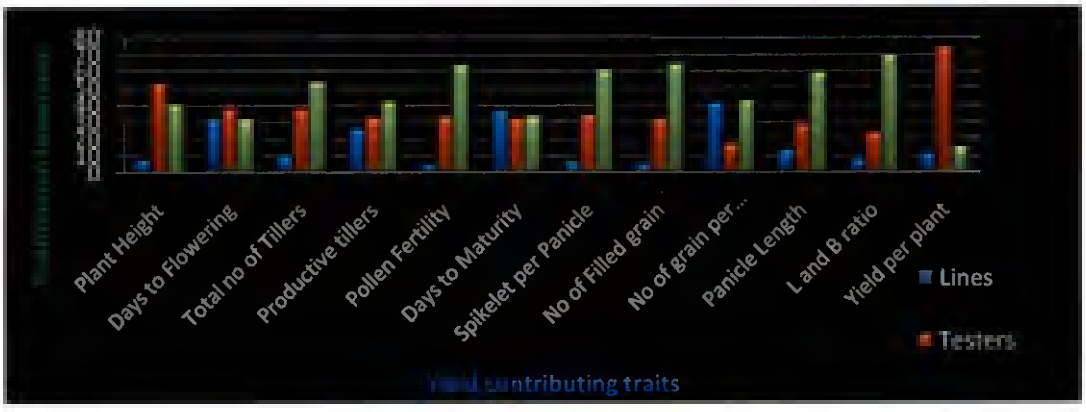


Fig 08: Percent contribution of 12 characters to total variance

Table 31: SCA score for yield and yield attributing traits with GCA status of parents in hybrids.

Crosses	PH (cm)	TTPP	DF	NPTP	PL	PF	NSPP	NFGP	DM	L:B	NGP	GYP	**Total**	SCA **status**	**GCA status of crosses**
KanchanaxRemya	0	0	0	1	0	-1	1	1	1	1	1	1	6	H	LXH
KanchanaxJayathi	0	-1	-1	1	0	1	1	1	1	1	0	1	5	H	LXH
KanchanaxSwarnaprabha	0	1	1	-1	-1	1	1	1	1	0	1	1	6	H	LXH
KanchanaxManupriya	0	-1	1	1	1	-1	1	1	1	1	0	1	6	H	LXL
KanchanaxAnnapoorna	0	-1	0	1	1	0	-1	-1	-1	1	0	-1	-2	L	LXH
KanchanaxKanakom	0	-1	0	0	0	-1	1	1	1	1	-1	0	1	H	LXL
KanchanaxVarsha	0	0	1	-1	-1	1	-1	-1	-1	-1	-1	-1	-6	L	LXH
KanchanaxMattatriveni	0	1	1	1	1	1	**1**	**1**	**1**	1	0	1	10	H	LXL
KanchanaxNeeraja	-1	1	-1	-1	-1	-1	1	1	1	1	1	1	2	H	LXH
KanchanaxAiswarya	0	1	-1	-1	1	0	-1	-1	-1	-1	-1	-1	-6	L	LXL
KanchanaxPavizham	1	0	-1	-1	1	1	-1	-1	-1	-1	-1	-1	-5	L	LXH
ArunaxRemya	0	-1	0	0	1	1	1	-1	0	-1	1	1	2	H	LXH
ArunaxJayathi	0	-1	-1	-1	0	1	-1	-1	-1	-1	0	-1	-7	L	LXH
ArunaxSwarnaprabha	0	1	1	1	0	-1	1	1	1	1	0	1	7	H	LXH
ArunaxManupriya	0	1	-1	-1	-1	0	-1	-1	-1	-1	-1	-1	-8	L	LXH
ArunaxAnnapoorna	0	0	0	-1	-1	1	1	1	1	-1	0	1	2	H	LXL
ArunaxKanakom	0	0	-1	-1	0	-1	**-1**	**-1**	-1	-1	1	-1	-7	L	LXH
ArunaxVarsha	0	-1	**1**	**1**	**1**	1	1	1	1	**1**	1	**1**	9	H	LXL
ArunaxMattatriveni	0	1	-1	-1	**-1**	-1	-1	1	1	-1	-1	-1	-5	L	LXH
ArunaxNeeraja	0	1	1	1	0	1	1	1	1	1	-1	1	8	H	LXL
ArunaxAiswarya	0	1	1	1	1	1	1	1	1	1	**-1**	-1	7	H	LXH
ArunaxPavizham	0	1	1	0	1	0	1	1	1	-1	1	-1	5	H	LXL
JyothixRemya	0	1	1	1	0	-1	1	1	1	1	**1**	1	8	H	LXH
JyothixJayathi	0	0	0	0	-1	-1	1	1	1	1	-1	1	2	H	HXH
JyothixSwarnaprabha	0	-1	-1	0	1	1	0	0	1	-1	-1	-1	-2	L	HXH
JyothixManupriya	0	1	-1	-1	0	1	-1	-1	-1	-1	1	-1	-4	L	HXH

JyothixAnnapoorna	1	1	-1	-1	0	1	-1	-1	-1	1	1	-1	-1	L	HXL
JyothixKanakom	1	1	1	0	1	0	1	1	1	0	0	1	8	H	HXH
JyothixVarsha	-1	0	-1	0	0	1	1	1	1	1	0	0	3	H	HXL
JyothixMattatriveni	0	-1	0	1	0	1	-1	-1	-1	-1	1	-1	-3	L	HXH
JyothixNeeraja	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	-1	-10	L	HXL
JyothixAiswarya	0	-1	1	0	0	-1	1	1	1	0	1	-1	2	H	HXH
JyothixPavizham	-1	1	1	1	0	0	1	1	1	1	0	1	7	H	HXL
BharathyxRemya	0	-1	-1	-1	-1	-1	-1	-1	1	-1	1	-1	-7	L	LXH
BharathyxJayathi	0	1	1	1	1	1	0	1	1	-1	1	0	7	H	LXH
BharathyxSwarnaprabha	0	1	-1	-1	0	-1	-1	-1	-1	-1	0	-1	-7	L	LXH
BharathyxManupriya	-1	0	1	1	0	-1	1	1	1	1	-1	1	4	H	LXL
BharathyxAnnapoorna	0	1	1	0	0	-1	1	1	1	0	0	1	5	H	LXH
BharathyxKanakom	0	0	1	1	-1	1	1	1	1	0	-1	1	5	H	LXL
BharathyxVarsha	0	1	-1	-1	0	-1	-1	-1	-1	-1	0	-1	-7	L	LXH
BharathyxMattatriveni	-1	-1	-1	-1	1	1	1	1	1	1	1	1	4	H	LXL
BharathyxNeeraja	1	-1	1	1	0	-1	-1	-1	-1	1	-1	-1	-3	L	LXH
BharathyxAiswarya	1	-1	1	0	1	-1	1	1	1	1	-1	1	5	H	LXL
BharathyxPavizham	0	-1	-1	1	1	0	-1	-1	-1	1	1	0	-1	L	LXH

PH- Plant height, TTPP- Tillers no of tillers/plant, DF- Days to flowering, NPTP- Number of productive tillers/plant, PL- Panicle length, PF%- Pollen fertility %, NSPP- Number of spikelet/panicle, NFGP- Number of filled grain/panicle, DM- Days to maturity, L:b- Length-breadth ratio, NGP- Number of grain/panicle, GYP- Grain yield/plant, H- High, L- Low.

Table 32: Components of varaiance for 12 traits in rice

Sl. No.	Characters	σ^2GCA	σ^2SCA	σ^2GCA/ σ^2SCA	σ^2A	σ^2D
1	Plant Height (cm)	2.01	45.15	0.04	3.44	488.20
2	Days to Flowering	0.61	11.02	0.05	0.61	11.02
3	Total no of Tillers	0.21	1.79	0.12	0.43	1.79
4	Number of productive tillers	0.07	2.47	0.03	0.07	2.47
5	Pollen Fertility%	0.03	7.05	0.004	0.03	7.05
6	Days to Maturity	5.18	7	0.74	5.18	17.37
7	Number of spikelet per Panicle	3.44	488.20	0.007	3.44	591.82
8	Number of Filled grain	1.41	340.89	0.004	1.41	340.89
9	Number of grain per Panicle	12.32	418.80	0.02	12.32	418.80
10	Panicle Length (cm)	0.39	3.58	0.10	0.78	3.58
11	Grain length and breadth ratio	0.004	0.02	0.17	0.004	0.02
12	Yield per plant (g)	0.08	6.290	0.01	0.08	6.29

Table 33: Proportional contribution (%) of lines, testers and line × tester interaction to total variance in the rice hybrids

Sl. No.	Characters	Lines	Testers	Line x Tester
1	Plant Height (cm)	7.458	52.316	40.225
2	Days to Flowering	31.463	37.019	31.516
3	Total no of Tillers	9.289	37.149	53.562
4	Number of productive tillers	25.827	32.158	42.014
5	Pollen Fertility%	3.950	32.748	63.301
6	Days to Maturity	35.78	31.51	32.71
7	Number of Spikelet per Panicle	5.757	33.868	60.374
8	Number of Filled grain	4.736	31.334	63.928
9	Number of grain per Panicle	41.207	16.495	42.296
10	Panicle Length (cm)	12.942	28.175	58.882
11	Grain length and breadth ratio	7.356	23.203	69.440
12	Yield per plant (g)	11.274	73.722	15.003

4.7 IDENTIFICATION OF HETEROTIC HYBRIDS FROM CROSS BETWEEN MAINTAINERS AND RESTORERS FOR SEED YIELD AND YIELD ATTRIBUTES

Heterosis of forty four crosses developed through 11 male parents Vs 4 female parent were estimated for twelve characters as mid parental heterosis (relative heterosis), better parent heterosis (heterobeltiosis) and heterosis over check Uma as a commercial check variety (standard heterosis). The results of heterosis estimation for different characters are described below. All the results for heterosis estimation over mid parent, better parent and over commercial check for different traits has been presented in Table 34,35,36,37,38 and 39.

4.7.1 Plant height

The hybrids Kanchana x Pavizham exhibited highest significant negative heterosis over both mid parent (-31.78%) as well as better parent (-33.28%). Fifteen hybrids showed significant negative heterosis heterosis over check Uma. The hybrid Kanchana x Pavizham also recorded highest significant negative heterosis over check Uma.

4.7.2 Days to flowering

All most all the crosses showed significantly high negative heterosis for days to flowering. However, highest value of negative heterosis over mid parental (-12.25%) and better parental (-22.22%) value has been found for the cross Kanchana x Neeraja. Thirty three crosses showed significant negative heterosis over the commercial parent while the highest negative value showed by the cross Kanchana x Mattatriveni (-14.227%).

4.7.3 Total number of tillers/plant

The hybrids Jyothi x Pavizham and Aruna x Mattatriveni registered highest positive significant heterosis of 39.91 per cent and 35.30 per cent over mid parent and better parent respectively. The hybrid Aruna x Varsha (58.08%) recorded significant positive heterosis over Uma.

4.7.4 Number of Productive tillers/Plant

Twenty six crosses showed significant positive heterosis over mid parent. Whereas seventeen crosses showed significant positive heterosis for this trait over better parent. The cross Jyothi x Pavizham showed highest positive heterosis value over both mid parent (64.9 %) and better parent (43.87%).Total 13 crosses showed significant positive heterosis over check variety Uma, while the cross Aruna x Varsha showed maximum heterosis (43.646%) over check variety.

4.7.5 Pollen fertility

Twenty three crosses showed significantly positive heterosis for pollen fertility over mid parent, whereas 9 crosses showed significantly positive heterosis over better parent. Cross Jyothi x Varsha (14.48%) was found to have highest heterosis over midparent and cross Bharathy x Jayathi (9.27%) observed highest heterosis over check Uma. A total of 7 crosses showed significantly positive heterosis over check. Among them Kanchana x Annapoorna (6.39%) had highest heterosis over check.

4.7.6 Days to Maturity

Twenty nine crosses showed significantly negative heterosis for Days to Maturity, whereas 39 crosses showed significantly negative heterosis over better parent. A total of 36 crosses showed significantly negative heterosis over check Uma. The hybrid Aruna x Swarnaprabha had highest significant negative heterosis over mid parent (-32.6%), better parent (-35.7) and over commercial check (-31.1%).

4.7.7 Number of spikelets/panicle

Twenty-seven hybrids had significant positive heterosis over mid-parent, where as 19 hybrids had significant positive heterosis over betterparent. A to tal of 18 crosses showed significant positive heterosis over commercial check Uma. Hybrid Aruna x Varsha showed highest significant positive heterosis over midparent (28.69), better parent (26.13) and also over check (9.31).

4.7.8 Number of filled grains/panicle

Total 36 hybrids found to have positive significant heterosis over midparent. Thirty hybrids had significant positive heterosis over better parent and 21 hybrids had significant heterosis over check variety. The cross Aruna x Varsha showed highest significant positive heterosis over midparent (41.61), better parent (25.6) and also over check (12.8).

4.7.9 Number of grains/panicle

Aruna x Varsha registered highest positive significant heterosis over mid parent (28.69) and better parent (26.13) was registered by the hybrid. Hybrid Jyothi x Remya (17.93%) recorded highest significant positive heterosis over Uma.

4.7.10 Panicle Length

Total nine crosses showed positive significant heterosis over both mid parent and better parent. While eleven cosses showed positive significant heterosis over commercial check. The hybrid Aruna x Varsha alone recorded highest significant positive heterosis over mid parent (22.24), better parent (15.05) and commercial check (19.42)

4.7.11 Grain length/breadth ratio

Tthe hybrid Jyothi x Manupriya (18.32 recorded highest positive significant heterosis over mid parent recorded for. The hybrid Jyothi x Kanakom registered highest positive significant heterosis of 9.6 per cent over better parent and hybrid Jyothi x Neeraja registered highest positive significant heterosis of 33.33 per cent over commercial check Uma.

4.7.12 Grain yield/ plant

Total 34 hybrids hybrids found to have positive significant heterosis over mid parent, 31 hybrids over better parent and 29 hybrids over commercial check. Cross Bharathy x Annapoorna registered highest significant positive heterosis over both mid parent (38.31) and better parent (30.88). Hybrid Aruna x Varsha registered highest positive significant heterosis over commercial check Uma (30.46)

Plate 17: Three superior hybrid having higher standard heterosis

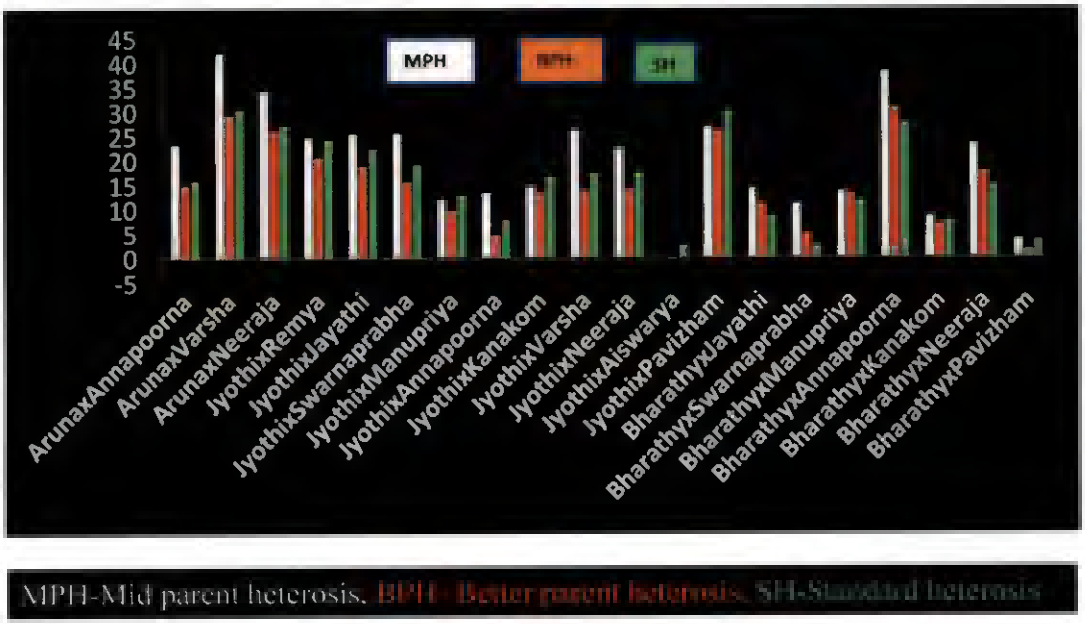


Fig 09: Percentage of heterosis for grain yield in F_1 hybrids

Table 34: Heterosis (%) of hybrids for Plant height and Days to flowering

		Per see performce	Plant height (cm)			Per see perormanc	Days to flowering		
			Heterosis over		Standard heterosis over		Heterosis over		Standard heterosis over
SL No	Crosses		MP	BP	UMA		MP	BP	UMA
1	KanchanaxRemya	102.32	-0.198	-1.989	4.911	82.33	-7.605**	-10.614**	-9.074***
2	KanchanaxJayathi	110.52	-2.661	-12.586**	13.318*	81.66	-9.062**	-12.65**	-9.810**
3	KanchanaxSwarnaprabha	101.35	4.556	9.290	3.920	79.66	-8.029**	-8.569**	-12.019**
4	KanchanaxManupriya	99.08	4.720	7.686	1.592	80	-6.602**	-7.096**	-11.651**
5	KanchanaxAnnapoorna	104.04	1.752	6.391	6.678	84.66	0.463	-1.676**	-6.497**
6	KanchanaxKanakom	105.33	0.166	-3.951	8.000	82.66	-3.376**	-3.999**	-8.706**
7	KanchanaxVarsha	88.97	-13.707**	-15.711**	-8.776	81	-2.278**	-5.934**	-10.546**
8	KanchanaxMattatriveni	85.11	-14.996**	-15.440*	-12.734*	77.66	-6.675**	-9.805**	-14.227**
9	KanchanaxNeeraja	96.27	-6.978	-9.464	-1.291	86.66	-12.256**	-22.226**	-4.288**
10	KanchanaxAiswarya	98.08	-1.933	-2.550	0.567	84	-8.915**	-14.57**	-7.233**
11	KanchanaxPavizham	70.25	-31.783**	-33.289**	-27.974**	82.66	-6.473**	-8.824**	-8.706**
12	ArunaxRemya	96.64	-3.516	-7.433	-0.915	88.66	-1.419**	-3.738**	-2.079**
13	ArunaxJayathi	111.70	0.468	-11.656*	14.525**	85	-6.221**	-9.091**	-6.129**
14	ArunaxSwarnaprabha	105.26	1.384	-5.791	7.929	82.33	-5.856**	-6.201**	-9.074**
15	ArunaxManupriya	88.64	-12.782**	-17.419**	-9.118	80.33	-7.117**	-8.480**	-11.282**
16	ArunaxAnnapoorna	101.67	-1.803	-8.529	4.241	81.66	-.046	-6.961**	-9.810**
17	ArunaxKanakom	104.67	1.822	-4.559	7.317	83.33	-3.536**	-5.062**	-7.969**
18	ArunaxVarsha	95.88	-4.818	-9.161	-1.688	86.66	3.518**	-1.265	-4.288**
19	ArunaxMattatriveni	96.67	-1.118	-2.945	-0.885	82.33	-2.048**	-6.201**	-9.074**
20	ArunaxNeeraja	84.42	-16.517**	-20.605**	-13.438*	89	-10.647**	-20.132**	-1.711
21	Arunaxaiswarya	97.54	-0.118	-1.858	0.006	85.66	-7.940**	-12.881**	-5.392**
22	ArunaxPavizham	81.26	-19.233**	-22.830**	-16.682**	91.33	2.367**	0.735	0.865**
23	JyothixRemya	91.31	-14.489**	-16.357**	-6.377	82	-9.029**	-10.976**	-9.442**
24	JyothixJayathi	102.49	-12.999**	-18.940**	5.082	84	-7.523**	-10.160**	-7.233**

25	JyothixSwarnaprabha	102.55	-7.150	-8.216	5.150	90.33	3.061**	2.457*	-0.239
26	JyothixManupriya	84.59	-21.860**	-22.516**	-13.271*	79.66	-8.095**	-9.641**	-12.019**
27	JyothixAnnapoorna	85.55	-22.335**	-23.027**	-12.28*	79.33	-7.000**	-10.019**	-12.387**
28	JyothixKanakom	76.43	-30.151**	-30.310**	-21.637**	81.66	-5.679**	-7.372**	-9.810**
29	JyothixVarsha	98.34	-8.402	-9.918*	0.830	83.33	-0.695	-5.482**	-7.969**
30	JyothixMattatriveni	81.04	-22.360**	-25.762**	-16.904**	90	6.825**	2.079**	-0.607
31	JyothixNeeraja	89.84	-16.622**	-17.704**	-7.884	93.66	6.146**	15.944**	3.441**
32	JyothixAiswarya	87.45	-16.132**	-19.890**	-10.331	93	-0.268	-5.424**	2.705**
33	JyothixPavizham	89.00	-17.007**	-18.476**	-8.749	82.33	-7.922**	-9.191**	-9.074**
34	BharathyxRemya	90.18	-13.173**	-13.615**	-7.532	91.66	-3.700**	-6.716**	1.233
35	BharathyxJayathi	112.00	-2.507	-11.413**	14.839**	85.33	-1.003	-13.161**	-5.761**
36	BharathyxSwarnaprabha	100.71	-6.345	-9.866*	3.260	86.33	-6.868**	-12.144**	-4.656**
37	BharathyxManupriya	101.71	-3.437	-5.236	4.289	86.33	-5.887**	-12.144**	-4.656**
38	BharathyxAnnapoorna	103.31	-3.662	-7.048	5.929	85.33	- 5.558**	-13.161**	-5.761**
39	BharathyxKanakom	101.18	-4.992	-7.736	3.745	87.66	-4.329**	-10.787**	-3.184**
40	BharathyxVarsha	85.37	-18.262**	-19.121**	-12.468*	85.66	-3.709**	-12.822**	-5.392**
41	BharathyxMattatriveni	99.00	-2.431	-4.194	1.507	93.33	4.517**	-5.020**	3.073**
42	BharathyxNeeraja	73.44	-29.943**	-30.931**	-24.696**	100.33	-4.308**	-9.961**	10.804**
43	BharathyxAiswarya	82.25	-18.852**	-20.403**	-15.667**	96.66	-1.662	-1.695	6.755**
44	BharathyxPavizham	83.31	-20.137**	-20.883**	-14.580**	89.33	-5.434*	-9.091**	-1.343

* Significant at 0.05 level, ** Significant at 0.01 level

Table 35: Heterosis (%) of hybrids for Total number of tillers/plant and Productive tillers/plant

		Per see performance	Total number of tillers/plant			Per see performance	Number of productive tillers/plant		
			Heterosis over		Standard heterosis over		Heterosis over		Standard heterosis over
SL No	Crosses		MP	BP	UMA		MP	BP	UMA
1	KanchanaxRemya	12.73	1.623	-3.439	23.233**	9.64	-1.094	-12.311	6.519
2	KanchanaxJayathi	13.04	8.092	14.189**	26.266**	10.15	-13.967**	-19.482**	12.191
3	KanchanaxSwarnaprabha	14.11	20.347**	7.080	36.656**	10.11	-0.247	-8.035	11.712
4	KanchanaxManupriya	12.22	0.604	-7.307	18.296**	9.11	0.330	-17.132*	0.662
5	KanchanaxAnnapoorna	12.45	-19.117**	-29.283**	20.555**	11.66	2.071	-9.091	28.913**
6	KanchanaxKanakom	12.41	-2.038	-5.815	20.200**	8.57	10.477**	-22.013**	-5.267
7	KanchanaxVarsha	14.16	15.808**	7.434*	37.108**	7.22	21.208**	-34.324**	-20.221**
8	KanchanaxMattatriveni	13.99	14.962**	6.169	35.495**	8.66	9.691**	21.164**	-4.235
9	KanchanaxNeeraja	13.12	4.196	-0.480	27.008**	10.29	18.853**	-6.337	13.775
10	KanchanaxAiswarya	10.39	-14.840*	-21.138**	0.645	6.11	33.261**	-44.421**	-32.486**
11	KanchanaxPavizham	10.88	-6.819	-17.421**	5.388	6.88	22.008**	-37.356**	-23.904**
12	ArunaxRemya	12.15	-2.448	-6.847	17.650**	9	0.935	-3.571	-0.552
13	ArunaxJayathi	12	-15.034*	-21.053**	16.166**	8.26	-24.685**	-34.470**	-8.692
14	ArunaxSwarnaprabha	14.22	21.938**	8.993*	37.657**	12.26	31.793**	31.393**	35.506**
15	ArunaxManupriya	10.73	-11.163	-17.757**	3.872	6.83	-17.212**	-26.821**	-24.530**
16	ArunaxAnnapoorna	11.73	-23.475**	-33.390**	13.552*	7.88	-28.842**	-38.545**	-12.854
17	ArunaxKanakom	10.35	-17.874**	-20.644**	0.225	7.66	-12.348**	-17.857*	-15.285
18	ArunaxVarsha	16.33	34.274**	25.166**	58.083**	13	56.000**	39.286**	43.646**
19	ArunaxMattatriveni	8.44	30.286**	35.309**	-18.296**	6.66	23.954**	28.571**	-26.335**
20	ArunaxNeeraja	13.48	7.666*	3.347	30.525**	12.39	58.255**	32.821**	36.979**
21	Arunaxaiswarya	11.26	-7.221*	-13.669**	9.035*	7.66	-7.948*	-17.893*	-15.322**
22	ArunaxPavizham	14.49	24.810**	11.114**	40.335**	9.66	20.833**	3.571	6.813
23	JyothixRemya	13.88	16.434**	15.883**	34.430**	12.04	38.032**	34.562**	33.075**
24	JyothixJayathi	14.12	3.936	-7.061	36.753**	12	11.317**	-4.837	32.596**

25	JyothixSwarnaprabha	13.61	22.342**	13.630**	31.816**	12.44	36.540**	34.136**	37.495**
26	JyothixManupriya	12.04	4.301	0.501	16.586**	8.39	4.199	-6.182	-7.219
27	JyothixAnnapoorna	12.52	-15.386**	-28.904**	21.200**	9.50	-12.716**	-25.922**	5.046
28	JyothixKanakom	13.15	8.930*	8.110*	27.331**	10.58	23.748**	18.287*	16.979*
29	JyothixVarsha	13.42	15.391**	11.989**	29.912**	11.11	36.459**	24.134**	22.762**
30	JyothixMattatriveni	11.77	1.713	-1.752	13.972*	12.33	43.829**	37.803**	36.279**
31	JyothixNeeraja	13.26	10.605*	10.528**	28.396**	11	43.948**	22.905**	21.546**
32	JyothixAiswarya	12.18	4.953	1.669	17.941**	8.73	7.336*	-2.458	-3.535
33	JyothixPavizham	15.50	39.910**	29.402**	50.112**	12.87	64.909**	43.873**	42.283**
34	BharathyxRemya	11.22	-2.941	-5.476	8.615*	7.11	14.952**	16.353*	-21.436**
35	BharathyxJayathi	14.73	11.380*	-3.092	42.594**	9.66	-7.185*	-23.341**	6.813
36	BharathyxSwarnaprabha	12.43	15.485**	10.489**	20.329**	9.22	5.392	-0.611	1.878
37	BharathyxManupriya	11.44	2.326	1.689	10.745**	7.83	1.776	-4.745	-13.480*
38	BharathyxAnnapoorna	11.83	-18.018**	-32.822**	14.520**	8	-24.003**	-37.662**	-11.602
39	BharathyxKanakom	12.93	10.434*	6.274	25.169**	9.83	20.024**	19.586**	8.618
40	BharathyxVarsha	11	-2.338	-2.453	6.485	7	9.987*	14.842*	-22.651**
41	BharathyxMattatriveni	10.26	-8.431	-8.770*	-0.645	5.76	29.801**	29.886**	-36.316**
42	BharathyxNeeraja	13.18	-13.434*	-9.889*	27.654**	10.76	47.916**	30.941**	18.931*
43	BharathyxAiswarya	10.88	-3.158	-3.230	5.388	5.66	-27.097**	-31.103**	-37.421**
44	BharathyxPavizham	12	11.975*	6.667	16.166**	9.27	24.631**	12.855*	2.504

* Significant at 0.05 level, ** Significant at 0.01 level

Table 36: Heterosis (%) of hybrids for Pollen fertility and Days to maturity.

		Per see performance	Pollen fertility (%)			Per see performance	Days to maturity		
			Heterosis over		Standard heterosis over		Heterosis over		Standard heterosis over
SL No	Crosses		MP	BP	UMA		MP	BP	UMA
1	KanchanaxRemya	81.33	-0.258	-1.744*	1.010	111.69	-2.768**	-7.729**	-2.020**
2	KanchanaxJayathi	81.33	1.310	-1.744*	1.010	107.33	-7.061**	-12.221**	-5.847**
3	KanchanaxSwarnaprabha	79.33	-4.473**	-4.785**	-1.473	104.66	-3.176**	-3.711**	-8.187**
4	KanchanaxManupriya	83	1.002	0.270	3.079**	104.81	-3.480**	-3.579**	-8.061**
5	KanchanaxAnnapoorna	85.66	4.657**	3.491**	6.391**	104.66	-0.727	-3.711**	-8.187**
6	KanchanaxKanakom	79.66	-2.081*	-3.757**	-1.059	107.66	-3.648**	-6.203**	-5.555**
7	KanchanaxVarsha	76.66	-4.827**	-7.381**	-4.785**	106.55	-3.196**	-4.388**	-6.532**
8	KanchanaxMattatriveni	79.66	-1.713	-3.757**	-1.059	104.17	-0.773*	-4.161**	-8.616**
9	KanchanaxNeeraja	83.66	2.464*	1.075	3.907**	112.94	-8.650**	-18.498**	-0.926*
10	KanchanaxAiswarya	75.33	-6.261**	-8.992**	-6.441**	109.11	-5.591**	-10.889**	-4.289**
11	KanchanaxPavizham	84.33	3.198**	1.881	4.735**	108.44	-4.780**	-8.927**	-4.874**
12	ArunaxRemya	82	1.878	1.653	1.838	112.66	-2.783**	-6.928**	-1.169**
13	ArunaxJayathi	81.33	2.659*	0.826	1.010	111.84	-32.614**	-35.796**	-31.134**
14	ArunaxSwarnaprabha	80	-2.431*	-3.985**	-0.645	112.33	2.949**	1.448**	-1.461**
15	ArunaxManupriya	78	-3.848**	-4.384**	-3.129**	107.11	-2.275**	-3.269**	-6.043**
16	ArunaxAnnapoorna	79.33	-1.815	-1.977*	-1.473	106.33	-0.108	-3.971**	-6.725**
17	ArunaxKanakom	78.33	-2.455*	-2.893**	-2.715*	109.33	-3.037**	-4.751**	-4.093**
18	ArunaxVarsha	84.33	6.080**	4.545**	4.735**	118	6.223**	5.883**	3.508**
19	ArunaxMattatriveni	69.33	-13.333**	-14.050**	-13.893**	108.66	2.512**	-1.863**	-4.678**
20	ArunaxNeeraja	80.66	0.083	0.045	0.182	114.16	-8.546**	-17.735**	0.146
21	Arunaxaiswarya	79	-0.391	-2.066*	-1.887	109.93	-5.710**	-10.220**	-3.570**
22	ArunaxPavizham	83.66	3.721**	3.719**	3.907**	112.66	-1.945**	-5.380**	-1.169**
23	JyothixRemya	81	13.836**	0.859	0.596	112.77	-0.368	-6.837**	-1.073**

24	JyothixJayathi	77	10.168**	-1.011	-4.371**	112.42	-1.217**	-8.061**	-1.385**
25	JyothixSwarnaprabha	82	12.854**	-1.584*	1.838	112.15	5.391**	4.329**	-1.619**
26	JyothixManupriya	80	11.439**	-1.933*	-0.645	103.85	-2.858**	-4.265**	-8.903**
27	JyothixAnnapoorna	81	13.340**	0.082	0.596	103.66	-0.080	-1.582**	-9.064**
28	JyothixKanakom	80.33	13.191**	0.488	-0.231	107.33	-2.477**	-6.493**	-5.847**
29	JyothixVarsha	80.33	14.489**	2.553**	-0.231	110.77	2.203**	-0.598*	-2.827**
30	JyothixMattatriveni	77	8.962**	-2.941**	-4.371**	103.33	0.027	-1.899**	-9.356**
31	JyothixNeeraja	79.33	11.319**	-1.490*	-1.473	109.54	10.177**	20.951**	-3.909**
32	JyothixAiswarya	77.66	10.989**	-0.368	-3.543**	112.59	1.134**	8.042**	-1.230**
33	JyothixPavizham	81	13.554**	0.417	0.596	115.33	2.790**	-3.141**	1.169**
34	BharathyxRemya	80	1.768**	-0.386	-0.645	116.33	-4.829**	-5.742**	2.046**
35	BharathyxJayathi	85	9.892**	9.273**	5.563**	117.88	-4.039**	-4.483**	3.409**
36	BharathyxSwarnaprabha	80.66	0.689	-3.185**	0.1821	109.33	-5.306**	-11.414**	-4.093**
37	BharathyxManupriya	80.33	1.376	-1.524*	-0.231	110.66	-4.555**	-10.333**	-2.923**
38	BharathyxAnnapoorna	82.33	4.323**	1.730*	2.252*	114.83	1.806**	-6.960**	0.728
39	BharathyxKanakom	76	-3.094**	-4.933**	-5.613**	113.95	-4.324**	-7.670**	-0.040
40	BharathyxVarsha	80	3.064**	2.128**	-0.645	111	-5.477**	-10.063**	-2.631**
41	BharathyxMattatriveni	82	4.964**	3.361**	1.838	112.23	- 0.105	-9.067**	-1.552**
42	BharathyxNeeraja	80.66	2.471*	0.166	0.182	112.66	- 13.994**	-18.697**	-1.169**
43	BharathyxAiswarya	79.66	2.886*	2.198**	-1.059	115.44	- 6.091**	-6.463**	1.266**
44	BharathyxPavizham	80.33	1.963	-0.409	-0.231	112.22	- 7.445**	-9.07**	-1.561**

* Significant at 0.05 level, ** Significant at 0.01 level

Table 37: Heterosis (%) of hybrids for Spikelet/ panicle and Number of filled grains/panicle

		Per see performance	Number of spikelet/panicle			Per see performance	Number of filled grain/panicle		
			Heterosis over		Standard heterosis over		Heterosis over		Standard heterosis over
SL No	Crosses		MP	BP	UMA		MP	BP	UMA
1	KanchanaxRemya	162.44	1.504**	-0.115	-2.728**	138.68	8.370 **	2.379**	0.497**
2	KanchanaxJayathi	169.44	1.904**	-3.236**	1.463**	143.73	15.546 **	12.029**	4.152**
3	KanchanaxSwarnaprabha	168.11	0.713**	-4.698**	0.664*	143.25	27.542 **	18.896**	3.806**
4	KanchanaxManupriya	158.59	6.944**	0.733**	-5.031**	135.05	11.340 **	10.600**	-2.135**
5	KanchanaxAnnapoorna	137.33	-12.575**	-12.773**	-17.764**	110.38	-3.208**	-8.383**	-20.009**
6	KanchanaxKanakom	153.11	-2.487**	-2.752**	-8.317**	129.91	7.767 **	7.707**	-5.857**
7	KanchanaxVarsha	145.62	-1.746**	-7.510**	-12.802**	124.42	14.982 **	3.265**	-9.840**
8	KanchanaxMattatriveni	158.22	-2.888**	-6.049**	-5.257**	136.05	11.638 **	10.385**	-1.410**
9	KanchanaxNeeraja	173.22	0.450*	-7.588**	3.724**	144.11	16.272 **	13.119**	4.427**
10	KanchanaxAiswarya	99	-35.609**	-37.120**	-40.718**	79	-35.812**	-37.135**	-42.753**
11	KanchanaxPavizham	141.42	-11.672**	-13.118**	-15.317**	113	-10.391**	-14.212**	-18.115**
12	ArunaxRemya	157.62	2.568**	-3.077**	-5.612**	134.48	3.677 **	-0.721*	-2.545**
13	ArunaxJayathi	153.48	-4.023**	-12.348**	-8.091**	128.69	2.032 **	0.312	-6.741**
14	ArunaxSwarnaprabha	179.33	11.688**	1.663**	7.383**	152.73	33.903 **	23.199**	10.673**
15	ArunaxManupriya	140.84	-0.776**	-2.688**	-15.664**	127.37	3.519 **	2.743**	-7.702**
16	ArunaxAnnapoorna	174.44	15.732**	11.302**	4.457**	144.23	24.565 **	16.343**	4.514**
17	ArunaxKanakom	89.5	-40.594**	-42.843**	-46.407**	74.22	-39.311**	-40.131**	-46.217**
18	ArunaxVarsha	182.55	28.693**	26.134**	9.313**	155.70	41.616 **	25.600**	12.830**
19	ArunaxMattatriveni	98.33	-37.195**	-41.610**	-41.117**	121.11	-2.024**	-2.307**	-12.239**
20	ArunaxNeeraja	180.22	8.510**	-3.854**	7.916**	153.26	21.944 **	20.304**	11.060**
21	Arunaxaiswarya	149.18	1.218**	-0.578*	-10.666**	125.64	0.658 **	-0.021	-8.956**

22	ArunaxPavizham	167.61	9.013**	2.971**	0.365	143.13	11.956 **	8.662**	3.717**
23	JyothixRemya	178.55	13.971**	9.789**	6.916**	151.60	15.187 **	11.917**	9.859**
24	JyothixJayathi	178.28	9.443**	1.814**	6.758**	150.11	17.241 **	17.002**	8.775**
25	JyothixSwarnaprabha	177.70	8.658**	0.743*	6.411**	149.88	29.255 **	17.307**	8.613**
26	JyothixManupriya	154.45	6.575**	2.493**	-7.512**	129.58	3.715 **	1.417**	-6.099**
27	JyothixAnnapoorna	151.33	-1.548**	-3.443**	-9.381**	137.28	16.653**	7.445**	-0.516**
28	JyothixKanakom	175.11	13.973**	11.829**	4.856**	144.29	16.184 **	12.932**	4.562**
29	JyothixVarsha	177.63	22.643**	17.873**	6.365**	149.66	33.805**	17.132**	8.451**
30	JyothixMattatriveni	93.38	-41.471**	-44.549**	-44.081**	76.33	-39.183**	-40.259**	-44.686**
31	JyothixNeeraja	177.23	4.826**	-5.449**	6.125**	147.94	15.954 **	15.783**	7.202**
32	JyothixAiswarya	155.66	3.517**	3.296**	-6.788**	130.92	3.317 **	2.465**	-5.128**
33	JyothixPavizham	181.77	15.977**	11.675**	8.84**	154.94	19.417**	17.628**	12.275**
34	BharathyxRemya	142.88	-11.855**	-12.140**	-14.439**	123.26	-5.472**	-9.006**	-10.678**
35	BharathyxJayathi	173.66	3.158**	-0.828**	3.988**	144.37	13.846 **	12.531**	4.618**
36	BharathyxSwarnaprabha	159.46	-5.636**	-9.600**	-4.512**	135.25	17.876**	7.915**	-1.990**
37	BharathyxManupriya	166.19	10.529**	2.859**	-0.481	142.33	15.040 **	13.561**	3.137**
38	BharathyxAnnapoorna	180.93	13.683**	11.978**	8.341**	153.90	32.145**	22.798**	11.526**
39	BharathyxKanakom	164.15	3.188**	1.595**	-1.704**	140.58	14.320 **	12.170**	1.874**
40	BharathyxVarsha	142.87	-4.925**	-11.576**	-14.447**	122.18	10.442 **	-2.513**	-11.461**
41	BharathyxMattatriveni	156.45	-5.175**	-7.098**	-6.315**	124.57	0.225	-0.606	-9.729**
42	BharathyxNeeraja	176.44	1.106**	5.870**	5.652**	144.28	14.182 **	13.258**	4.555**
43	BharathyxAiswarya	168.33	8.034**	4.182**	0.798**	126.51	0.810 *	0.676	-8.321**
44	BharathyxPavizham	153.87	-5.119**	-5.468**	-7.860**	129.55	0.796 *	-1.647**	-6.123**

* Significant at 0.05 level, ** Significant at 0.01 level

Table 38: Heterosis (%) of hybrids for No of Grain/panicle and Panicle length

		Per see performance	Number of Grain/panicle			Per see performance	Panicle length (cm)		
			Heterosis over		Standard heterosis over		Heterosis over		Standard heterosis over
SL No	Crosses		MP	BP	UMA		MP	BP	UMA
1	KanchanaxRemya	2.74	1.435 **	-0.182	2.742**	21.33	-5.431**	-5.689*	-4.478*
2	KanchanaxJayathi	7.03	1.704 **	-3.426**	7.031**	22.29	-10.658**	-18.685**	-0.149
3	KanchanaxSwarnaprabha	6.18	0.513	-4.887**	6.187**	22.22	-5.312	9.083**	-0.49
4	KanchanaxManupriya	0.37	6.944 **	0.733*	0.377	21.15	-3.615	-5.957**	-5.269*
5	KanchanaxAnnapoorna	-15.18	-14.697**	-4.890**	-15.189**	19.40	-18.285**	-22.376**	-13.106**
6	KanchanaxKanakom	-3.27	-2.663**	-2.928**	-3.270**	21.04	-10.517**	-14.249**	-5.762**
7	KanchanaxVarsha	-7.86	-1.776**	-7.537**	-7.862**	19.36	-15.203**	-16.453**	-13.285**
8	KanchanaxMattatriveni	0.13	-2.888**	-6.049**	0.139	22.36	-0.379	-0.563	0.164
9	KanchanaxNeeraja	9.54	0.373 **	-7.659**	9.548**	22.36	-1.779**	-2.951**	0.149
10	KanchanaxAiswarya	-37.36	-35.635**	-37.146**	-37.367**	18.39	-20.806**	-23.241**	-17.614**
11	KanchanaxPavizham	-10.70	-11.876**	-13.319**	-10.700**	19.04	-9.375**	-15.338**	-14.718**
12	ArunaxRemya	-0.34	2.460 **	-3.179**	-0.341	21.17	-1.664	-6.367**	-5.164*
13	ArunaxJayathi	-2.85	-4.023**	-12.348**	-2.856**	20.40	-14.747**	-25.577**	-8.613**
14	ArunaxSwarnaprabha	13.43	11.620 **	1.601**	13.430**	24.23	17.870 **	8.256**	8.508**
15	ArunaxManupriya	-10.86	-0.776**	-2.688**	-10.860**	18.93	-8.761**	-10.779**	-15.226**
16	ArunaxAnnapoorna	10.40	15.732 **	11.302**	10.407**	20.02	-11.801**	-19.816**	-10.344**
17	ArunaxKanakom	-43.40	-40.652**	-42.899**	-43.409**	21.79	-3.112**	-11.179**	-2.388
18	ArunaxVarsha	15.54	28.693 **	26.134**	15.540**	26.66	22.240 **	15.058**	19.420**
19	ArunaxMattatriveni	-37.83	-37.271**	-41.681**	-37.839**	17.55	-1.299*	-5.608**	-21.402**

20	ArunaxNeeraja	14.17	8.616 **	-3.759**	14.175**	24.72	17.695 **	11.081**	10.703**
21	Arunaxaiswarya	-5.57	1.218 **	-0.578	-5.578**	21	-5.448*	-12.378**	-5.956**
22	ArunaxPavizham	6.08	9.013 **	2.971**	6.082**	22.55	12.788 **	10.251**	0.985
23	JyothixRemya	17.93	18.939 **	14.575**	17.932**	25.27	7.661**	3.863**	13.181**
24	JyothixJayathi	12.83	9.443 **	1.814**	12.839**	23.44	-9.416**	-14.515**	4.970**
25	JyothixSwarnaprabha	12.47	8.658**	0.743*	12.472**	23.22	-4.784	-4.992**	3.985*
26	JyothixManupriya	-2.24	6.575 **	2.493**	-2.244**	20.60	-9.883**	-15.315**	-7.717**
27	JyothixAnnapoorna	-4.35	-1.687**	-3.579**	-4.354**	21.22	-13.967**	-15.109**	-4.970**
28	JyothixKanakom	10.82	13.973 **	11.829**	10.829**	22.71	-7.039**	-7.430**	1.731
29	JyothixVarsha	12.42	22.643 **	17.873**	12.424**	23.25	-2.126	-4.452**	4.120*
30	JyothixMattatriveni	-41.10	-41.680**	-44.747**	-41.107**	19.60	-12.815**	-16.260**	-12.210**
31	JyothixNeeraja	11.99	4.663 **	-5.596**	11.995**	23.25	-1.850**	-4.452**	4.120*
32	JyothixAiswarya	-1.68	3.295**	3.075**	-1.689**	21.11	-12.588**	-13.247**	-5.463**
33	JyothixPavizham	17.29	18.246 **	13.860**	17.299**	26.14	19.210 **	7.452**	17.092**
34	BharathyxRemya	-9.70	-11.994**	-12.279**	-9.708**	19.18	-6.847**	-8.873**	-14.076**
35	BharathyxJayathi	9.70	2.960 **	-1.018**	9.700**	21.19	-13.591**	-22.708**	-5.090**
36	BharathyxSwarnaprabha	0.75	-5.799**	-9.756**	0.751*	21.11	-8.363*	-13.625**	-5.463**
37	BharathyxManupriya	5.11	10.456 **	2.791**	5.118**	19.64	7.064 **	6.487**	-12.046**
38	BharathyxAnnapoorna	14.30	13.473**	11.772**	14.301**	20.04	-14.032**	-19.816	-10.240**
39	BharathyxKanakom	3.89	3.188 **	1.595**	3.894**	21.84	- 5.400**	-11.002	-2.194
40	BharathyxVarsha	-9.57	-4.925**	-11.576**	-9.573**	19.11	- 14.707**	-17.546**	-14.420**
41	BharathyxMattatriveni	-0.97	-5.175**	-7.098**	-0.978**	24.54	11.451 **	9.520	9.911**
42	BharathyxNeeraja	11.39	0.852 **	-6.107**	11.390**	24.18	8.259	4.947	8.299**
43	BharathyxAiswarya	6.11	7.606 **	3.769**	6.118**	20.88	-8.392**	-12.851*	-6.463**
44	BharathyxPavizham	-2.75	-5.255**	-5.603**	-2.751**	23.75	15.401 **	9.800	6.374**

* Significant at 0.05 level, ** Significant at 0.01 level

Table 39: Heterosis (%) of hybrids for Grain length/Breadth ratio and Grain yield.

		Per see performance	Grain length-breadth ratio			Per see performance	Grain yield (g)		
			Heterosis over		Standard heterosis over		Heterosis over		Standard heterosis over
SL No	Crosses		MP	BP	UMA		MP	BP	UMA
1	KanchanaxRemya	2.65	-6.028**	-10.774**	16.666**	27.49	11.654**	11.308**	7.661**
2	KanchanaxJayathi	2.62	-2.420*	-3.321**	15.238**	29.22	21.155**	18.311**	14.434**
3	KanchanaxSwarnaprabha	2.82	-0.992	-5.777**	25.079**	28.81	23.052**	16.651**	12.829**
4	KanchanaxManupriya	2.83	0.354	-2.749**	25.23**	26.40	5.831**	4.788**	3.393**
5	KanchanaxAnnapoorna	2.86	-1.940*	-7.634**	26.825**	25.26	7.611	2.253**	-1.096
6	KanchanaxKanakom	2.41	-3.526**	-10.82**	5.555**	26.06	3.467**	1.493**	2.062**
7	KanchanaxVarsha	2.46	-6.641**	-9.225**	7.619**	24.51	7.029**	-0.756	-4.006**
8	KanchanaxMattatriveni	2.69	-2.767**	-1.703*	18.730**	26.36	9.915**	6.733**	3.236**
9	KanchanaxNeeraja	2.95	-2.317**	-10.87**	30.952**	29.26	23.599**	18.446**	14.565**
10	KanchanaxAiswarya	2.79	0.7220	1.454*	23.333**	22.76	-10.409**	-12.838**	-10.858**
11	KanchanaxPavizham	1.99	-14.77**	-26.56**	-14.76**	23.52	-7.260**	-9.629**	-7.883**
12	ArunaxRemya	2.8	0.7194	-12.5**	23.809**	26.26	4.379**	1.915*	2.819**
13	ArunaxJayathi	2.8	-5.405	-12.5**	23.809**	25.48	3.360**	1.100	-0.221
14	ArunaxSwarnaprabha	2.75	-12.140**	-14.06**	21.428**	31.78	32.707**	23.351**	24.445**
15	ArunaxManupriya	2.91	-5.761**	-8.854**	29.365**	25.73	0.981	-0.129	0.756
16	ArunaxAnnapoorna	2.93	-8.189**	-8.333**	30.158**	29.61	23.349**	14.916**	15.935**
17	ArunaxKanakom	2.76	-0.479	-13.541**	22.222**	25.33	-1.523	-1.682	-0.809
18	ArunaxVarsha	2.68	-7.789**	-16.145**	18.253**	33.32	42.160**	29.314**	30.462**
19	ArunaxMattatriveni	2.71	-11.001**	-15.312**	19.523**	22.83	-6.892**	-11.397**	-10.610**
20	ArunaxNeeraja	2.83	-14.011**	-11.458**	25.396**	32.49	34.256**	26.119**	27.238**
21	Arunaxaiswarya	2.84	-6.732**	-11.25**	25.714**	25.51	-1.670**	-2.335**	-0.117
22	ArunaxPavizham	2.48	-4.853**	-22.395**	8.730**	24.44	-5.637**	-6.120**	-4.306**

23	JyothixRemya	2.42	-20.785**	-18.518**	5.714**	31.73	24.808**	20.641**	24.262**
24	JyothixJayathi	2.42	1.8207**	-8.438**	5.873**	31.28	25.523**	18.931**	22.500**
25	JyothixSwarnaprabha	2.6	1.960**	-15.217**	14.285**	30.42	25.599**	15.636**	19.107**
26	JyothixManupriya	2.88	14.645**	-0.916	27.777**	28.84	11.998**	9.643**	12.933**
27	JyothixAnnapoorna	2.96	13.448**	-5.016**	31.746**	27.53	13.423**	4.663**	7.804**
28	JyothixKanakom	2.50	13.939**	9.620**	9.841**	29.8	14.637**	13.279**	16.679**
29	JyothixVarsha	2.57	10.300**	1.314	12.857**	29.96	26.383**	13.900**	17.319**
30	JyothixMattatriveni	2.91	18.323**	6.318**	29.365**	25.73	3.805**	-2.179**	0.756
31	JyothixNeeraja	3	10.497**	-9.365**	33.333**	30.01	22.615**	14.078**	17.501**
32	JyothixAiswarya	2.83	14.942**	2.905**	25.396**	26.21	-0.258	-0.355	2.636**
33	JyothixPavizham	2.07	2.134**	-4.454**	-10.79**	33.22	26.939**	26.280**	30.070**
34	BharathyxRemya	2.82	14.208**	-11.66**	25.079**	24.36	-1.496*	-2.234*	-4.594**
35	BharathyxJayathi	2.8	-4.436**	-12.5**	23.80**	27.72	14.415**	11.248**	8.561**
36	BharathyxSwarnaprabha	2.85	-8.064**	-10.937**	26.190**	26.17	11.256**	5.029**	2.492**
37	BharathyxManupriya	2.78	-9.298**	-13.125**	22.857**	28.56	13.959**	13.333**	11.824**
38	BharathyxAnnapoorna	2.65	-16.27**	-17.187**	16.666**	32.62	38.318**	30.881**	27.721**
39	BharathyxKanakom	2.63	-4.121**	-17.604**	16.031**	27.49	8.655**	7.047**	7.648**
40	BharathyxVarsha	2.64	-8.217**	-17.395**	16.349**	24.14	4.895**	-3.130**	-5.468**
41	BharathyxMattatriveni	2.9	-3.814**	-9.37**	28.571**	25.27	4.890**	1.418**	-1.031*
42	BharathyxNeeraja	2.76	-15.262**	-13.541**	22.222**	29.36	23.448**	17.801**	14.956**
43	BharathyxAiswarya	2.87	-4.809**	-10.312**	27.142**	25.53	0.046	-2.246**	-0.026
44	BharathyxPavizham	2.31	-10.335**	-27.708**	0.634	26.47	3.905**	1.690**	3.654**

* Significant at 0.05 level, ** Significant at 0.01 level

Discussion

5. DISCUSSION

Today, rice (*Oryza sativa* L.) provides 50 to 80% of calorie intake of over 75% of Asian population and more than three billion of world population. In India, it is grown in an area of around 43.5 m.ha with a production of 105 million tonnes. However to ensure food supply for the ever increasing population, there is a need to enhance the current annual rice production to 120 million tonnes by the year 2020. So in order to narrow the gap between production and demand, increase in productivity is the only option left (Yashitola *et al.*, 2002). Hybrid rice technology is the most viable option for sustaining the global production (Alahgholipour *et al.*, 2007)

Hybrid rice technology particularly utilizing the Cytoplasmic Genetic Male Sterility (CGMS) has now been widely adopted. Identification of restorer line, maintainer lines and development of promising maintainer lines in to CMS lines forms an integral part of hybrid rice technology. Nuclear genes are required to restore pollen fertility to CMS lines. Restorer line carrying the restorer genes (*Rf3, Rf4, Rf5, Rf6 and Rf7*) to restore fertility is indispensable for the development of hybrid varieties (Virmani *et al.*, 2003, Ahmadikhah and Alavi 2009). Hybrid rice technology aims to increase the yield potential of rice beyond the level of high-yielding varieties (HYVs) by exploiting the phenomenon of hybrid vigour or heterosis. Combining ability analysis is one of the powerful tools available to estimate the combining ability effects and aids in selecting the desirable parents and crosses for the exploitation of heterosis.In India during the year 2011-12, hybrid rice had occupied an area of 1.3 m.ha and an additional rice production of 1.5 to 2.5 m.tonnes was added to our food basket through this technology. So in view of these, present investigation was carried out to identify maintainer and restorers from Kerala rice varieties, locating heterotic combiners from the probable restorer and maintainer lines by assessing general and specific combining ability effects of restorers and maintainers and identification of heterotic hybrids for the state of Kerala.

5.1 MOLECULAR CHARACTERIZATION OF *Rf* LOCI USING SSR MARKERS

Hybrid breeding based on CMS/*Rf* system achieved great success worldwide (Cao and Zhan, 2014). At least 90% of the rice hybrids use the wild abortive cytoplasmic source (Yao *et al.*, 1997). Molecular marker technology assists screening a large number of cultivars and breeding lines within a short period and avoiding test crossing, thus saving resources and time. Bazrkar *et al* in the year 2008 used SSR markers linked to fertility restorer genes (*Rf3* and *Rf4*) for identification of potential restorer lines for WA-CMS system, thus avoiding routine testcrossing. Sheeba *et al.*, (2009) reported that the selection accuracy in a set of 21 restorer lines with RM6100 from *Rf4* was 94.4%.

Rosamma *et al.* (2003) reported that most of the selected varieties give higher yield in Kerala condition. These high yielding semi dwarf rice varieties inspired farmers to enhance the coverage of cultivable rice land under high yielding varieties and thereby boosting productivity. They had also reported that these varieties are resistant to different biotic and abiotic stresses which can be used as a source of resistance. Red and bold kernelled rice is most preferred among Kerala farmers (Leenakumari *et al.*, 1998). Rosamma *et al.*, (2003) also reported that most of these varieties (Table 09) had red and bold kernelled grains except PTB 9, Neeraja and Swarnaprabha.

It was observed that different restoring fertility (*Rf*) gene is present separately in almost all the rice varieties *viz;* PTB 9, Remya, Jayanthi, Swarnaprabha, Manupriya, Bharathy, Uma, Annapoorna, Jyothi, Karthika, Kanakom, Varsha, PTB 32, Kanchana, Mattatriveni, Neeraja, PTB 10, Aruna, Aiswarya and Pavizham. Totally seven varieties had two *Rf* genes, four varieties had three *Rf* genes and only one variety showed the presence of all the *Rf* genes.Totally four lines *viz.*, Remya, PTB 9, Manupriya and Swarnaprabha had four genes (*Rf3*, *Rf4*, *Rf5* and *Rf6*) in common whereas variety Aruna, Uma and Bharathy had three genes (*Rf3*, *Rf4* and

Rf6) in common. Huang *et al.,* (2003), Bazrkar *et al.,* (2008), Mishra *et al.,* (2003) and Singh *et al.,* (2005) employed similar set of SSR marker for screening of *Rf* gene.

The varieties (Remya, PTB 9, Manupriya, Swarnaprabha, Aruna, Uma and Bharathy) having more number of *Rf* gene can be given more preference in hybrid seed production programme as restorers after field evaluation of their F_1 with A lines (CMS) to confirm their restoring ability.

5.2 VALIDATION OF THE RESTORATION OF FERTILITY AND IDENTIFICATION OF RESTORERS AND MAINTAINERS.

Out of 21 Kerala rice varieties, three were found to be maintainer for IR58025A, one for UPRI95-17A, four for CRMS31A and two for CRMS32A. Only one pollen parent Kanchana identified as potential maintainer for all four male sterile lines (Table 11). Jyothi and Aruna were identified as maintainer for two CMS lines namely IR58025A and CRMS31A and Bharathy was identified as maintainer for CRMS 31A and CRMS 32A. Altogether four lines were identified as maintainers from this experiment. These potential maintainers can be utilized for the development of new CMS lines by using them as recurrent parent in backcross programme. Several researchers *viz;* Kumar *et al.,* 2010, Akhter *et al.,* 2008, Ingale *et al.,* 2008, Jayashudha and Sharma 2010 and Waghmode and Mehta 2011 reported the development of CMS lines by repeated backcrossing from maintainer lines.

Line Remya was an effective restorer for all the four CMS lines (IR58025A, UPRI95-17A, CRMS31A and CRMS 32A). Jayathi behaved as effective restorer for UPRI95-17A, CRMS31A and CRMS 32A. Aiswarya found to be effective restorer for IR58025A, UPRI95-17A and CRMS 32A. Tester Swarnaprabha and Kanakom was an effective restorer for CRMS31A and CRMS32A. Annapoorna effectively restored fertility in CMS lines UPRI95-17A, and CRMS 32A and Neeraja in UPRI95-17A, CRMS31A. Similar findings had also reported by Kumar *et al.,* (2002), Hariprasanna *et al* 2005, and Upadhyay and Jaiswal 2012. They reported that the

genotypes acting as restorers may also behave as partial restorer against a similar source of CMS lines. Rosamma and Vijaykumar (2005) reported that rice genotypes expressed differential fertility reactions when crossed with different CMS lines having WA cytoplasm.

No rice hybrid has been released from Kerala, the main reason is non availability of restorers and maintainers from Kerala rice varieties specific for their quality traits. The varieties which were identified as maintainers and restorers in this study are having had red kernelled grains with bold size along with resistance to different biotic and abiotic stress (Table 21). As the red kernelled grains being mostly preferred by the local farmers in Kerala, these lines can be used in hybrid seed production programme to get good quality hybrid. The sterile hybrids from F_1 can be backcrossed to the locally adapted maintainers (Jyothi, Kanchana, Bharathy and Bharathy) to develop locally adaptable CMS lines with red kernels for future hybrid development.

5.2.1 Rf loci and restoration of fertility

Remya a restorer for all the 4 CMS lines had 4 genes *viz; Rf3, Rf4, Rf5* and *Rf6*. Kanchana which was maintainer for all the crosses had only one gene *Rf3*. But jayathi which was a restorer for three CMS sources had only one gene *Rf3*. None of the genes of the *Rf* loci was found specific to the restorers. But in most of the cases restorers had more number of *Rf* genes with few exceptions.

5.3 STUDY OF INHERITANCE PATTERN OF RESTORER GENE FOR WA CYTOPLASM THROUGH BULK SEGREGANT ANALYSIS (BSA)

5.3.1 Inheritance of Fertility Restoration (*Rf*) Gene

The finding based on pollen and spikelet fertility showed that fertility restoration in UPRI95-17A x Remya, UPRI95-17A x Jayathi, UPRI95-17A x Annapoorna, and UPRI95-17A x Pavizham is governed by two genes with dominant type of interaction. It appears that the restoration ability of restorers governed by two independent major genes and the interaction of allele between these two genes

produces normal dihybrid ratio. In the case of UPRI95-17A x Aiswarya, it appears that the restoration ability of restorers governed by two independent major genes and the interaction of allele between these two genes produces typical masking gene interaction. The inheritance of fertility restoration in WA type CMS has been extensively investigated. Most investigators tended to agree that restoration of WA type CMS is controlled by two nuclear genes (*Rf3*, *Rf4*) (Zhang *et al.,* 1997; Yao *et al.,* 1997; Zhang *et al.,* 2002). When dominant allele of these two genes is present together they produce homozygous fertile expression, but alone these two allele with recessive alleles of other gene produce different phenotype, where as reccesive allele of both the gene produces sterile phenotype. This is the case for four crosses UPRI95-17A X Remya, UPRI95-17A X Jayathi, UPRI95-17A X Annapoorna, and UPRI95-17A X Pavizham giving a segregation ratio 9:3:3:1 in F_2 generation. While two independent dominant genes control the fertility restoration in the genotype Aiswarya. One of the genes is strong enough to produce complete fertile plants in dominant homozygous or heterozygous condition despite of dominant or recessive allele of other gene whereas the other dominant genes in dominant homozygous or heterozygous condition in presence of recessive condition of previous gene produces partial fertile plants and the recessive homozygous condition of both the genes produces complete sterile plants.

The information on genetic control of fertility restoration of cytoplasmic male sterile lines facilitates the formulation of effective breeding plans for efficient transfer of fertility restorer genes to promising breeding lines that will contribute to the development of superior restorer lines. The genetics of fertility restoration of WA cytoplasm has been shown to follow monogenic inheritance (Shen *et al.* 1998), digenic inheritance (Bharaj *et al.*, 1991, Govindraj and Virmani, 1988) and trigenic inheritance with several types of interaction (Sarker *et al.,* 2002, Kumar and Chakrabarti 1983 and Huang *et al.,* 1987). Most of the results indicated the

inheritance governed by two genes with several types of interaction that vary from genotype to genotype.in this study also digenic inheritance was prominent.

5.3.2 Bulk Segregant Analysis (BSA)

Co-segregation was found between marker RM 1 and the trait in the crosses *viz.,* UPRI95-17A x Remya, UPRI95-17A x Jayathi and UPRI95-17A x Neeraja, indicating that *Rf* gene carried by these lines is located on short arm of chromosome 1. Similarly co-segregation between marker RM 1 and the trait was found in crosses, UPRI95-17A x Remya, UPRI95-17A x Neeraja and UPRI95-17A x Pavizham indicating that *Rf* gene carried by these lines is located chromosome 10. No co-segregation was found between the *Rf* gene and the marker locus of RM1 and RM 171 in the F_2 generation of crosses UPRI95-17A x Pavizham and UPRI95-17A x Jayathi respectively, indicating that *Rf* gene carried by this line is located on a chromosome other than chromosome 1 or adequately far from that locus.

In the previous experiment, we have found that rice varieties Remya, Jayathi and Neeraja had restoring fertility gene *Rf3* where as Remya, Neeraja and Pavizham had restoring fertility gene *Rf4* when screened with SSR marker RM 1 and RM171 respactively. Bazrkar *et al.*, in the year 2008 and Kiani, 2015 had reported that microsatellite marker RM 1 and RM 171 is linked to *Rf3* gene and *Rf4* gene respectively. Inheritance studies in this experiment also showed that fertility resoration is controlled mainly by two major genes. In the present investigation it has been seen that *Rf3* and *Rf4* gene is co-segregating in the F_2 generation of cross between UPRI95-17A x Remya, UPRI95-17A x Jayathi, UPRI95-17A x Neeraja and UPRI95-17A x Remya, UPRI95-17A x Neeraja and UPRI95-17A x Pavizham respectively, this means the marker locus and fertility restoration gene are tightly linked in those fertile parental lines. Similar result had also been reported by Ahmadikhah *et al.*, (2007), Alavi *et al.*, (2009) and Boopathi *et al.*, (2013) while studying inheritance pattern of *Rf* gene in rice.

5.4 DIVERSITY ANALYSIS AMONG 21 RICE VARIETIES

5.4.1 Variability parameters

The knowledge of genetic variability present in a given crop species for the character under improvement is of paramount importance for the success of any plant breeding programme. Information on coefficient of variation is useful in measuring the range of variability present in the characters. Heritability and genetic advance are important selection parameters for selection of characters which relates to yield improvement. Genotypic coefficient of variation (GCV) along with heritable estimates would provide a better picture on the amount of genetic advance to be expected by phenotypic selection (Burton, 1953). It is suggested that genetic gain should be considered in conjunction with heritability estimates (Johnson *et al.*, 1955). Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone.

Genotypic and phenotypic co-efficients of variation was found to be moderate for pollen fertility, number of spikelets/panicle, number of filled grains/panicle, grain length and breadth ratio and grain yield/plant. Estimates of PCV were slightly higher than the corresponding GCV estimates for characters all the characters studied indicating that the characters are under the influence of environment. Therefore, selection on the basis of phenotype alone can be effective for the improvement of these traits.

The estimates of heritability act as predictive instrument in expressing the reliability of phenotypic value. Therefore, high heritability helps in effective selection for a particular character. Heritability in broad sense is the ratio of genotypic variance to the phenotypic variance and is expressed in percentage. In the present study all characters show high heritability of more than 60 % for all the characters eecept total number of tillers/plant. High heritability for quantitative characters indicates the scope of genetic improvement for these characters through selection.

If the value of heritability in broad sense is high, it indicates that the character is least influenced by the environment, the selection for improvement of such character sometimes may not be useful, because broad sense heritability is based on total genetic variance which includes both fixable (additive) and nonfixable (dominance and epistasis) variances. On the other hand, if the value of genetic advance is high, it shows that the character is governed by additive genes and selection will be rewarding for improvement of such trait. So high heritability accompanied with high genetic advance indicates most likely that the heritability is due to additive gene effect and selection may be effective.

In the present investigation, high heritability coupled with high genetic advance as per cent of mean was observed for pollen fertility (h^2-98, GA5%-25.34), number of spikelets/panicle (h^2-99.8, GA5%-21.49), number of filled grains/panicle (h^2-99.7, GA5%-30.75), length and breadth ratio ratio (h^2-75, GA5%-23.97), number of grain/panicle (h^2-98.9, GA5%-21.71) and grain yield/plant (h^2-87.28, GA5%-20.44). Thus, these traits are predominantly under the control of additive gene action and hence these characters can be improved by selection. Similar result reported by Ghosh and Sharma (2012) and Paikhomba *et al.*, (2014) for pollen fertility, Subbaiah *et al.*, (2011), Ramanjaneyulu *et al.*, (2014) and Ketan and Sarker (2014) for Spikelet per panicle, by Debnath *et al.*, (2015), Paikhomba *et al.*, (2014) and Anis *et al.*, (2016) for filled grain per panicle, Subbaiah *et al.*, (2011) and Ketan and Sarkar (2014) for length and breadth ratio, Subbaiah *et al.*, (2011) and Debnath *et al.*, (2015) for number of grain per panicle and Subbaiah *et al.*, (2011) and Paikhomba *et al.*, (2014) grain yield per plant.

5.4.2 Correlation analysis

Crop yield is the end product of the interaction of a number of other, often interrelated attributes. A thorough understanding of the interaction of characters among themselves had been of great use in plant breeding. The efficiency of selection for yield mainly depends on the direction and magnitude of association between yield

and its component characters and also among themselves. Character association provides information on the nature and extent of association between pairs of metric traits and helps in selection for the improvement of the character. Phenotypic and genotypic correlations were worked out on yield and yield contributing characters in 21 genotypes. In general, genotypic correlations were found to be higher than phenotypic correlations, indicating that though there is strong inherent association between characters studied, its expression is lessened due to influence of environment

Grain yield per plant recorded a significant positive correlation with total number of tillers (0.517P, 0.660G), days to flowering (-0.624p, -0.674g), number of productive tillers per plant (0.500P, 0.889G), panicle length (0.610P, 0.745G), number of spikelets/panicle (0.866P, 0.927G), number of filled grains/panicle (0.847P, 0.914G) and number of grains per panicle (0.825P, 0.860G). This indicated that all these characters were important for yield improvement. Similar kind of association was revealed by Augustina *et al.,* (2013) and Ramanjaneyulu *et al.,* (2014) for total number of tillers/plant, Lakshmi *et al.,* (2014) for productive tillers, Vanisree *at al.,* (2013) for panicle length, Rai *et al.,* (2014) and Dhurai *et al.,* (2014) for spikelet/panicle, Vanisree *et al.,* (2013) and Sritama *et al.,* (2015) number of filled grains/panicle and Augustina *et al.,* (2013) for numbers of grains/panicle.

Hence, these characters could be considered for selection for higher yield as these were mutually and directly associated with grain yield. The characters like plant height, pollen fertility and days to maturity were also positively associated with grain yield per plant and it indicated that these characters could also be considered for selection for higher yield.

The study of phenotypic correlation showed that selection of plants with more number of total tillers, productive tillers, spikelets/panicle, filled grain/panicle, number of grains/panicle and increased panicle length would result in improvement of yield.

5.4.3 Path analysis

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

As a guideline for interpretation of results of path analysis, the following broad points may be kept in view (Singh and Chaudhary, 1977).

If the correlation coefficient between a causal factor and the effect is almost equal to its direct effect, then correlation explains the true relationship and a direct selection through this trait will be effective.

If the correlation coefficient is positive and the direct effect is negative or negligible, the indirect effects seem to be the cause of positive correlation. In such situations, the indirect causal factors are to be considered simultaneously for selection.

Correlation coefficient may be negative but the direct effect is positive and high. Under these circumstances, a restricted simultaneous selection model is to be followed *i.e* restrictions are to be imposed to nullify the undesirable indirect effects in order to make use of the direct effect.

If correlation coefficient is negative and direct effect is also negative, then the selection based on that character has to be dropped.

The residual effect determines how best the causal factors account for the variability of the dependent factor. If the residual effect is high, some other factors which have not been considered here need to be included in this analysis to account fully for the variation in yield.

Path coefficient analysis in this study revealed that number of grains per plant (0.633) exerted the highest positive direct effect on grain yield followed by number of filled grains per panicle, number of spikelets/panicle, total number of tillers/plant and days to maturity. The similar results were reported by Vanisree *et al.,* (2013) and Ratna *et al.,* (2015) for filled grain/panicle, Lakshmi (2013) and Vanisree *et al.,* (2013) for spikelet/panicle, Rai *et al.,* (2014) total tillers/panicle and Khan *et al.,* (2009) and Lakshmi (2013) for days to maturity. The negative direct effect on grain yield was found in pollen fertility, panicle length, plant height, productive tillers/plant, days to flowering and length and breadth ratio. The similar result were reported by Fiyaz *et al.,* (2011), Padmaja *et al.,* (2011) for panicle length, Debnath *et al.,* (2015) and Ratna *et al.,* (2015) for plant height, Rai *et al.,* (2014) and Debnath *et al.,* (2015) for productive tillers, Vanisree *et al.,* (2013) and Debnath *et al.,* (2015) for days to flowering and Rajeswari *et al.* (2010) and Lakshmi (2013) for grain length and breadth ratio.

Traits such as number of grain per plant, filled grains per panicle, spikelets/panicle, total number of tillers/plant and days to maturity exerted positive direct effect as well as significant positive correlation with dependant variable grain yield. Two characters productive tillers/plant and panicle length exerted negative direct effect but significant positive indirect effect on grain yield. These two characters exerted positive direct effect through total tillers/plant, days to flowering, spikelet/panicle, filled grain/paniclelength and breadth ratio and number of grain/panicle.

Path analysis revealed that number of grains per plant, number filled grains per panicle, spikelet/panicle, total number of tillers/plant, days to flowering, days to

maturity and length and breadth ratio are the most important characters which could be used as selection criteria for effective improvement on grain yield.

5.4.4 Divergence analysis

It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters. The inter-cluster D^2 values ranged widely with minimum distance between clusters III and IV and maximum distance between clusters II and VI indicating high diversity among the genotypes of these clusters. It is reported that genotypes within the cluster with high degree of divergence would produce more desirable breeding materials for achieving maximum genetic advance. Therefore the maximum amount of heterosis is expected from the crosses with parents belonging to the most divergent clusters as has been reported by Prasad *et al.,* (2013), Allam *et al.,* (2014), Kumar *et al.,* (2014) and Bharathi *et al.,* (2016). Hence, it is desirable to select the genotypes from the cluster showing high inter-cluster distance in breeding programme for obtaining the desireable segregants. The inter-cluster distances were higher than the intra-cluster distances indicating the presence of wider genetic diversity between the clusters rather than within the clusters.

It is observed that no cluster contained at least one genotype with all the desirable traits, which ruled out the possibility of selecting directly one genotype for immediate use. Therefore, hybridization between the selected genotypes from divergent clusters is essential to judiciously combine all the targeted traits.

Genotype groups in cluster V were with low grain yields per plant associated with low pollen fertility, less spikelet/panicle and less no of filled grain/panicle and Genotypes in cluster VIII had grain yields per plant associated with higher plant height and higher number of productive tillers/plant. Higher yielding clusters were VIII, VI, I. The variety PTB 9 from cluster VIII, Swarnaprabha from cluster VI and Uma from cluster I having high mean values for grain yield per plant may be directly used for adaptation or may be

used as parents in future hybridization programme as has been reported Kumar *et al.*, (2014) and Bharathi *et al.*, (2016).

Among the 11 quantitative characters studied the most important character contributing to the divergence was number of filled grains/panicle followed by grain yield/plant, number of grains/panicle, number of spikelets/panicle, number of grains/panicle, plant height and pollen fertility and these results have conformity with reports of Allam *et al.*, (2014), Rajput *et al.*, (2014), Kumar *et al.*, (2014) and Bharathi *et al.*, (2016). In order to select genetically diverse genotypes the material should be screened for the above mentioned important traits.

5.5 IDENTIFICATION OF HETEROTIC HYBRIDS BETWEEN CMS LINES AND IDENTIFIED RESTORERS

Semi-dwarf plant height (80-100cm) is desirable for recording high yield in rice variety as vigour in plant height may lead to unfavorable grain/straw ratio and optimum yield due to lodging (Tiwary *et al.*, 2011). Significant negative heterosis were found in four hybrids over mid parent (IR58025A x Manupriya, IR58025A x Swarnaprabha, UPRI95-17A x Remya and IR58025A x Varsha), better parent (CRMS32A x Remya, CRMS32A x Kanakom, CRMS31A x Kanakom, IR58025A x Manupriya) and standard check (CRMS32A x Kanakom, IR58025A x Manupriya, CRMS31A x Kanakom, CRMS32A x Remya). Expression of significant negative heterosis for plant height was reported by Tiwary *et al.*, (2011), Aditya Kumar *et al.*, (2012) and Dwivedi and Pandey (2012).

Four hybrids UPRI95-17A x Neeraja, UPRI95-17A x Remya, UPRI95-17A x Aiswarya and CRMS31A x Kanakom exhibited highest significant positive heterosis for number of tillers per plant over the check variety Uma as well as midparent. Hybrids UPRI95-17A x Remya, UPRI95-17A x Neeraja, UPRI95-17A x Aiswarya and IR58025A x Manupriya exhibited the highest positive significant heterosis over better parent for the trait number of tillers /plant. Among them three hybrids (UPRI95-17A x Neeraja, UPRI95-17A x Remya and CRMS31A x Kanakom) showed

the highest significant positive heterosis over standard check. Highly significant heterosis for this trait is closely associated with high grain yield per plant resulting high productivity as also noticed by Tiwary *et al.*, (2011).The positive heterosis for above traits were reported by Dwivedi and Pandey (2012).

Generally productive tillers per plant is positively correlated with the yield, therefore hybrids with positive heterosis for this trait is desirable. Hybrids viz; CRMS31A x Kanakom, CRMS32A x Mattatriveni, UPRI95-17A x Aiswarya and UPRI95-17A x Neeraja registered the highest significant heterosis over midparent where as hybrids CRMS31A x Kanakom, UPRI95-17A x Neeraja and IR58025A x Manupriya registered significant heterosis over better parent for number of productive tillers/plant. Four hybrids UPRI95-17A x Aiswarya, UPRI95-17A x Neeraja, UPRI95-17A x Remya and IR58025A x Manupriya manifested significant heterosis over standard check variety. Two hybrids UPRI95-17A x Aiswarya and UPRI95-17A x Neeraja recorded significant heterosis over mid parent, better parent as well as commercial check. Similar result had been reported by Amudha *et al.*, (2010) and Cahndiraka and Thiyagarajan (2010).

Pollen fertility is one of the constraints in hybrid rice breeding programme, which affects the yield considerably. Only one single hybrid (CRMS31A x Jayathi) registered significant heterosis over mid parent, better parent and standard variety, where as hybrid IR58025A x Manupriya registered significant heterosis over mid parent and better parent for pollrn fertility. Expression of significant positive heterosis for pollen fertility was reported by by Amudha *et al.*, (2010), Dwivedi and Pandey (2012) and Behera (2016).

Number of spikelets per panicle is one of the important yield contributing trait and number of fertile spikelets directly contribute to the seed yield, hence hybrids with positive heterosis for this trait are desirable. In the present study, almost all the hybrids showed positive heterosis for number of spikelet. Two hybrids UPRI95-17A x Remya and UPRI95-17A x Aiswarya registered significant heterosis, heterobeltiosis

and standard heterosis for this trait. Two hybrids viz; IR58025A x Mnupriya and UPRI95-17A x Remya registered high significant positive heterosis and standardard heterosis for the trait spikelet fertility percentage. None of the hybrid registered significant positive heterosis over better parent for spikelet fertility percentage. The Significant heterosis in the hybrids for spikelets per panicle was reported by Vanaja and Babu (20040, Kumar *et al.*, (2012) and Dwivedi and Pandey (2012). Significant heterosis for spiklet fertility was reported by Veeresha *et al.*, (2015) and Behera (2016).

Higher number of filled grains per panicle positively correlated with the yield, therefore hybrids with positive heterosis for number of filled grains/panicle are desirable. The hybrids UPRI95-17A x Remya and UPRI95-17A x Neeraja registered high significant positive heterosis, heterobeltiosis and standardard heterosis where as hybrids UPRI95-17A x Aiswarya and CRMS31A x Swarnaprabha registered high significant positive heterosis over better parent and standard check. Smilar significant heterosis for filled grain per panicle was reported by Cahndiraka and Thiyagarajan (2010) and Roy (2013).

Grain yield is a complex and dependent trait. The hybrid CRMS31A x Kanakom registered high significant positive heterosis, heterobeltiosis and standardard heterosis for grain yield per plant. Hybrids UPRI95-17A x Aiswarya and UPRI95-17A x Neeraja registered high significant positive heterosis over midparent and standard check. The Significant heterosis in the hybrids for Grain yield per plant was reported by Roy (2013), Veeresha *et al.*, (2015) and Behera (2016).

The check Uma used in this study is a highly stable and higher yielding commercial rice variety in the state. But the yield of this variety comparably less than hybrid rice. Therefore the present study undertook to develop hybrid rice having characteristic similar to Kerala rice variety and which can yield more than the local rice varieties. The present study resulted in identification of promising hybrids

UPRI95-17A x Aiswarya, UPRI95-17A x Neeraja, UPRI95-17A x Remya and CRMS31A x Kanakom based on high mean grain yield per plant and high standard heterosis over standard check Uma (26.67%, 24.31%, 23.36% and 18.46% respectively). All the high yielding hybrids had red kernel except hybrids developed from male parent Neeraja.

5.6 LOCATING HETEROTIC COMBINERS FROM THE PROBABLE RESTORERS AND MAINTAINERS

5.6.1 Analysis of variance

The analysis of variance revealed highly significant difference among the genotypes for all the 12 quantitative traits studied, thereby justifying the selection of parents for the study. The mean sum of squares due to crosses was highly significant, indicating the diverse performance of different cross combinations. The mean sum of squares due to parents versus crosses was highly significant for all traits revealing the presence of heterosis due to the significant difference in the mean performance of hybrids and parents. Significant differences were also observed for all traits studied in testers (male) and lines (females) indicating the predominance of non-additive gene action.

The GCA variances were significant for plant height, days to maturity, number of spikelet/panicle and number of grain/panicle indicating operation of additive gene action. On the other hand, SCA variances were highly significant for all characters except for total number of tillers, productive tillers and grain length/breadth ratio indicating the predominance of non-additive gene action. These results were in agreement with earlier reports of Anand Kumar *et al.*, (2004), Fiaz *et al.*, (2006), Jagadeesan and Ganesan (2006), Chakraborty *et al.*, (2009), Bagheri and Jelodar (2010) and Mirarab *et al.*, (2011).

5.6.2 General Combining Ability Effects

The potentiality of a strain to be used as a parent in hybridization may be judged by comparing the *per se* performance of the parents, the F_1 value (heterosis)

and the combining ability effects. However, most of the time *per se* performance of a parent alone is not always a true indicator of its potentiality in hybrid combination. Therefore, general combining ability, which is also the breeding value of the parent, has proved as a useful tool for choosing the parents for hybridization. Among the parents with significant *gca* effects, the ones with higher magnitude of *gca* effects were considered as superior to those with lower magnitude.

The summary of general combining ability effects of the parents revealed that none of the parent was found to be good general combiner for all the characters. Among the lines, Jyothi had significant *gca* effects in desired direction for nine traits, plant height, total number of tillers, productive tillers, days to maturity, number of spikelet/panicle, number of filled grain, number of grains/panicle, panicle length and yield/plant. The line Kanchana was a good source of favorable genes for days to flowering, total no of tillers, pollen fertility and days to maturity whereas the line Aruna was found to be good combiner for days to flowering, panicle length and grain length/breadth ratio in desirable direction. The line Bharathy was a good combiner for pollen fertility, number of spikelet/panicle, no of filled grain, no of grain/panicle and L/B ratio.

Among the eleven testers, Swarnaprabha was found to be a good general combiner for days to flowering, total number of tillers, number of productive tillers, days to maturity, number of spikelets/panicle, number of filled grains/panicle, panicle length, length/breadth ratio and yield/plant and tester Neeraja for plant height, total number of tillers, number of productive trillers, pollen fertility, number of spikelet/panicle, number of filled grains/panicle, panicle length, grain length and breadth ratio and yield/plant. Tester Jayathi was found to be good general combiner for days to flowering, total number of tillers, productive tillers, pollen fertility, number of spikelet/panicle, number of filled grain, number of grain/panicle and yield/plant, whereas Annapoorna was found to be good general combiner for days to flowering, pollen fertility, days to maturity, number of spikelet/panicle, no of filled grain/panicle, length and breadth ratio and yield/plant. The tester Varsha was a good

combiner for days to flowering, total number of tillers, productive tillers, spikelet/panicle, number of filled grains/panicle, panicle length, length and breadth ratio and yield/plant. While tester Pavizham was found to be good general combiner for plant height, total no of tillers, productive tillers, pollen fertility, number of spikelet/panicle, no of filled grain, no of grain/panicle, length and breadth ratio and panicle length. The tester Remya was found to be good general combiner for pollen fertility, number of spikelets/panicle, number of filled grain, panicle length and grain length-breadth ratio. High overall good general combiners suggesting their ability to transmit additive genes in the desirable direction for the traits under study. Superiority of female and male parents based on *gca* effects was also reported by Swamy *et al.*, (2003), Panwar (2005), Sao and Motiramani (2006), Rashid *et al.*, (2007), Chakraborty *et al.*, (2009), Kumar and Reddy (2011) and Patil *et al.*, (2011).

Perusal of findings indicated that lines Jyothi was observed good general combiner for yield and yield contributing traits. Barrathy was observed as good general combiner for pollen fertility, number of spikelet/panicle, number of filled grains/panicle and number of grains/panicle. Among testers Swarnaprabha, Neeraja, Annapoorna, Varsha and Jayathi, were good general combiners for yield and yield contributing characters. They could be considered as the best combining parents of the present study in yield attributes and hence could be utilized in the future breeding programme.

5.6.3 Specific Combining Ability Effects

Grain yield is a complex character dependent upon the contribution of various component characters affecting directly or indirectly. The existence of total genetic variability and magnitude as well as nature of gene effects in the population under improvement to a large extent would dictate the choice of breeding methodology. The range of *sca* effects for grain yield per plant varied from- 0.067 (Jyothi x Varsha) to 5.420 (Aruna x Varsha).

In case of specific combing ability effects, none of the hybrids exhibited favorable *sca* effect for all the characters. Yield/plant is ultimate goal of rice breeding

and hybrid development programme. For grain yield/plant eighteen hybrids showed significant *sca* effects for seed yield per plant, of which the best ten hybrid combination were Aruna x Varsha followed by Jyothi x Pavizham, Bharathy x Annapoorna, Kanchana x Mattatriveni, Aruna x Swarnaprabha, Jyothi x Remya, Kanchana x Jayathi, Aruna x Neeraja, Bharathy x Manupriya and Kanchana x Remya and these were good specific combiners based on high and significant *sca* effects.

The cross combination Aruna x Varsha was the best specific combination for acceptable and significant *sca* effects for yield/plant and other desireable characters like total number of tillers, productive tillers, pollen fertility, number of spikelets/panicle, number of filled grains/panicle, number of grains/panicle, panicle length and grain length/breadth ratio. Kanchana x Mattatriveni was another best specific combination for acceptable and significant SCA for grain yield/plant and other desireable characters like days to flowering, total number of tillers, productive tillers, pollen fertility, number of spikelet/panicle, number of filled grain/panicle, no of grain/panicle and panicle length (Table 30). Some other cross combination for acceptable and significant SCA effects for grain yield/plant and other desirable characters are Jyothi x Kanakom, Jyothi x Remya, Kanchana x Jayathi, Aruna x Swarnaprabha, Aruna x Neeraja, Bharathy x Manupriya and Bharathy x Annapoorna.

High *sca* effect results mostly from dominance and interaction effects existing between the hybridizing parents. High *sca* effects for grain yield using line x tester analysis have earlier been reported by Roy and Mandal (2001), Singh and Kumar (2004), Rashid *et al.*, (2007), Thakare *et al.*, (2010). Saidaiah *et al.*, (2010) reported high SCA effect for grain yield also and most of the remaining traits over environments. Similar kind of results was reported by Mirarab *et al.*, (2011).

Out of forty four crosses the top three crosses exhibiting high *sca* effects selected for each character and GCA status of parents of such hybrids are presented as either low or high in. Total 5 hybrids had significant negative effect for Plant height. Out of 5 hybrids Jyothi x Kanakom, Kanchana x Pavizham and Bharathy x Neeraja were the superior hybrids and Kanchana x Pavizham (L x H) had both desirable *sca*

effects and high *per se* performance. Among top three crosses, Kanchana x Mattatriveni (L x L) also had both higher *sca* effect and *per se* performance in desirable direction for days to flowering. Totally nineteen crosses exhibited desirable positive significant *sca* effect for total number of tillers/plant and among them Aruna x Varsha (L X H) had both desirable SCA and high per see performance followed by Bharathy x J ayathi (L X H) and Kanchana x Mattatriveni (L X L). A total of fifteen crosses were found to be significant for productive tillers/plant. Among them three crosses having highest significant SCA were Aruna x Varsha, Kanchana x Annapoorna, and Jyothi x Mattatriveni. Hybrid Aruna x Varsha (L X H) had both high *sca* effect as well as per see performance.

Sixteen hybrids had positively significant *sca* effect for pollen fertility. 3 hybrids which showed highest *sca* effect were Aruna x Varsha (L X H), Bharathy x Mattatriveni (L X L) and Bharathy x Jayathi (L X H). For Days to maturity total sixteen hybrids showed negative sca effect. Among them Bharathy x Kanakom (L x L) had highest *sca* effect (-3.55). The cross combination viz; Kanchana x Mattatriveni, Aruna x Varsha and Bharathy x Mattatriveni recorded highest significant positive *sca* effect. Among them hybrid Aruna x Varsha had both higher sca effect and higher per see performance. Among three best crosses Aruna x Varsha (L x L) had both higher SCA and per see performance, where as two other crosses had higher *sca* effect but moderate per se performance. All three best hybrids Kanchana x Mattatriveni, Jyothi x Kanakom and Bharathy x Aiswarya had high *sca* effect and moderate per se performance for total grain/panicle. Among them Kanchana x Mattatriveni (L x L) had highest *sca* effect (28.71). For Panicle length among the three best hybrids Aruna x Varsha (L x L) had both highest *sca* effect and highest per see performance. Among top three crosses Kanchana x Neeraja (L x H) had higher *sca* effect and highest per se performance for grain Length/Breadth ratio. With respect to grain yield, top three crosses for high *sca* effect exhibited highest to moderate grain yield. Hybrid Aruna x Varsha (L x L) had highest sca effect as well as per see performance.

Based on the *sca* effects of the hybrids, 26 out of 44 hybrids had high (H) overall SCA status across the twelve traits. Hence, these crosses could be utilized for exploitation of heterosis and the remaining 18 had low (L) overall sca status. The high (H) performing hybrids belonged to H × H, H × L, L × H and also L × L type of parental combinations suggesting the action of additive, non additive gene action and also overdominance and epistasis, respectively. Several workers including Swamy *et al.,* (2003), Jagadeesan and Ganesan (2006), Rahimi *et al.,* (2010), Saidaiah *et al.,* (2010) and Tiwary *et al.*, (2011) identified good specific combiners for different yield attributing traits based on high *sca* effects in desirable direction.

Three hybrids (Jyothi x Jayathi, Jyothi x Aiswarya and Jyothi x Kanakom) out of 26 had expressed high overall SCA status involving H x H type of general combiners (Table 39). This interaction between positive and positive alleles in high x high combiners can be fixed in subsequent generations if no repulsion phase linkages are involved. Similar conclusion was reported by Shivani *et al.,* (2009) in their study on combining ability for grain quality characters in indica/indica hybrids of rice. Eleven crosses had both the parents with low overal GCA status (Low × Low), which has been attributed to over dominance and epistasis interaction as suggested by Swamy *et al.*, (2003), Singh *et al.,* (2005) and Dalvi and Patel (2009). In remaining crosses high *SCA* was mainly either due to high x low (12) or low x high (17) combining parents, which further substantiate the operation of non-additive gene action (additive x dominance and dominance x dominance epistatic interaction).

5.6.4 **Proportional contribution of parents**

Testers appeared to contribute to the bulk of the variation observed in hybrids which was higher for yield/plant, plant height, total number of tillers/plant, days to flowering, number of spikelets/panicle, pollen fertility, productive tillers and number of filled grains. Per cent contribution of the line × tester interaction effect to the variation observed in hybrids was higher for grain length and breadth ratio, number of filled grain/panicle, pollen fertility, number of spikelet/panicle and panicle length.

Whereas the lines contributed less towards the variation in the hybrids compared to testers and line x tester interaction.

The contribution of Line x Tester towards the total variance was found higher than lines and Testers. The greater contributions of lines x testers interaction for all the characters than testers except days to maturity indicates higher estimates of specific combining ability variance effects These results are in agreement with the findings of Rashid *et al.*, (2007). Greater contribution of Line x tester interactions than that of lines and testers except for number of spikelets per panicle was also reported by Ghara, *et al.*, (2012).). Greater contribution of testers than lines x tester's interaction and thus higher estimates of variance due to *gca* effects was reported by Faiz *et al.*, (2006) and Saleem *et al.*, (2010

In this study the lines chosen are varieties which are identified as maintainers for the CGMS system. The testers are the varieties identified as restorers for the CGMS system. The line in the best hybrid can be used for the development of male sterile line by repeated backcrossing. So that the hybrid can be released as hybrid rice with provision for easy commercial hybrid seed production using CGMS system.

5.7 IDENTIFICATION OF HETEROTIC HYBRIDS FROM CROSS BETWEEN MAINTAINERS AND RESTORERS

In the present investigation heterosis over mid parent, better parent and standard heterosis over check were estimated for yield and yield attributing traits for forty-four hybrids and the results have been discussed below. Out of forty four crosses the top three exhibiting high heterosis been selected for each character.

Out of forty four hybrids, three hybrids Kanchana x Pavizham, Bharathy x Neeraja and Jyothi x Kanakom recorded significant standard heterosis, mid parent heterosis and heterobeltiosis for dwarfness. Expression of significant negative heterosis for plant height was reported by Tiwary *et al.*, (2011), Kumar *et al.*, (2012) and Dwivedi and Pandey (2012).

Early flowering hybrids are desirable as they fit well in multiple cropping systems and can escape terminal drought. The heterosis in negative direction was

considered to be desirable for this trait. Among 44 hybrids 32 were found negatively significant over midparent, 38 over better parent and 35 over commercial check for Days to flowering, while three hybrids Kanchana x Mattatriveni, Jyothi x Kanchana and Jyothi x Manupriya registered highest negative heterosis for early flowering. Earlier workers including Joshi (2001), Umakantha *et al.*, (2002), Munisonnappa and Vidyachandra (2007), Saidaiah *et al.*, (2010), Tiwary *et al.*, (2011) and Dwivedi and Pandey (2012) also noticed significant negative heterosis for earliness.

Out of forty four cross 17 crosses showed significant positive heterosis over mid parent, 12 crosses over better parent and total 36 crosses over commercial check for total number of tillers per plant. Eleven hybrids indicated significantly positive heterosis as well as heterobeltiosis. Over-dominant type of gene action was suggested for them. Out of them three hybrids Aruna x Varsha, Jyothi x Pavizham and Aruna x Pavizham were important based on higher mean performance and highly significant heterosis over midparent, better parent and standard check Uma. Similarly three hybrids viz; Aruna x Varsha, Jyothi x Pavizham and Jyothi x Swarnaprabha were important based on higher mean performance and high significant positive relative heterosis, heterobeltiosis and standard heterosis for number of productive tillers per plant. Over-dominant type of gene action was suggested for them. Four crosses viz; Kanchana x Kanakom, Kanchana x Varsha, Kanchana x Aiswarya and Kanchana x Pavizham recorded significant positive heterosis over mid parent but significant negative heterosis over commercial parent, indicating partial dominance. These results are in agreement with earlier findings *viz;* Vanaja and Babu (2004), Chandiraka *et al.*, (2010), Vanisree *et al.*, (2011) and Veeresha *et al.*, (2015).

Pollen fertility is one of the constraints in hybrid rice breeding programme, which affects the yield considerably. Twenty four crosses showed significant positive heterosis over midparent whereas only 9 crosses registered positive significant heterosis over better parent. Four crosses Jyothi x Swarnaprabha, Jyothi x Manupriya, Jyothi x Mattatriveni and Jyothi x Neeraja registered significant positive heterosis over mid parent but negative heterosis over better parent, indicating partial

dominance. Total 8 crosses registered significant positive heterosis over commercial check. Only 4 crosses found to have significant heterosis over mid parent, better parent and Uma, indicating over dominant type of gene action. Among these 4 cross 3 crosses (Kanchana x Annapoorna, Bharathy x Jayathi and Kanchana x Pavizham) had significant heterosis of all types as well as higher mean value. These results are in agreement with earlier findings Bagheri and Jelodar (2010) and Dar (2013).

Among forty four crosses twenty four crosses registered significant negative heterosis over mid parent, better parent as well as over commercial check variety for the character Days to maturity. Out of 24 hybrids three hybrids Aruna x Jayathi, Kanchana x Swarnaprabha and Kanchana x Manupriya recorded highest significant relative heterosis, heterobeltiosis as wellas standard heterosis. Similar result was reported by Roy (2013).

Generally larger panicle is associated with higher number of grains per panicle, therefore hybrids with positive heterosis are desirable. Total 10 crosses registered significant positive heterosis over mid, where as sevn crosses recorded significant positive heterosis over better parent. While 12 crosses registered significant positive heterosis over commercial variety. Among these crosses only 6 were found positively significant for all three types of heterosis. Over-dominant type of gene action was suggested for these 6 crosses. Among them 3 hybrids (Aruna x Varsha, Jyothi x Pavizham and Jyothi x Remya) reported higher significant heterosis in all 3 types of heterosis. The heterosis for above triats was also reported by Joshi (2001), Umakantha *et al.*, (2002) and Dwivedi and Pandey (2012).

Number of spikelets per panicle is one of the important yield contributing traits and number of fertile spikelets directly contribute to the seed yield, hence hybrids with positive heterosis for these traits are desirable. Total six hybrids recorded significant positive heterosis over mid parent but significant negative heterosis ove better parent, indicating partial dominat gene action. As many as twelve hybrids showed significant positive heterosis over mid parent, better parent and Uma,

indicating over-dominant gene action. Among them three hybrids Aruna xVarsha, Jyothi x Pavizham and Bharathy x Annapoorna registerd higher significant heterosis in all the three types of heterosis. The Significant heterosis in the hybrids for spikelets per panicle was also reported by Joshi (2000), Munisonnappa and Vidyachandra (2007), Tiwary *et al.*, (2011), Kumar *et al.*, (2012) and Dwivedi and Pandey (2012).

A total of 22 crosses showed significant positive heterosis over mid parent, better parent as well as commercial check for filled grain/panicle. Among these 22 hybrids, 3 hybrids Aruna x Varsha, Jyothi x Pavizham and Bharathy x Annapoorna registered highest significant heterosis in all 3 types of heterosis. These results are in agreement with earlier findings (Joshi, 2001, Raju *et al.,* 2014 and Bhatti *et al.,* 2015) .Out of 44 crosses total 12 crosses registered significant positive heterosis over mid parent, better parent and commercial parent for number of grains/panicle. Among these 3 hybrids Jyothi x Remya, Jyothi x Pavizham and Aruna x Varsha recorded highest positive significant heterosis for all 3 types of heterosis. These results are in agreement with earlier findings (Raju *et al.*, 2014 and Bhatti *et al.*, 2015)

Grain yield is an important economic trait which is a complex quantitative character influenced directly or indirectly by many component traits. In the present investigation, the mean *per se* performance of hybrids for yield character was significantly greater than the parental means for most of the crosses (mid parent-33, better parent-30 and commercial variety-25.54). Two crosses Jyothi x Manupriya and Bharathy x Varsha reported significant positive heterosis over mid parent but significant negative heterosis over better parent. Partial dominant type of gene action was suggested for them. Total 28 crosses reported significant heterosis over mid parent, better parent and check variety.overdominant type of gene action was suggested for them. Among these, 3 crosses viz; Aruna xVarsha, Jyothi x Pavizham and Bharathy x Annapoorna registered highest significant heterosis in all three types of heterosis. Joshi (2000), Umakantha *et al.*, (2002), Tiwary *et al.*, (2011), Kumar *et*

al., (2012) and Dwivedi and Pandey (2012) also reported different levels of heterosis for seed yield per plant in their respective studies.

The degree of heterosis varied from cross to cross and from character to character. For plant height and days to 50% flowering and days to maturity negative heterosis is desirable but for rest of the characters positive heterosis was desirable. Positive heterosis ranges from 0.22-58.08%, 0.9-64%, 0.08-140.36-28.69%, 0.22-41.6%, 0.3-28.6%, 0.14-22.24% and 0.7-42% for total number of tillers/plant, number of productive tillers/plant, pollen fertility, number of spikelets/panicle, number of filled grains/panicle, no of grains/panicle, panicle length and grain yield respectively. Negative heterosis ranges from -0.06 to -33.28%, -0.04 to -22.22% and -0.04 to -35.79% for plant height, days to flowering and days to maturity. The highest heterosis for grain yield was observed in cross Aruna x Varsha associated with the significant and desirable heterosis for total no of tillers, Productive tillers, number of spikelets/panicle, number of filled grains/ panicle, number of grains/panicle and panicle length. Desirable and significant heterosis for grain yield was found in nine crosses associated with higher heterosis for most of the yield related traits. However, significant and desirable heterosis was observed in 11 crosses for total number of tillers/plant, 11 crosses for number of productive tillers, 12 crosses for number of spikelets/panicle, 22 crosses for filled grains/panicle, and 12 crosses for number of grains/ panicle and 6 crosses for panicle length.

Keeping in view mean performance, heterosis and heterobeltiosis estimates, 7 hybrids (Bharathy x Annapoorna, Jyothi x Pavizham, Aruna x Varsha, Aruna x Neeraja, Jyothi x Remya, Jyothi x Jayathi and Aruna x Swarnaprabha) having better mean yield performance over the most popular variety can be recommended as potential hybrids. Similar type of observation was also reported in sunflower by Swamy *et al.*, (2003), Bagheri (2010), Tiwary *et al.*, (2011) and Chauhan *et al.*, (2015).

The present study conducted in five different experiments could reveal the reliability of using molecular markers in identifying restorers and maintainers and

could identify heterotic combinations of Kerala rice varieties with available CGMS sources. These combinations can be used for the direct release of hybrids after the standardisation of hybrid seed production technology for Kerala. The heterotic combinations of line x testers (maintainers x restorers) can be used for hybrid development after incorporating male sterile cytoplasm into the maintainer to suit the CGMS system. This study could give a green signal for hybrid rice technology for Kerala.

Summary

6. SUMMARY

Rice is a staple food of more than 60% of the world's population. Among the available innovative and immediately adoptable technologies, hybrid rice technology offers an opportunity to enhance rice production and productivity and thereby ensure a steady supply of food as per projection made for 2025. Hybrids have 15-20% yield advantage over high yielding semi dwarf rice varieties and increases yield around 1.0 to 1.5 t/ha which is significant to increase production (Virmani and Kumar, 2004). Future food security of major rice growing countries lies in the development of hybrid rice varieties which has potential to increase production and productivity with good quality. To achieve targeted food production of increasing population as well as decreasing cultivated area, hybrid rice technology is very useful. The success of future hybrid rice programme depends upon identification of parents having good combining ability with higher magnitude of heterosis and good restorer and maintainer capabilities. Identification of restorer lines having R*f* gene is crucial for hybridization programme. Molecular marker technology offers fast and accurate identification of restorers. . So present project "Genotyping of *Rf* (Restoring fertility) loci of rice varieties of Kerala using molecular markers" was undertaken to identify heterotic maintainers and restorers from Kerala rice varieties and development of superior hybrids.

Thirteen microsatellite primers that were reported to be linked with fertility restoring genes in different chromosomal locations (chromosomes 1, 7, 10, and 12) were employed for polymorphism survey between 21 different rice varieties of Kerala. Genomic DNA was isolated from the 3-4 week old seedlings of the 21 rice varieties by using Quiagen Dneasy Minikit. The quality and quantity of DNA was checked both by agarose gel electrophoresis, UV spectrophotometer and its quantity was checked by taking the ratio of absorbance at 260nm and at 280.

It has been observed that different restoring fertility gene (*Rf*) gene is present separately in almost all the rice varieties *viz;* PTB 9, Remya, Jayathi, Swarnaprabha, Manupriya, Bharathy, Uma, Annapoorna, Jyothi, Karthika, Kanakom, Varsha, PTB 32, Kanchana, Mattatriveni, Neeraja, PTB 10, Aruna, Aiswarya and Pavizham. Total seven varieties had two *Rf* gene, four varieties had 3 *Rf* gene and only one variety showed the presence of all the four *Rf* genes.Total four lines *viz.,* Remya, PTB 9, Manupriya and Swarnaprabha had four gene (*Rf3*, *Rf4*, *Rf5* and *Rf6*) in common whereas variety Aruna, Uma and Bharathy had three gene (*Rf3*, *Rf4* and *Rf6*) in common. No correlation between the *Rf* genes and the male sterility restoration could be deduced from the result.

Eighty four cross was made between twenty one Kerala rice varieties (as male) and four CMS lines for the validation of restoration and identification of restorers and maintainers. Pollen parents Jyothi, Kanchana and Aruna were identified as potential maintainers and pollen parent Remya, Manupriya, Varsha and Aiswarya were identified as restorers against IR58025A. Rice variety Kanchana identified as maintainer against UPRI95-17A and Remya, Jayathi, Annapoorna, Neeraja, Aiswarya and Pavizham found to be restorer in cross with UPRI95-17A. Among 21 pollen parents, 4 lines were identified as maintainers (Bharathy, Jyothi, Kanchana and Aruna) and five lines (Remya, Jayathi, Swarnaprabha, Kanakom and Neeraja) as restorers against against CMS line CRMS31A. Bharathy and Kanchana identified as maintainer lines against CMS line CRMS32A. Remya, Jayathi, Annapoorna, Kanakom, Mattatriveni and Aiswarya were identified as effective restorers against CRMS32A. Variety Kanchana was a maintainer with all the male sterile sources studied and Remya was a restorer for all the four CMS lines used in this study.

Harvested F_2 seed of crosses between UPRI9517A and identified restorers Remya, Jayathi, Annapoorna, Aiswarya and Pavizham were sown in nursery and after 25 days they were transplanted in field. Based on pollen and spikelet fertility plants

were classified into complete fertile, partial fertile, partial maintainer and complete sterile. Goodness of fit for R*f* loci to expected segregation ratio was tested by means of chi-square analysis. The finding based on pollen and spikelet fertility showed that fertility restoration in UPRI95-17A X Remya, UPRI95-17A X Jayathi, UPRI95-17A X Annapoorna, and UPRI95-17A X Pavizham was governed by two genes with dominant type of interaction. In the case of UPRI95-17A x Aiswarya, it appeared that the restoration ability of restorers were governed by two independent major genes and the interaction of allele between this two gene produced typical masking gene interaction.

Co-segregation was found between marker RM 1 and the trait in the crosses (UPRI95-17A x Remya, UPRI95-17A x Jayathi and UPRI95-17A x Neeraja), indicating that R*f* gene carried by these lines is located on short arm of chromosome 1. Similarly co-segregation between marker RM 1 and the trait was found in crosses 171 (UPRI95-17A x Remya, UPRI95-17A x Neeraja and UPRI95-17A x Pavizham) indicating that *Rf* gene carried by these lines is located chromosome 10. No co-segregation was found between the R*f* gene and the marker locus of RM1 and RM 171 in the F_2 generation of crosses UPRI95-17A x Pavizham and UPRI95-17A x Jayathi respectively, indicating that *Rf* gene carried by this line is located on a chromosome other than chromosome 1 or adequately far from that locus.

The observation on 12 metric traits from the 21 genotypes were subjected to genetic analysis to study variability parameters, correlation of different parameters with yield, path analysis and genetic divergence. Coefficients of variation indicated that the estimates of PCV were slightly higher than the corresponding GCV estimates for characters indicating that the characters were under influence of environment. In the present investigation, high heritability coupled with high genetic advance as per cent of mean was observed for pollen fertility, number of spikelet/panicle, filled grains/panicle, length and breadth ratio, number of grains/panicle and grain yield/plant. Thus, these traits are predominantly under the control of additive gene

action and hence these characters can be improved by selection. The study of phenotypic correlation showed that selection of plants with more number of total number of tillers, number of productive tillers, number of spikelet/panicle, filled grain/panicle, number of grain/panicle and increased panicle length would result in improvement of yield. Path analysis revealed that number of grain per plant, filled grains per panicle, number of spikelet/panicle, total number of tillers/plant, days to flowering, days to maturity and length and breadth ratio of grain were the most important characters which could be used as selection criteria for effective improvement on grain yield.

Twenty one genotypes were grouped into eight clusters based on D^2 values such that the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. From the inter cluster D^2 values of the eight clusters, it was found that the highest divergence occurred between cluster II and VI followed by cluster I and V, cluster IV and VIII, cluster V and VI, cluster V and VIII and cluster IV and V suggesting that the crosses involving varieties from these clusters would give wider and desirable recombination.

It is observed that no cluster contained at least one genotype with all the desirable traits, which ruled out the possibility of selecting directly one genotype for immediate use. Therefore, hybridization between the selected genotypes from divergent clusters is essential to judiciously combine all the targeted traits. . The variety PTB-9 from cluster VIII, Swarnaprabha from cluster VI and Uma from cluster I having high mean values for grain yield per plant may be directly used for adaptation or may be used as parents in future hybridization programme. Among the 11 quantitative characters studied the most important character contributing to the divergence was filled grains/panicle followed by grain yield/plant, number of grain /panicle, number of spikelets/panicle, number of grains/panicle, plant height and pollen fertility.

The check Uma used in this study is a highly stable and higher yielding commercial rice variety in the state. But the yield of this variety was comparably less

than hybrid rice. Therefore the present study undertook to develop hybrid rice having characteristic similar to Kerala rice variety and which can yield more than the local rice varieties. The present study resulted in identification of promising hybrids UPRI95-17A x Aiswarya, UPRI95-17A x Neeraja, UPRI95-17A x Remya and CRMS31A x Kanakom based on high mean grain yield per plant and high standard heterosis over standard check Uma. All the high yielding hybrids had red kernel except hybrids developed from male parent Neeraja.

Four identified maintainers (Kanchana, Jyothi, Aruna and Bharathy) and 11 restorers (Remya, Jayathi, Swarnaprabha, Manupriya, Annapoorna, Kanakom, Mattatriveni, Varsha, Aiswarya, Neeraja and Pavizham) were crossed in L x T fashion and combining ability of parental lines were carried out in F_1 generation. The GCA variances were significant for plant height, days to maturity, number of spikelet/panicle and no of grain/panicle indicating operation of additive gene action. On the other hand, SCA variances were highly significant for all characters except for total number of tillers, number of productive tillers and grain length/breadth ratio indicating the predominance of non-additive gene action. Perusal of findings indicated that line Jyothi was observed good general combiner for yield and yield contributing traits. Barrathy was observed as good general combiner for pollen fertility, number of spikelets/panicle, number of filled grains/panicle and number of grain/panicle. Among testers Swarnaprabha, Neeraja, Annapoorna, Varsha and Jayathi, were good general combiners for yield and yield contributing characters. So these lines could be considered as the best combining parents of the present study in yield attributes and hence could be utilized in the future breeding programme.

In case of specific combining ability effects, none of the hybrids exhibited favorable *sca* effect for all the characters. The cross combination Aruna x Varsha was the best specific combination for acceptable and significant *sca* effects for yield/plant and other desireable characters like total number of tillers, number of productive

tillers, pollen fertility, number of spikelet/panicle, number of filled grain/panicle, number of grain/panicle, panicle length and grain length/breadth ratio.

Based on the *sca* effects of the hybrids, 26 out of 44 hybrids had high (H) overall SCA status across the twelve traits. Hence, these crosses could be utilized for exploitation of heterosis and the remaining 18 had low (L) overall SCA status. The high (H) performing hybrids belonged to H × H, H × L, L × H and also L × L type of parental combinations suggesting the action of additive, non additive gene action and also overdominance and epistasis, respectively. Three best hybrids Aruna x Varsha (L x L), Jyothi x Pavizham (H x H) and Kanchana x Mattatriveni (L x L) were identified based on SCA effect for yield as well as other yield contributing characters and mean performance.

In the present investigation heterosis over mid parent, better parent and standard heterosis over check were estimated for yield and yield attributing traits for forty-four hybrids. The mean *per se* performance of hybrids for yield character was significantly greater than the parental means for most of the crosses. Keeping in view of mean performance, heterosis and heterobeltiosis estimates, 7 hybrids (Bharathy x Annapoorna, Jyothi x Pavizham, Aruna x Varsha, Aruna x Neeraja, Jyothi x Remya, Jyothi x Jayathi and Aruna x Swarnaprabha) having better mean yield performance over the most popular variety Uma can be recommended as potential hybrids.

In this study the lines chosen were varieties identified as maintainers for the CGMS system. The testers were the varieties identified as restorer for the CGMS system. The lines in the best hybrid combination could be used for the development of male sterile line by repeated backcrossing, that the hybrid could be released as hybrid rice with provision for easy commercial hybrid seed production using CGMS system.

References

7. REFERENCES

Ahmadikhah, A. and Alavi, M. 2009. A coldinducible modifier QTL affecting fertility restoration of WA CMS in rice. *International Journalof Genetics and Molecular Biology.* 1(5): 089-093.

Ahmadikhah, A. and Karlov, G. I. 2006 Molecular mapping of the fertility-restoration gene Rf4 for WA-cytoplasmic male sterility in rice. *Plant Breeding.* 125:363–367

Ahmadikhah, A., Karlov, G. I., Nematzadeh, G., and Bezdi, G. K. 2007. Inheritance of the fertility restoration and genotyping of rice lines at the restoring fertility (*Rf*) loci using molecular markers. *International Journal of Plant Production.* 1(1): 13-21

Akagi, H., Yokozeki, Y., Inagaki, A., Nakamura, A. and Fujimura, T. 1996. A codominant DNA marker closely linked to the rice nuclear restorer gene, Rf-1, identified with inter-SSR fingerprinting. *Genome.* 39: 1205-1209.

Akhter, M., Zahid, M.A., Ahamd, M. and Haider, Z. 2008. Selection for restorers and maintainers from the test crosses for the development of rice hybrids. *Pakistan J. Sci.* 60(3-4): 100-102.

Akter, A., Hasan, M.J., Begum, H., Kulsum, M.U., and Hossain, M.K. 2010. Combining ability analysis in rice (*Oryza Sativa* L.). *Bangladesh J. Pl. Breed. Genet.* 23(2): 07-13.

Alahgholipour, M., Rabiei, B., Hosseini, M., Dorosti, H., and Mohammadi, M. 2007. Study of General and Specific Combining Ability of Traits in Parental Lines of Hybrid Rice. *J. Agric.* 9: 1-12.

Alavi, M., Ahmadikhah, A., Kamkar, B., and Kalateh, M. 2009. Mapping *Rf3* locus in rice by SSR and CAPS markers. *Int. J. Genet. Mol. Biol.* 1 (7):121-126.

Ali, M., Hossain, M.A., Hasan, M.J., and Kabir, M.E. 2014. Identification of Maintainer and Restorer Lines in local Aromatic Rice (*Oryza sativa*). *Bangladesh J. Agril. Res.* 39(1): 1-12

Allam, C.R., Jaiswal, H.K., and Qamar, A. 2014. Divergence analysis for yield and quality traits in some indigenous basmati rice genotypes [*Oryza sativa*

L.]. *International Journal of Applied Biology and Pharmaceutical Technology.* 5 (4): 257-263.

Allard, R.W. 1960 Principles of plant breeding. John Willey and Sons inc. New York, 485p.

Amudha, K., Thiyagarajan, K., Robin, S., Prince, S.J.K., Poornima K.R., and Suji, K.K. 2010. Heterosis under aerobic condition in hybrid Rice. Electronic Journal of Plant Breeding. 1(4): 769-775

Anandakumar, C.R., and Subramaniam, S. 1992. Genetics of fertility restoration in hybrid rice. *Theoretical and Applied Genetics.* 83:994–996.

Anis, G., Sabagh, A.E.L., Ghareb, A., and Rewainy, I.E. 2016. Evaluation of promising lines in rice (Oryza sativa L.) to agronomic and genetic performance under Egyptian conditions. *International Journal of Agronomy and Agricultural Research.* 8 (3): 52-57

Anonymous, 2008. *Annual report, 2007-08.* Directorate of Rice Research, Hyderabad. 2p.

Anonymous, 2009. Draft proceedings of 44th Annual Rice Group meeting, DRR, *Approaches to Rice Breeding.* IRRI. pp. 35-51.

Arunachalam, V. 1976. Evaluation of diallel cross by graphical and combining ability methods. *Indian Journal of Genetics and Plant Breeding.* 36: 358-366.

Arunachalam, V., and Bandyopadhyay, A. 1979. Are 'multiple cross multiple pollen hybrids' an answer for productive populations in *Brassica campestris var. brown sarson*? I. Methods for studying 'mucromphs'. *Theor. Applied genet.* 4: 203-217.

Athwal, D.S., and Virmani, S.S. 1972. Cytoplasmic male sterility and hybrid breeding in rice. '*Rice Breeding'*. IRRI, Philippines. pp. 615-620.

Augustina, U.A., Iwunor, O.P., and Ijeoma, O.R. 2013. Heritability and character Correlation among some Rice Genotypes for yield and yield components. *J. Plant Breed. Genet.* 1(02): 73-84.

Babu, N.N., Shivakumar, N., and Hittalmani, S. 2010. Pollen fertilioty Vs Spikelet fertility in F2 of a CMS based hybridsin Rice (*Oryza sativa* L.) under Aerobic condition. *Electronic J. Plant Breeding*. 1(4): 789-793.

Bagheri, N., and Jelodar, N.B. 2010. Heterosis and combining ability analysis for yield and related-yield traits in hybrid rice. *Int. J. Bio*., 2(2):222-231.

Bazrkar, L., Ali, A.J., Babaeian, N.A., Ebadi, A.A. Allahgholipour, M., Kazemitabar, K., and Nematzadeh, G. 2008. Tagging of Four Fertility Restorer Loci for Wild Abortive-Cytoplasmic Male Sterility System in Rice (*Oryza sativa* L.) Using Microsatellite Markers. *Euphytica.* 164: 669-677.

Bedi, S., and Sharma, D. 2014. Identification of maintainers and restorers for CMS lines of rice. *Advance Research Journal of Crop Improvement.* 5 (2): 190-193.

Behera, M. 2016. Study of Combining Ability and Heterosis for Development of Aromatic Hybrids in Rice (*Oryza sativa* L.). Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur ,Chhattisgarh.

Bharaj, T.S, Bains S.S., Sidhu, G.S., and Gagneja, M.R. 1991. Genetics of fertility restoration of 'wild abortive' cytoplasmic male sterility in rice (*Oryza sativa* L.). *Euphytica.* 56, 199–203.

Bharathi, G., Krishna Veni, B., Lal Ahamed M., and Jaya Lalitha, K. 2016. Studies on Genetic Divergence in High Yielding Rice (*Oryza sativa* L.) Genotypes. *Journal of Rice Research.* 9 (2):6-10.

Bhati, P.K., Singh, S.K., Singh, R., Sharma, A., and Dhurai, S.Y. 2015. Estimation of heterosis for yield and yield related traits in rice (*Oryza sativa* L.). *Sabrao j. breed. genet.* 47 (4) 467-474.

Bhatti, S., Pandey, D.P., and Singh, D. 2015. Combining ability and heterosis for yield and its component traits in rice [*Oryza sativa* (L.)]. *Electronic Journal of Plant Breeding*. 6(1): 12-18.

Bisne, R., and Motiramani, N.K. 2005. Identification of maintainers and restorers using WA source cytoplasmic male sterile lines in rice. *International Rice Research Notes.* 30 (1): 14-15.

Boopathi, N.M., Swapnashri, G., Kavitha, P., Sathish, S., Nithya, R., Ratnam, W., and Kumar, A. 2013. Evaluation and Bulked Segregant Analysis of Major Yield QTL *qtl12.1* Introgressed into Indigenous Elite Line for Low Water Availability under Water Stress. *Rice Science*. 20(1): 25−30

Burton, G.W., and De vane, E.H. 1953. Estimating heritability in tall Fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal*. 45: 478-481.

Cahndiraka, R., and Thiyagarajan, K. 2010. Heterotic expression of two line hybrid in rice (*Oryza sativa* L.). *Electronic J. Plant Breeding*. 1(4): 1070-1078.

Cao, L.Y., and Zhan, X.D. 2014. Chinese experiences in breeding three line, two-line and super hybrid rice. *In*: Yan W G, Bao J S. Rice: Germplasm, Genetics and Improvement. Rijeka, Croatia: *InTech*. 279–308.

Chakraborty, R., Chakraborty, S., Dutta, B.K., and Paul, S.B., 2009, Combining ability analysis for yield and yield components in bold grained rice (*Oryza sativa* L.) of Assam. *Oryza*. 3(4): 281-283.

Chakravorty, A., and Ghosh, P. D. 2012. Genetic divergence in landraces of rice (*O. sativa* L.) of West Bengal, India. *Journal of Crop and Wee*. 8(2):23-28.

Chaturvedi, H.P., Talukdar, P., and Changkija, S. 2010. Combining Ability Analysis for Yield and Yield Components in Rice (*Oryza Sativa* L.) *International Journal of Agriculture, Environment and Biotechnology* . 3(3).

Chaudhary, R.C. 1984. Introduction to Plant Breeding. New Delhi, Oxford and IBH.

Chauhan, D.A. Patel, R.R., and Bhimani, G.J. 2015. Heterosis on grain yield and qualitative traits in hybrid rice (*oryza sativa l.).The Ecoscan*. 9(1&2): 499-502.

Dalvi, V.V., Patel, D.U. 2009. Combining ability analysis for yield and its components in hybrid rice. *Madras Agric. J.* 93:17-25.

Dar, S.H. 2013. Heterosis and Combining Ability Analysis for Yield and Component Traits in Rice (*Oryza sativa* L.). PhD thesis submitted to Sher e-Kashmir University of Agricultural Sciences and Technology of Kashmir

Das, P., Mukherjee, B., Santra, C.K., Mukhopadhyay, S. and Dasgupta, T. 2013. Evaluation of Genotypes for fertility restoring and maintaining behaviors in Rice (*Oryza Sativa L.*). *International Journal of Scientific & Technology Research* .2 (11): 228-232).

Debnath, K., Das, B., Sikder, S., and Sarkar, K.K. 2015. Assessment of Genetic Variability Character Association and Path Coefficient for Yield and its Component Characters in Rice. *The ecoscan.* 9(1&2): 455-459.

Dellaporta, R.P., Wood, J., and Hicks, J. D. 1983. A plant DNA mini preparation: version II. *Plant Molecular Biology Reports.* 1:19–21.

Dhurai, S.Y., Bhati, P.K. and Saroj, S.K. 2014. Studies on genetic variability for yield and quality characters in rice (Oryza sativa L.) under integrated fertilizer management. The Bioscan. 9(2): 745-748

Durai, A.A., and Nadarajan, N. 2007. New restorers for WA-cytoplasmic genic male sterile (CMS) lines in rice (*Oryza sativa* L.). *Agricultural Science Digest.* 27 (3): 170-173.

Dwivedi, D.K., and Pandey, M.P. 2012. Gene Action and Heterosis for Yield and Associated Traits in Indica and Tropical Japonica Crosses of Rice (*Oryza sativa* L.) Involving Wide Compatibility Gene(s). *International Journal of Plant Breeding and Genetics.* 6: 140-150.

El-Namaky, R., Sedeek, S., Dea Moukoumbi, Y., Ortiz, R., and Manneh, B. 2016. Microsatellite-Aided Screening for Fertility Restorer Genes (*Rf*) Facilitates Hybrid Improvement. *Rice Science.* 23(3): 1-6.

Faiz, F.A., Sabar, M., Awan, T.H., Ijaz, M., and Manzoor, Z. 2006, Heterosis and combining ability analysis in basmati rice hybrids. *J. Anim. Pl. Sci.* 16(1-2): 56-59.

Fiyaz, A., Ramya, R., Chikkalingaiah, K.T., Ajay, B.C., Gireesh, C., and Kulkarni, R.S. 2011.Geneticvariability, correlation and path coefficient

analysis studies in rice (*Oryza sativa* L.) under alkaline soil condition. *Electronic Journal of Plant Breeding*. 2(4):531-537.

Fujii, S., and Toriyama, K. 2005, Molecular mapping of the fertility restorer gene for ms-CW-type cytoplasmic male sterility of rice. *Theor. Applied Genet.* 111(4): 696-701.

Ganesen, K.N., and Rangaswamy, M. 1997. Heterosis in rice hybrids bred with wild abortive source of CMS lines. *Crop Res.* 13: 603-607.

Gao, M. 1981. A Preliminary analysis of the genotype of hybrid shen rice with wild rice cytoplasm. *Acta Genetica Sinica*. 8: 66-74.

Ghara, A.G., Nematzadeh, G., Bagheri, N., Ebrahimi. A., and Oladi, M. 2012. Molecular and cytological evaluation of male sterile and restorer lines in hybrids rice. *International Research Journal of Applied and Basic Sciences*. 3 (1): 183-189.

Ghosh, S.C., and Sharma, D. 2012. Genetic parameters of agro morpho-physiological traits in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*. 3(1):711-714.

Govindaraj, K., and Virmani, S.S. 1988. Genetics of Fertility Restoration of WA Type Cytoplasmic Male Sterility in Rice. *Crop Sci.*, 28: 787–792.

Gupta, S. K., 2000. Plant Breeding: Theory and Techniques. Published by Updesh Purohit for Agrobios, India.

Hanson, M.R., and Bentolila. S. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell*. 16:154–169.

Hariprasana, K., Zaman, F.U., Singh, A.K., and Tomar, S.M.S. 2006. Analysis of combining ability status among parents and hybrids in rice. *Indian Journal of Genetics*. 66: 28-30.

Hariprasanna, K., Zaman, F.U., and Singh, A.K. 2005. Identification of versatile fertility restorer genotypes for diverse CMS lines of rice (*Oryza sativa* L.). *Oryza*. 42 (1): 20-26.

Hayes, K., Immer, I.R., and Badsmith, D.C. 1955. "Methods in Plant Breeding", McGraw Hill Company Inc., New York. Inc. New York. 468-471p.

Hayman, B.I. 1954. The theory and analysis of diallel crosses. *Genetics.* 39: 789-809.

Hossain, S., Singh, A.K., and Fasih-uz-Zaman. 2010. Genetics of fertility restoration of 'WA'-based cytoplasmic male sterility system in rice (*Oryza sativa*) using indica/japonica derivative restorers. *Science Asia.* 36: 94–99.

Huang, C.S., Tseng, T.H., Chen, C.G., and Chern, C.G. 1987. Genetic analysis of fertility restoration in cytoplasmically male sterile indica rice. *J Agr Res.* 36:137–50.

Huang, J., Hu, J., Xu, X., Li, S., Yi, P., Yang, D., Ren, F., Liu, X., and Zhu, Y. 2003. Fine mapping of the nuclear fertility restorer gene for HL cytoplasmic male sterility in rice. *Academia Sinica Taipei.* 44:285–289.

Ingale, B.V., Waghmode, B.D., and Shodawadekar, S.S. 2008. Identification of restorers and maintainers for different CMS lines of rice. *Madras Agriculture Journal.* 95: 266-277.

Islam, A., Mian M.A.K., Rasul, G., Bashar, K., and Johora, F.T. 2015. Development of Component Lines (CMS, Maintainer and Restorer lines) and their Maintenance Using Diversed Cytosources of Rice. *J Rice Res.* 3 (3):1-5.

Jagadeesan, S. ,and Ganesan, J. 2006, Combining ability in rice (*Oryza sativa* L.). *Indian J. Agric. Res.* 40 (2): 139 – 142

Jaiswal, H.K., and Sharma, P. 2009. Identification of basmati maintainers and restorers of WA cytoplasmic male sterile lines in rice. *International Rice Research Note.*3: 0117-4185.

Jayasudha, S., and Sharma D. 2010. Identification of restorers and maintainers for CMS lines of rice (Oryza sativa. L) under shallow low land condition. *Elec. J. Plant Breed.*1:311–314

Jing, R., Li, X., Yi, P., and Zhu, Y. 2001. Mapping Fertility Restoring Genes of Rice WA Cytoplasmic Male Sterility Using SSLP Markers. *Bot. Bull. Acad. Sin.* 42: 167–171.

Johnson, H.W., Robinson , H.F., and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soybean. *Agronomy Journal.* 47(7): 314-318.

Jones, J.W. 1926. Hybrid vigor in rice. *Journal of American Society.* 18: 423-428.

Joshi, B.K. 2000. Heterosis for yield and yield components in rice. *Nepal Agric. Res. J.* 4 & 5:6-12.

Joshi, B.K. 2001. Heterosis for Yield and Yield Components in Rice. *Nepal Agric. Res. J.* 5: 6-12.

Kemothorne, Q. 1957. An introduction to genetic statistics. John Willey & Sons. Lowa State College Press, Ames. pp 14-48.

Ketan, R., and Sarkar, G. 2014. Studies on variability, heritability, genetic advance and path analysis in some indigenous *Aman* rice (*Oryza sativa* L.). *Journal of Crop and Weed.* 10(2):308-315.

Khan, A.S., Imran, M., and Ashfaq, M. 2009. Estimation of genetic variability and correlation for grain yield components in rice. *American-Eurasian J. Agric. & Environ. Sci.* 6 (5): 585-590.

Khan, M.A., Malik, S., Singh, S. 2012. Identification of maintainers and restorers for development of potential rice (*Oryza sativa L.*) hybrids for tarai region. *Vegetos: An International Journal of Plant Research.* Vol. 25(1): 48-51.

Khatibani, L.B., Ali, A., Babaiyan, N.A., Ebadi, A.A., Tabar, S.K.K., and Zandi,P. 2009. Mapping of fertility restorer genes (*Rf)* using microsatellite markers (SSR) in rice. *Iranian J. Field Crop Sci.* 40(1): 11-18.

Khush, G.S. 1974. Disease and insect resistance in rice. In: RC King (ed) *Handbook of Genetics*, 2: Plenum Press, New York and London, pp 31-58.

Khush, G.S. 2005. What it will take to feed 2.0 billion rice consumers in 2030.*Plant Mol.Biol.* 59: 1-6.

Khush, G.S. 2007. Rice breeding for 21st century. *Book on Science, Technology and Trade for Peace & Prosperity.* pp. 64-65.

Kiani, G. 2015. Validation of SSR Markers Linked to Restoring Fertility (*Rf*) Genes and Genotyping of Rice Lines at *Rf* Loci. *J. Agr. Sci. Tech.* 17: 1931-1938.

Komori, T., Yamamoto, T., Takemori, N., Kashihara, M., Matsushima, H., and Nitta, N. 2003. Fine genetic mapping of the nuclear gene, *Rf*- 1, that restores the BT-type male sterility in rice (*Oryza sativa L.*) by PCR-based markers. *Euphytica.* 129: 241–247.

Kumar, A., Singh, N. K., and Sharma, V. K. 2002. Restorers and maintainers for cytoplasmic male sterile (CMS) lines in rice. *Journal of Applied Biology.* 12(1/2): 17-19.

Kumar, A., Singh, N.K., and Chaudhory, V. K. 2004. Line x Tester analysis for grain yield and related characters in rice. *Madras Agric. J.* 91(4-6): 211-214.

Kumar, A., Singh, N.K., and Sharma, V.K. 2006. Combining ability analysis for identifying elite parents for heterotic rice hybrids. *Oryza.* 43(2): 82-86.

Kumar, A., Singh, S., and Singh, S.P. 2012. Heterosis for Yield and Yield Components in Basmati Rice. *Asian Journal of Agricultural Research.* 6(1): 21-29.

Kumar, M., Verma, O.P., Kumar, K., and Verma, G.P. 2010. Fertility restoration of rice genotypes for CMS lines under saline-alkali situation. *Oryza.* 47(4): 265-268.

Kumar, S, Dwivedi S.K., Singh, S.S., Jha, S.K., Lekshmy, S., Elanchezhian, R., Singh, O.N., Bhatt, B.P. 2014. Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. *SABRAO J. Breed. Genet.* 46 (2): 217-230.

Kumar, S., and Chakarabarti, S.N. 1983. Genetic and cytogenetic analysis of spikelet sterility in indica japonica crosses in *Oryza sativa* L., *Indian J. Genet. Plant breed.* 60: 441-50.

Kumar, S., Singh, H.B., Sharma, J.K. 2007. Combining ability analysis for grain yield and other associated traits in rice. *Oryza.* 44(2): 108-114.

Kumar, V.P., and Reddy, C.V.C.M. 2011. Study the fertility restoration of elite indica tropical japonica derivatives for WA based indica CMS lines in rice. *Plant Archives.* 11(1): 331-333.

Lakshmi, B.V., Kumar, M.V., Srinivas, B., and Seetharamaiah, K.V. 2008. Line × tester analysis of combining ability analysis in rice (*Oryza sativa* L.). *Ressearch on Crops.* **9**(3): 640-643.

Lakshmi, M.V. 2013. Genetic divergence analysis in rive (*Oryza sativa L.).* M.Sc theisis submitted to Acharya N. G. Ranga Agricultural University, Hydedrabad.

Lakshmi, M.V., Suneetha, Y., Yugandhar, G., and Lakshmi, N. V. 2014. Correlation Studies in Rice (*Oryza sativa* L.). *International Journal of Genetic Engineering and Biotechnology.* 5 (2):121-126.

Leenakumari, S., Valarmathi, G., Tessy J., Knakamony, M.T., and Nayar, N. K. 1998. Rice Varieties of Kerala as restorers and maintainers for wild abortive cytoplasmic rice. *Theoretical and Applied Genetics.* 83:994–996.

Li, L.J., Zhou, H.P., Zhan, X.D., Zhuang, J.Y., Cheng, S.H., and Cao, L.Y. 2007. Mapping of rice fertility-restoring gene for Yinshui cytoplasmic male sterility in a restorer line R68. *Chin J Rice Sci.* 21(5): 547–549.

Lin, S.C., and Yuan, L. P. 1980. Hybrid rice breeding in China. International Rice Research Institute, p35-51.

Lingaiah, N., Venkanna, V., and Cheralu, C. 2014. Genetic Variability Analysis in Rice (*Oryza sativa* L.). *Int. J. Pure App. Biosci.* 2(5): 203-204

Liu, X.Q., Xu , X., Tan, Y.P., Li , S.Q., Hu, J., Hung, J.Y., Yang, D.C., Li, Y.S. and Zhu ,Y.G. 2004. Inheritance and molecular mapping of two fertility-restoring loci for Honglian gametophytic cytoplasmic male sterility in rice (*Oryza sativa L.*). *Mol. Gen. Genomics.* 271: 586–594.

Lush, J.L. 1949. Heritability of quantitative characters for farm animals. *Proc. 8th Int. Cong. Of Genetics. Suppl. Vol. Hereditas.* 356-375.

Mackill, D.J., and Ni, J. 2001. Molecular mapping and marker assisted selection for major-gene traits in rice. In: Khush GS, Brar DS, Hardy B, eds. Rice genetics IV. Los Baños (Philippines). *Int. Rice Res. Inst.* 137-15

Mahalanobis, P.C. 1963. On the Genaralised Distance In Science, India. 2:49 55.

Malarvizhi, D., Thiyagarajan, K., Manonmani, S., and Deepa Sankar, P. 2003. Fertility restoration behaviour of promising cms lines in rice (*Oryza sativa* L.). *Indian J. Agric. Res.* 37 (4): 259-263.

Mehmood, T., Shabbir, G., Sarafraz, M., Sadiq, M., Bhatti, M.K., Mehdi, S.M. Jamil, M., and Hasan, G. 2002. Combining ability studies in rice (*Oryza sativa* L.) under salinized soil conditions. *Asian Journal of Plant Science.* 1: 88-90.

Mirarab, M., Ahmadikhah, A. and Pahlavani, M.H. 2011. Study on combining ability, heterosis and genetic parameters of yield traits in rice. *Afr. J. Biotechnol.* 10(59):12512-12519.

Mishra, B., 2003. Rice research in India- major achievements and future thrusts. Presented in training on "*Advances in hybrid rice technology*" Directorate of Rice Research, Hyderabad, pp 1-15.

Mishra, G.P., R. Singh, R.K., Mohapatra, T., Singh, A.K., Prabhu, K.V., Zaman, U.V., and Sharma, R.K. 2003. Molecular mapping of a gene for fertility restoration of wild abortive (WA) cytoplasmic male sterility using a basmati rice restorer line. *Journal of Plant Biochemistry and Biotechnology.* 12: 37- 42.

Mishra, G.P. 2001. Inheritance of fertility restoration and identification of molecular markers for fertility restorer gene(s) in Basmati rice. MSc thesis submitted to IARI, New Delhi.

Moukoumbi, Y.D., Ortiz, R., Manneh, B. 2016. Microsatellite-aided screening for fertility restorer genes (Rf) facilitates hybrid improvement. Rice Science. 23(3).

Munisonnappa, S., and Vidyachandra, B. 2007. Standard heterosis in newly developed rice hybrids. *Karnataka J. Agric. Sci.* 20(2): 379-380.

Nematzadeh, A., and Kiani, G. 2010. Genetic analysis of fertility restoration genes for WA type cytoplasmic male sterility in Iranian restorer rice line DN-33-18. *Afr J Biotech.* 9: 6273–6277.

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., Kumar, A., Chaurasia, A. K., and Rai, P. K. 2014. Assessment of Genetic Parameters for Yield and Yield Components in Hybrid Rice and Parents. *J Rice Res*. 2(1): 117-119.

Pandey, R., and Tripathi, R.S. 2006. Heterosis breeding in hybrid rice. *Oryza*. 43(2): 88-93.

Panse, V.G., and Sukhatme, P.V. 1967. "Statistical methods for agricultural research workers", ICAR, New Delhi

Panwar, L.L. 2005. Line x Tester analysis of combining ability in rice. *Indian Journal of Genetics*. 65:51-52.

Patil, S.R., Vashi, R.D., Patil, P.P., and Shinde, D. A. 2011. Combining ability in rice (*Oryza Sativa* L.). *Plant Archives*. 11(1):439-442.

Pearson, K. 1900. "On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling". *Philosophical Magazine, Serie*. 5. 50(302): 157–175.

Perera, U.I.P., Bentotal, A.P., Ratnasekara, D., and Senanayake, S.G.J.N. 2013. Heterosis in F_1 generations of two Indica rice crosses for growth and yield characteristics. *The Journal of Agricultural Sciences*. 8 (3):136-141.

Pradhan, S.B., Ratho, S.N., and Jachuck, P.J. 1992. Restorers and maintainers for five CMS lines. *Int. Rice Res. Newsl*. 17(5): 8.

Pradhan, S.K., Bose, L.K., and Mani, S.C. 2006. Basmati type restorers and mainatiners for two cytosterile lines of rice (*Oryza sativa* L.). *Indian J. Genet*. 66(4): 335-336.

Prasad, S., Krishna, G. R., Rao, K.V.S., Padmaja, L.V., and Chaitanya, U. 2013. Diversity analysis of indica rice *(oryza sativa* l.) genotypes against low and high temperature stress. *Journal of Agriculture and Veterinary Science*. 4 (6): 34-39.

Rahimi, M., Rabiei, B., Samizadeh, H., and Kafi Ghasemi, A. 2010. Combining ability and heterosis in rice (*Oryza sativa* L.) cultivars. *J. Agr. Sci. Tech.* 12: 223-231.

Rai, S., Suresh, B.G., Rai, P.K., Lavanya, G.R., Kumar, R., and Sandhya. 2014. Genetic Variability, Correlation and Path Coefficient Studies for Grain Yield and Other Yield Attributing Traits in Rice *(Oryza Sativa* L.). *International Journal of Life Sciences Research.* 2 (4): 229-234.

Rajeswari, S., Robin, S., Chandirakala, R., Premalatha, N and Muthuramu, S. 2010. Genetic analysis and character association of quality in rice. *Oryza.* 47 (2): 86-90.

Rajput, A.S., Suresh B.G., and Bhatti, M. 2014. Genetic diversity of irrigated medium duration of rice genotypes suited for eastern plain zone of U.P. *Journal of Agriculture and Veterinary Science.*7 (3): 42-45

Raju, C.D., Kumar, S.S., Raju, C.S., and Srijan, A. 2014. Combining ability Studies in the Selected Parents and Hybrids in Rice (*Oryza sativa* .L). *Int. J. Pure App. Biosci.* 2 (4): 271-279.

Ramanjaneyulu, A.V., Gouri shankar, V., Neelima, T.L. and Shashibhusahn, D. 2014. Genetic analysis of rice (*Oryza sativa* L.) genotypes under aerobic conditions on alfisols. *SABRAO Journal of Breeding and Genetics.* 46 (1):99-111.

Rao, C.R. 1952. *Advanced statistical methods in biometrical research.* John Wiley and Sons Inc., New Yark, pp. 236-272.

Rao, Y.S. 1988, Cytohistology of cytoplasmic male sterile lines in hybrid rice. In: Smith WH, Bostian LR, Cervantes EP (eds) Hybrid rice. International Rice Research Institute, Manila, 115–128p.

Rashid, M., Cheema. A.A., and Ashraf, M. 2007. Line × Tester analysis in basmati rice. *Pak. J. Bot.* 39(6): 2035-2042.

Rahimi, M., Rabiei, B., Samizadeh, H., and Ghasemi, A.H. 2010. Combining ability and heterosis in rice (*Oryza sativa* L.). *J.Agri.Sci.Tech.* 12: 223-231.

Ratna, M., Begum, S., Husna, A., Dey, S.R., and Hossain, M.S. 2015. Correlation and path coefficients analyses in basmati RICE. *Bangladesh J. Agril. Res.* 40(1): 153-161.

Ratnakar, P., Lal, S.S., and Upadhyay, D.K. 2009. Exploitation of heterosis in rice (*Oryza sativa* L.). *Int. J. Plant Sci.* 4(1): 20-23.

Reddy, J.N. 2002. Combining ability for yield and its components in low land rice (*Oryza sativa* L.). *Indian J. Genet.* 62(3): 251-252.

Richharia, R.H. 1962. Clonal propagation as a practical means of exploiting hybrid vigor in rice. *Nature.* 194: 598

Rongbai, L., Lishu, L., Sumei, W., Yanping, W., Yingzhi, C., Delang, B., Lang, Y., Fengkuan, H., Weili, L., and Xiangjun, Z. 2005. The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (*Oryza rufipogon* Griff.). *Mol. Plant Breed.* 4: 365–371.

Rosamma, C.A., and Vijayakumar, N.K. 2005. Maintainers and restorers for CMS lines of rice. *Journal of Tropical Agriculture.* 43 (1-2): 75-77.

Rosamma, C.A., and Vijaykumar, N.K. 2005. Heterosis and combining ability in rice (*Oryza sativa* L.) hybrids development for Kerala State. *Indian Journal of Genetics.* 65: 119-120.

Rosamma. S., Elsy, C.R., Balachandran, P.V., and Francies, R.M. 2003.Technical Bulletin, Pattambi Rice Varieties. RARS, Pattambi, Kerala Agricultural University.

Roy, B., and Mandal, A.B. 2001. Combining ability of some quantitative traits in rice. *Ind. J. Genet.* 61(2): 162-164.

Roy, C. 2013. Genetic study on CMS lines, fertility restoration, heterosis and combining ability in rice (oryza sativa l.). P.hD Thesis submitted to the G.B. Pant University of Agriculture & Technology, Pantnagar-263 145 (U.S. Nagar), Uttarakhand, India

Runchun, J., Yuqing, H., Qingyang, H., and Yingguo, Z. 2000. Analysis of the fertility restorer gene in the wild-abortive (WA) type cytoplasmic male sterility (CMS) system with the ISSR and SSLP markers. *Sci. Agri. Sini.,* 33(2): 10-15.

Saidaiah, P., Sudheer Kumar, S., and Ramesha, M.S. 2010. Combining ability studies for development of new hybrids in rice over environments. *J. Agri. Sci.* 2(2):225-233.

Saleem, M.Y., Mirza, J.I., and Haq, M.A. 2010. Combining ability analysis of some morphological traits in basmati rice. Pak. J. Bot., 42 (5): 3113-3123.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular Cloning: a laboratory manual. 2nd ed. N.Y., *Cold Spring Harbor Laboratory Press. 1659p.*

Sampath, S., and Mohanty, H.K. 1954. Cytology of semi-sterile rice hybrid. *Curr. Sci.* 23: 182-183.

Sao, A., and Motiramani, N. K. 2015. Combining ability analysis for yield and yield contributing traits using cytoplasmic male sterility fertility system restoration system in rice hybrids. *Jordan J. Agril. Sc.* 2(1): 29-34.

Sarial, A.K.; Singh, V.P., and Ram, K. 2006. Heterotic potential of basmati fertility restorers for grain yield and its components in rice (*Oryza sativa* L.). *Indian J. Genet.* 66(4): 293-302.

Sarker, C.K.G., Zaman, F.U., and Singh, A.K. 2002. Genetics of fertility restoration of 'WA' based cytoplasmic male sterility system in rice (Oryza sativa L.) using basmati restorer lines. *Indian J Genet Plant Breed.* 62:305–8.

Sarker, U., Biswas, P.S., Prasad, B., and Mian, M.A. 2002. Heterosis and genetic analysis in rice hybrids. *Pak. J. Bio. Sci.* 5(1):1-5.

Sattari, M., Kathiresan, A., Gregorio, G.B., and Virmani, S.S. 2008. Comparative genetic analysis and molecular mapping of fertility restoration genes for WA, Dissi and Gambiaca cytoplasmic male sterility systems in rice. *Euphytica.* 160:305–315.

Sattari, M., Kathiresan, A., Gregorio, G.B., Hernandez, J.E., Nas, T.M., and Virmani, S.S. 2007. Development and use of a two-gene marker-aided selection system for fertility restorer genes in rice. *Euphytica.* 153:35–42.

Seesang, J., Sripichitt, P., and Sreewongchai, T. 2014. Heterosis and inheritance of fertility-restorer genes in rice. *Sci. Asia.* 40: 48–52.

Senguttuvel, P., and Bapu, J.R.K. 2007. Combining ability analysis for yield and its components in rice. *Advance in Plant Science.* 20: 59-62.

Selvaraj, C.I., Nagarajan, P., Thiyagarjan, K., Bharathim, M., and Rabindran, R. 2011. Studies on heterosis and combining ability of well known blast resistant rice genotypes with high yielding varieties of rice (*Oryza sativa* L.). *Int. J. Plant Breed. Genet.* 5(2):111-129.

Shah, G., Sasidharan, N., Chakraborty, S., Trivedi, R., Ravikiran, R., and Deepti Davla, D. 2012. Genetic diversity and molecular analysis for fertility restorer genes in Rice (*Oryza sativa* L.) for wild abortive (*WA*) cytoplasm using microsatellite markers. *Journal of Agricultural Technology*, 8(1): 261-271.

Sharma, P.R., Khoyumthem, P., Singh, N.B., and Noren, K.S. 2005. Combining ability studies for grain yield and its component characters in rice (*Oryza sativa* L.). *Oryza.* 65(4): 287-289.

Sharma, R., and Malik, J.P.S. 2008. Line × tester studies on two line hybrids in rice (*Oryza sativa* L.). *Pantnagar J. Res.* 6(2): 293-95.

Sheeba, N.K., Viraktamath, B.C., Sivaramakrishnan, S., Gangashetti, M.G., Pawan, K., and Sundaram, R.M. 2009. Validation of molecular markers linked to fertility restorer gene(s) for WA-CMS lines of rice. *Euphytica.* 167: 217–227.

Shen, Y.W., Guan, Z.Q., Lu, J., Zhuang, J.Y., Zheng, K.L., Gao, M.W., Wang, X.M. 1998. Linkage analysis of a fertility restoring mutant generated from CMS rice. *Theor Appl Genetics.* 97:261– 266.

Shinjyo, C. 1975. Genetical studies of cytoplasmic male sterility and fertility restoration in rice (Oryza sativa L.). Ryukyus Univ., College Agric. Sci. Report, 22: 1-55p.

Shinjyo, C. and Omura, T. 1966. Cytoplasmic male sterility and fertility restoration in rice. *Sci. Bull. Coll. Agric. Univ. Ryukus.* 22 : 1-57.

Shivaprasad, G., Radha Krishna, K.V., Subba Rao, L.V., Padmaja, D., and Chaitanya, U. 2013. Diversity analysis of indica rice *(oryza sativa* l.)

genotypes against low and high temperature stress. *Journal of Agriculture and Veterinary Science.*4 (6): 34-39.

Shivani, D., Viraktamath, B.C., and Shobha Rani, N. 2009. Combining ability for grain quality characters in indica/indica hybrids of rice. *Oryza.* 46(2):152-155.

Shukla, S.K., and Pandey, M.P. 2008. Combinimg ability and heterosis over environments for yield and yield components in two line hybrids involving thermosensitive genic male sterile lines in rice (*Oryza sativa* L.). *Plant Breeding.* 127(1): 28-32.

Shull, G.H. 1952. Beginning of the heterosis concept. *In J.W.* Gowen Heterosis.

Singh S. K. 2016. Government of India, "Grain and Feed Annual. Grain report", p17.

Singh, A.K., Mahapatra, T., Prabhu, K.V., Singh, V.P., Zaman, F.U., Mishra, G.P., Nanada kumar, N., Joseph, M., Gopalakrishnan, S., Aparajita. G., Tyagi, N.K., Prakash, P., Sharma, R.K., Shab, U.S., and Singh, S.K. 2005. Application of molecular markers in rice breeding: progress at IARI In; Advances in marker assisted selection workshop. *Trainee's manual*, hand outs and references

Singh, A.K., Revathi, P., Pavani, M., Sundaram, R.M., Senguttuvel, P.K.B., Hari Prasad, A.S., Neeraja, C.N., Sravan Raju, N., Kotewara, Rao, P., Suryendra, P.J., Jayaramulu, K., and Viraktamath, B.C. 2014. Molecular Screening for Fertility Restorer Genes *Rf3* and *Rf4* of WA –CMS and Evaluation of F1 hybrids in Rice (*O. sativa* L.). *Journal of Rice Research.* 7: 1-2.

Singh, N.K., and Kumar, A. 2004. Combining ability analysis to identify suitable parents for heterotic rice hybrid breeding. *International Rice Research Notes.* 29: 21-22.

Singh R. K. and Chaudhary, B.D. 1979. Biometrical methods in quantitative genetic analysis. Kalyani publication, New Delhi, 120 p.

Singh, R.K., and Chaudary, B.D. 1985. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Pulishers, New Delhi, pp. 205-214.

Singh, R.K., and Chaudhary, B.D. 1977. Biometrical methods in quantitative genetic analysis, Kalyani Publishers, New Delhi pp. 57-58.

Singh, R.V., Verma, O.P., Dwivedi, J.L., and Singh R.K. 2006. Heterosis studies in rice hybrids using CMS-System. *Oryza*. 43(2): 154-56.

Sivasubramanian, S., and Madhavamenon, P. 1973. Combing ability in rice. *Madras Agricultural Journal*. 60: 419-421.

Soni, S., and Sharma, D. 2011. Study on heterosis for grain yield and its component traits for developing new plant type hybrids in rice (*Oryza sativa* L.). *Electronic J. Plant Breeding*. 2 (4): 543-548.

Sritama, K., Biswajit, P., and Sabyasachi, K. 2015. Study of Genetic Parameters and Character Association of Different Agro- Morphological Characters in some Paddy Genotypes for Saline and Non- Saline Belts of West Bengal, India. *Res. J. Agriculture and Forestry Sci.* 3 (5): 6-15.

Subbaiah, P.V., Sekhar, M.R., Reddy, K.H.P., and Reddy, N.P.E. 2011.Variability and genetic parameters for grain yield and its components and kernel quality attriburtes in cms based rice hybrids (oryza sativa l) *International Journal of Applied Biology and Pharmaceutical Technology*. 3(2): 603-609.

Sunil, C.I. 2006. Line x tester analysis for earliness yield and its components in rice (*Oryza sativa* L.) for the cauvery delta zone. *Indian J. Agric. Res.* 40(4): 255-261.

Suresh, R., and Anbuselvam, Y. 2006. Combining ability analysis for yield and its component traits in rice (*Oryza sativa* L.). *Research on crops* **7**: 709-713.

Suresh, P.B., Srikanth, B., Kishore, V.H., Rao, I.S., Vemireddy, L.R., Dharika, N., Sundaram, R.M., Ramesha, M.S., Rao, K.R.S.S., Viraktamath, B.C., and Neeraja, C.N. 2012. Fine mapping of *Rf3* and *Rf4* fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. *Euphytica*. 187: 421-435.

Swamy, M.H., Gururaja Rao, M.R., and Vidyachandra B. 2003. Studies on combining ability in rice hybrids involving new CMS lines.*Karnataka J. Agril. Sci.* 16 (2): 228-233.

Tan, X.L., Vanavichit, A., Amornsilpa. S., and Trangoonrung, S. 2008. Mapping of rice *Rf* gene by bulked line analysis. *DNA RES.* 5: 15- 18.

Tao, D., Xu, P., Li, J., Hu, F., Yang, Y., Zhou, J., Tan, X., and Jones, M. P. 2004. Inheritance and mapping of male sterility restoration gene in upland japonica restorer lines. *Euphytica.* 138: 247–254.

Teng L.S., and Shen, Z.T. 1994. Inheritance of fertility restoration for cytoplasmic male sterility in rice. Newsletters/Rice-Genetics.1-3.

Thakare, I.S., Mehta, A.M., Patel J.S., and Takle, S.R. 2010. Combining ability analysis for yield and grain quality traits in rice hybrids. *Journal of Rice Research.* 3(1):1-5

Tiwari, D.K., Pandey, P., Giri, S.P., and Dwivedi, J.L. 2011. Heterosis studies for yield and its components in rice hybrids using cms system. *Asian. J. Plant. Sci.* 10(1):29-42.

Torres E.A., and Geraldi I.O. 2007. Partial diallel analysis of agronomic characters in rice (*Oryza sativa* L.). *Genetics and Molecular Biology.* 605-613.

Umadevi, M., Veerabadhiran, P., Manonmani, S., and Shanmugasundaram, P. 2010. Identification of potential maintainers and restorers using cytoplasmic male sterile lines in rice. *Electronic Journal of Plant Breeding.* 1(4): 948-952.

Umakanta,S.,, Biswas, P.S., Prasad, B., and Mian, M.A. 2002. Heterosis and genetic analysis in rice hybrids. *Pak. J. Bio. Sci.* 5(1):1-5.

Upadhyay, M.N., and Jaiswal, H.K. 2012. Restorers and maintainers of WA cytoplasmic male sterile lines in rice. *Int. Rice Res. Notes* 37: 1-4.

Vanaja, T., and Babu, L.C. 2004. Heterosis for yield and yield components in rice (*Oryza sativa* L.). J. *Tropical Agri.* 42 (1-2): 43-44.

Vanaja, T., Luckins, C.B., Radhakrishnan, V.V., and Pushkaran, K. 2003. Combining ability analysis for yield and yield components in rice varieties of diverse origin. *Journal of tropical agriculture*. 41: 7-15.

Vanisree, S., Anjali, K., Raju, C.D., Raju, C.S. and Sreedhar, M. 2013. Heritability, Variability and Association analysis in scented Rice. *Journal of biolofgical and scientific opinion*.1 (4): 347-352.

Vanisree, S., Raju, C.S., Reddy, P.N., Sreedhar, M., and Krishna, L. 2011. Heterosis and gene effects for grain yield and physiological traits in rice (Oryza sativa l.). *Journal of Research ANGRAU*. 39(4):1-5.

Veeresha, B.A., Hanamaratti, N.G., and Salimath, P.M. 2015. Heterosis and Combining Ability Studies for yield and Productivity Traits in Rice: A Review. *International Journal of Current Agricultural Research*. 4(5): 120-126

Venkatesan, M., Anbuselvam, Y., Murugan, S., and Palaniraja, K. 2008. Heterosis for yield, its components and grain traits in rice (*Oryza sativa* L.). *Oryza*. 45(1): 76-78.

Venkateshwaralu, S., and Singh, R. B. 1982. Combining ability in Pigeanpea. *Indian J. Genet*. 42:11-14.

Verma, O.P., and Srivastava, H.K. 2004. Genetic component and combining ability analyses in relation to heterosis for yield and associated traits using three diverse rice-growing ecosystem. *Field Crops Research*. 88(2-3):91-102.

Vijayakumar, C.H.M., Ilyas Ahmed, M., Viraktamath, B.C., Ramesha, M.S., and Jauhar Ali, A. 2003. Genetic analysis and prediction of heterosis. In. Advances in Rice Genetics.,10p.

Viraktamath, B.C., Hari Prasad, A.S., Ramesha, M.S., and Ilyas, A.M. 2010. *Hybrid Rice in India*. Technical Bulletin No. 47, Directorate of Rice Research, Rajendranagar, Hyderabad, p43.

Virmani, S.S., Aquino, R.C., and Khush, G.S.1982. Heterosis breeding in rice (*Oryza sativa* L.). *Theor. Applied Genet*. 63:373-380.

Virmani, S.S., Mao, C.X. and Hardy, B. 2003. Hybrid rice for food security, poverty alleviation, and environmental protection. *Proceedings of the 4th International Symposium on Hybrid Rice*, Hanoi, Vietnam, pp407.

Virmani, S.S., Sun, Z.X., Mou, T.M., Jauhar, A.A., and Mao, C.X. 2003. Two-line Hybrid rice breeding manual. Los Baños (Philippines), International Rice Research Institute.

Virmani, S.S., Virakamath, B.C., Laral, C.L., Toledo, R.S., Lopez, M.T. and Manalo, J.O. 1997. Hybrid Rice Breeding Manual. Manila: *International Rice Research Notes*, pp151.

Waghmode, B.D., and Mehta, H. D. 2011. Genetics of fertility restoration of diverse cytosterile sources in rice (*Oryza sativa* L.). *Indian J. Genet. Plant Breed.* 71: 1-8.

Waza, S.A. and Jaiswal, H. K. 2016. Identification of elite Grain quality Restorers and Maintainers for WA CMS Lines of Rice (*Oryza sativa* L.). *J. Breed. Genet.* 48 (2) 145-153

Wilks, S. S. 1932. Certain generalizations in the analysis of variance, Biometrika 24:471-494.

Wright, S. 1921. Con-elation and causation. *Journal of Agricultural Research*. 20: 557-585.

Yao, F.Y., Xu, C.G., Yu, S.B., Li, J.X., Gao, Y.J., Li, X.H., and Zhang, Q.F. 1997. Mapping and Genetic Analysis of Two *Fertility Restorer* Loci in the Wild-abortive Cytoplasmic Male Sterility system of Rice (*Oryza sativa* L). *Euphytica.* 98: 183–187.

Yashitola, J., Thirumurugan, T., Sundaram, R.M., Naseerullah, M.K., Ramesha M.S., Sarma, N.P., and Sonti, R.V. 2002. Hybrids using microsatellite and STS markers. *Crop Sci.* 42:1369–1373.

Yuan, I.P., and Fu, X.Q. 1995. Investigation on the later generation of autotriploid Rice plants. *Science Bull. Fac. Agric*. Kyushu University. 11: 182-216.

Yuan, L.P. 1992. Development and prospect of hybrid rice breeding. *In* C.B. You and Z.L. Chen (eds.), Agricultural Biotechnology. Proc. Asian-Pacific

Conf. Agric. Biotechnol., China Sciences and Technology Press, Beijing, pp97-105.

Yuan, L.P. 1994. Increasing yield potential in rice by exploitation of heterosis. In: "Hybrid rice technology". IRRI, Manila, 1–6.

Yuan, L.P., and Fu, X.Q. 1995. Technology of hybrid rice production, *Rome FAO*, 84.

Zhang, G., Bharaj, T. S., Lu, Y., Virmani, S. S. and Huang, N. 1997. Mapping of the *Rf-3 Nuclear Fertility Restoring* Gene for WA Cytoplasmic Male Sterility in Rice Using RAPD and RFLP Markers. *Theor. Appl. Genet.* 94: 27–33.

Zhang, Q. Y., Liu, Y.G., and Mei, M.T. 2002. Molecular Mapping of the *Fertility* male sterile lines. *Int. Rice Res. Notes.* 22(2): 11-12.

Zhang, Q., B.Z., Shen, X.K., Dai, M.H., Mei, M.A., and Saghai, M.Z.B. 1994. Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. Proc Natl Acad Sci USA. 91: 8675–8679.

Zhu, X.D., Zhao, J., Yan, Q.Q., Zhou, Q.M., Peng, L.J., Xiao, M., Zen, X.D., and Peng, G.D. 2009. Study on combining ability of yield and related traits of hybrid rice with different types of cytoplasm. *Journal of Hunan Agricultural Unversity*. 35 (4): 352-356.

GENOTYPING OF *Rf* (RESTORING FERTILITY) LOCI OF RICE VARIETIES OF KERALA USING MOLECULAR MARKERS.

RAJIB DAS

(2013 - 21 - 107)

ABSTRACT

of the thesis submitted in partial fulfilment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2017

ABSTRACT

Hybrid rice technology aims to increase the yield potential of rice beyond the level of high yielding varieties by exploiting the phenomenon of hybrid vigour or heterosis. It is the only technology available now to break the yield plateau attained in rice. Commercial production of hybrid rice can be achieved through three line system of hybrid rice development. Identification of restorers and maintainers for the system is the initial step in hybrid rice breeding. So the present study entitled "Genotyping of *Rf* (Restoring fertility) loci of rice varieties of Kerala using molecular markers" was undertaken as an initial step for the development of hybrid rice for Kerala with the objectives to locate restorers and maintainers from Kerala rice varieties using molecular markers, validation of the restoration of fertility in cross with WA Cytoplasmic Male Sterile (CMS) lines, study of inheritance pattern of restorer gene and to locate heterotic combiners from the possible restorers and maintainers.

In the present study twenty one rice varieties were screened with 13 SSR markers linked to different R*f* genes i.e R*f3*, R*f4*, R*f5*, R*f6* and R*f7*. Three varieties had only one R*f* gene, seven varieties had two R*f* gene, four varieties had three R*f* gene. Rice varieties Remya, Manupriya and Swarnaprabha had four R*f* genes and PTB-9 had all the R*f* genes. For identification of maintainers and restorers from among the 21 rice varieties under study, these lines were crossed with 4 CMS lines (IR58025A, UPRI95-17A, CRMS31A and CRMS32A). Pollen and spikelet fertility of the hybrids recorded that Remya, Swarnaprabha, Manupriya, Varsha and Aiswarya were restorers for CMS line IR58025A, Remya, Jayathi, Annapoorna, Neeraja, Aiswarya and Pavizham were restorers for UPRI95-17A. Remya, Jayathi, Swarnaprabha, Kanakom and Neearaja were restorers for CRMS31A and Remya, Jayathi, Swarnaprabha, Annapoorna, Kanakom, Mattatriveni and Pavizham were restorers for CRMS32A. Rice variety Remya alone was found to be the restorer for all four CMS lines. Rice varieties Jyothi, Kanchana and Aruna were identified as maintainers for IR58025A. Only one variety Kanchana identified as maintainer for

UPRI95-17A. Bharathy, Jyothi, Kanchana and Aruna were identified as maintainers for CMS line CRMS31A, while Kanchana and Bharathy were identified as maintainers for CRMS32A. Kanchana alone was found to be the maintainer for all four CMS lines. In field validation Remya which had 4 R*f* genes (R*f3*, *Rf4*, R*f5* and R*f6*) was found to be a restorer for all the lines with WA Cytoplasm studied. All the restorers identified through field validation had either R*f3* or R*f4* gene which were reported as the major genes for fertility restoration.

Study of inheritance pattern of restorer gene was analysed in F_2 generation of the crosses between CMS lines and the restorers. It was found that in UPRI95-17A x Remya, UPRI95-17A x Jayathi, UPRI95-17A x Annapoorna, UPRI95-17A x Aiswarya and UPRI95-17A x Pavizham the restoration of fertility is governed by 2 dominant gene. Co-segregation of the molecular marker linked to R*f* loci and the trait of restoration of fertility in the segregating population was analysed through Bulk Segregant Analysis (BSA) and found co-segregation of marker RM1 with R*f3* gene and marker RM171 with R*f4* gene. This shows that the marker loci and fertility restoration genesR*f3* and R*f4* are tightly linked.

To assess the genetic parameters of the selected twenty one rice varieties they were grown in completely randomized block design with 2 replications in pots and observations were taken on 12 metric traits. The study revealed high heritability coupled with high genetic advance as per cent of mean for Pollen fertility, number of spikelets/panicle, number of filled grains/panicle, LB ratio, number of grains/panicle and grain yield/plant. Hence these traits are predominantly under the control of additive gene action and hence these characters can be improved by selection. Grain yield per plant recorded a significant positive correlation with total no of tillers, number of productive tillers per plant, panicle length, number spikelets/panicle, number of filled grains/panicle, number of filled grains per panicle. Divergence analysis grouped the rice varieties into eight clusters. Cluster II consisting of Mattatriveni and cluster VI consisting of Jayathi, Swarnaprabha, Kanakom and Aiswarya was the farthest.

243

The hybrid developed from 23 crosses between identified restorers and 4 CMS lines were evaluated for heterosis and identified promising hybrids were UPRI95-17A x Aiswarya, UPRI95-17A x Neeraja, UPRI95-17A x Remya and CRMS31A x Kanakom based on high mean grain yield per plant and high standard heterosis over standard check Uma.

In order to assess heterosis in different combination of the identified maintainers and restorers an L x T analysis was done with maintainers as the lines and restorers as the testers. Perusal of findings indicated that line Jyothi is a good general combiner as it recorded a high over all GCA status. The hybrid Aruna x Varsha was the best specific combiner followed by, Jyothi x Pavizham and Kanchana x Mattatriveni. Three crosses viz; Aruna x Varsha, Jyothi x Pavizham and Bharathy x Annapoorna registered high significant heterosis for grain yield per plant over mid parent, better parent and standard check Uma.

The present study could identify restorers and maintainers for 4 CMS lines from the Kerala rice varieties and also heterotic combination of restorers and maintainers. By reconstituting the identified maintainer with sterile cytoplasm of the CMS lines heterotic hybrids with grain qualities specific to Kerala can be developed. The superior hybrids obtained from identified restorers and CMS lines can be directly used for commercial release after yield trial.