IDENTIFICATION OF FERTILITY RESTORER GENE IN HYBRID RICE TECHNOLOGY THROUGH MARKER ASSISTED SELECTION

by

KOSANA ABHISHIKTH

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DECLARATION

I hereby declare that this thesis entitled **"Identification of fertility restorer gene in hybrid rice technology through marker assisted selection".** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellayani **Kosana Abhishikth**

Date: **(2009-11-150)**

CERTIFICATE

Certified that this thesis, entitled **"Identification of fertility restorer gene in hybrid rice technology through marker assisted selection".** is a record of research work done independently by **Mr. Kosana Abhishikth (2009-11-150)** under my guidance and supervision and that it has not part of previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Dr. V.G. Jayalekshmi
(Chairperson Advisory Committee)
Associate Professor
Dept:of plant Breeding & Genetics
College of Agriculture
Vellayani
Thiruvananthapuram-695522

CERTIFICATE

We the undersigned members of the advisory committee of **Mr. Kosana Abhishikth (2009-11-150)** a candidate for the degree of **Master of Science in Agriculture** agree that this thesis **"Identification of restorers for hybrid rice technology through molecular marker aided selection "** may be submitted by **Mr. Kosana Abhishikth (2009-11-150), in partial fulfillment** of the requirement for the degree.

Dr. V.L. Jayalekshmy
Associate Professor
Department of Plant Breeding & Genetics,
College of Agriculture, Vellayani,
Thiruvananthapuram.
(Chairman)

Dr. D.S Radha Devi

Professor and Head ,
Department of Plant Breeding & Genetics,
College of Agriculture, Vellayani
Thiruvananthapuram.
(Member)

Dr. Leena Kumari,

Professor ,
Rice Research Station,
College of Agriculture, Vellayani,
Thiruvananthapuram.
(Member)

Dr. Swapna Alex,
Associate Professor,
Department of Plant Biotechnology,
College of Agriculture, Vellayani,
Thiruvananthapuram.
(Member)

EXTERNAL EXAMINER

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List of abbreviations

ANOVA	Analysis of variance
BC_1F_1	First filial generation of first back cross
bp	Base pairs
BT	Bao tai
CB	Coimbattore
cm	Centi meter
CMS	cytoplasmic male sterile line
cm^2	Centimeter square
Co	Coimbattore
CRD	Completely randomised design
DNA	Deoxy ribonucleic acid
dNTPS	Deoxyribonucleotide triphosphate
Exx	Observed mean square for Erorrs
F_1	First filial generation
F_2	Second filial generation
F PRIMER	Forward primer
Fig	figure
g	Gram
GA	Genetic advance
GCV	Genotypic coefficients of vaariation
Gxx	Observed mean square for Genotypes
HL	Honglian

h	Horizantal
ha	Hactare
hrs	Hours
h^2	Heritability
i	Inclined
IRRI	International Rice Research Istitute
KAU	Kerala Agricultural University
M `	Molar
MAS	Marker assisted selection
Mm	Micro molar
Min	Minute
ml	Milli litre
nm	Nano moles
ng	Nano grams
PCR	Polymerase chain reaction
PCV	Phenotypic coefficients of variation
p.moles	Pico moles
Ptb	Pattambi
QTL	Quantitative trait loci
rpm	Revolutions per minute
R PRIMER	Reverse primer
Rf	Fertility restorer gene(s)
Rf WA	Fertility restorer gene(s) Wild Abortive
SDS	Sodium Dodecyl Sulphate
SSR	Simple sequence repeats
STS	Sequence Tagged Repeat

sec	Seconds
Tris HCl Hydrochloride	Tris (hydroxy methyl) aminomethane
TAE	Tris acetate EDTA buffer
Taq	*Thermus aquaticus*
TE	Tris EDTA buffer
TNAU	Tamilnadu Agricultural University
t/ha	Tonns per hactare
v	Vertical
WA	Wild Abortive
$\sigma^2 ex$	Envoronmental variance
$\sigma^2 gx$	Genotypic variance
$\sigma^2 px$	Phenotypic variance
°C	Degree centigrade
%	Per cent
µl	Micro litre
µM	Micro molar

Introduction

1. INTRODUCTION

Rice is the world's single most important food crop and a primary food for more than two third of the world's population. Production and consumption are concentrated in Asia where more than 90% of all rice is produced and consumed. Rice in Asia is typically grown by poor farmers on farms averaging 1 ha. During the last few decades, major progress has been made in increasing rice productivity. World rice production has more than doubled from 257 million tons in 1966 to 589 million tons in 2003. This has mainly been achieved through the application of principles of Mendelian Genetics and conventional plant breeding methods. The present world population of 6.1 billion is likely to reach 8.0 billion by 2030. To meet the growing food need and overcome malnutrition, rice varieties with higher yield potential and multiple resistance to biotic and abiotic stresses with improved nutritional quality are needed. Recent advances in Genetics offer new opportunities to achieve these objectives. (Khush et al.2003.)

In coming decades, to sustain the selfsufficiency achieved, the production of rice needs to be increased every year by almost 3 million tons, which is a difficult task, in view of the plateauing trend observed in yield potential of high yielding varieties, and decreasing and declining natural resource base. This increase in production needs to be achieved without disturbing the delicate environmental balance. Among the many genetic approaches being explored to break the yield barrier in rice, hybrid rice technology appears to be the most feasible and readily adopted one. Hybrid rice technology particularly utilizing the CMS system has now been widely adopted across several countries in Asia and in USA. Hybrid rice technology is the most viable option for sustaining the global rice production (Allahgholipour and Ali 2006). During 2006, hybrids were cultivated in India in about one million hectare and it is estimated that by 2010 and 2020 hybrids will be cultivated in more than 3 and 10 million ha respectively. In Kerala, rice is cultivated in 2.34 million ha but the area of cultivation of hybrid rice is nil. The lack of a 'hybrid' superior to the existing cultivars with preferred consumable qualities is the reason for the low impact of hybrid rice technology in Kerala.

At present, the approach widely adopted for commercial development of rice hybrids is the three-line system involving a cytoplasmic male sterile (CMS) line, a

maintainer line and a restorer line. Cytoplasmic male sterility (CMS) is a maternally inherited trait that results in the inability of the plant to produce fertile pollen. Pollen fertility is restored by nuclear-encoded genes called fertility restorer (*Rf*) gene. Identification of a promising 'restorer' is a challenge for hybrid rice technology.

The development of molecular maps and the complete sequencing of the rice genome have created many opportunities for the application of DNA markers in breeding and genetics. Marker-assisted selection (MAS) has become a practical tool in cultivar improvement. MAS is time-saving, very efficient, reliable, and consistent in dealing with traits whose phenotype is affected by the environment.

Development of a MAS procedure involving the restorer QTLs would significantly reduce the time and labour in making and evaluating test crosses in an active hybrid rice-breeding programme. In particular, the development of PCR based markers would empower researchers in local agricultural research systems to apply the technology in local hybrid rice breeding programmes and seed purity assessments.

Many PCR based markers have been reported as linked to *Rf* loci in rice but their validation in different populations and the different restorer lines needs to be done to characterize the allelic status of *Rf* loci in them. In this back ground, this study to locate restorers was undertaken with 19 rice varieties of Kerala and two restorers used in hybrid rice programme in TNAU.

The programme was planned and executed with the following objectives

- To compare the morphological characters of the genotypes with the known restorers of TNAU
- To study the allelic status of the Kerala varieties with respect to SSR markers linked to *Rf* loci
- To assess the possibility of using Marker Assisted Selection for screening for restorers

Review of Literature

2. REVIEW OF LITERATURE

. Rice is the most important food crop of the developing world. It provides up to two-thirds of the calories for more than 2 billion people in Asia. Rice is also a major source of protein for the masses of Asia. Demand for rice is expected to increase by about 3% per year over the next decade and beyond. In most Asian countries, prospects for increasing rice lands are very limited; moreover, their land-to-agricultural worker ratio (0.27) is the lowest in the world and is declining. Land to population ratios also are decreasing and most Asian countries must produce more rice on less land. Rice physiologists have indicated that the physiological yield potential of rice in the tropics in both wet and dry seasons is 2-3.5 t/ha higher than experimental yields obtained so far. Hybrid rice technology is considered as one of the promising, practical, sustainable and eco-friendly options to break the yield ceiling witnessed in rice (Virmani 1994).

2.1 Status of Hybrid rice research and Development

Hybrid rice has helped China to increase rice production by nearly 200 million tons from 1976 to 1991. Hybrid rice has a yield advantage of more than 30% over conventional pureline varieties. In the year 1991 the area under hybrid rice was about 17.6 million ha which is 55% of the total rice area in China and the production of hybrid rice was 66% of the total rice output (Yuan *et al.*., 1985).

Over the last 12 years, remarkable progress in development of hybrid rice technology has been made in India. First rice hybrids were developed and introduced to Indian agriculture in 1994. So far 13 public and 3 private sector hybrids have been released for general cultivation by the Central and State Variety Release Committees. Seed production of about 5-6 hybrids is being taken up on large scale predominantly

by the private seed sector. About 3500-4000 tons of hybrid seed is being produced annually and it is estimated that around 200,000 hectares are planted with hybrid rice annually in India. Area under hybrid rice will further increase after heterotic hybrids suitable for high productivity areas of Punjab, coastal regions of Andhra Pradesh and shallow low land areas are identified and an effective transfer of technology programme is undertaken in the target state (www. Hybridriceindia.org)

2.2 Heterosis in rice

Jones (1926) was the first in USA to report heterosis in rice which has been exploited by Chinese scientists in developing hybrid rice. Subsequently, other reports indicated significant heterosis for various agronomic traits in rice (Chang *et al.,* 1973, Davis and Rutger 1976, Virmani *et al.* 1981, Virmani and Edwards, 1983). Suggestions for developing F_1 rice hybrids to exploit heterosis commercially have been made by rice scientists in India (Richharia 1962, Siddiq *et al.,* 1972) China (Yuan 1966) U.S.(Stansel and Craigmdes 1966, Craigrmiles *et al.* 1968, Carnahan *et al.*. 1972), Japan (Shinjyo and O'mura, 1966) and IRRI (Athwal and Virmani, 1972). However, difficulties anticipated in hybrid seed production discouraged most of these researchers except the Chinese from continuing their efforts. China is the first country to commercially exploit heterosis in rice. Research on hybrid rice began in 1964. The genetic tools (cytoplasmic male sterile [CMS], maintainer, and restorer lines) essential to develop F_1 rice hybrids were successfully developed in 1973. Hybrids with strong heterosis were identified in 1974 and seed production techniques were primarily established in 1975. In 1976, hybrid rice was released to farmers. Since then, the area of hybrid rice in China has increased rapidly each year (Virmani 1994).

The presence of significant heterobeltiosis and standard heterosis for rice yield at IRRI has been reported by Virmani *et al.* (1982), Ponnuthurai *et al.* (1984), Virmani (1986,1987), Yuan and Virmani (1988), Yuan *et al.* (1989), Young and

Virmani (1990), Peng and Virmani (1991), and Virmani *et al.* (1991). Outside IRRI, similar results have been reported from South Korea (Koh 1987, Moon 1988), India (Parmasivan 1986, Ananda Kumar and Sreerangasamy 1986, Prakash and Mahadevappa 1987, Siddiq *et al.*. 1994), Indonesia (Suprihatno 1986, Subandi *et al.* 1987), Pakistan (Cheema and Awan 1985, Cheema *et al.* 1988), Malaysia (Osman *et al.* 1987), and Vietnam (Luat *et al.* 1985).

2.3 Male sterility systems in rice

In rice male sterility systems were broadly classified into three types 1.Genetic male sterility 2. Cytoplasmic male sterility and 3. Cytoplasmic-Genetic male sterility. Cytoplasmic genetic male sterility is the major system used to breed rice hybrids. Other systems (chemical male sterility, thermosensitive genic male sterility [TGMS], and photoperiod-sensitive genic male sterility [PGMS]) have not gained much popularity.

Various CMS sources discovered in rice were compiled by Virmani and Shinjyo (1988), and they proposed their interim designations. The symbols assigned were interim because studies on the interrelationship between various CMS sources are limited and it is possible that cytoplasmic factors derived from different rice cultivars may be genetically the same. They also proposed a model for identifying genetic differences among cytoplasm and restoring genes. Since then, new CMS source V20 B and Kalinga (Pradhan et a1 1990) and Oryza perennis (Acc. 104823) (Dalmacio *et al.* 1992) have been identified. Dalmacio *et al.*.1993 reported that V20 B is a maintainer of CMS-WA cytoplasm (the most extensively used CMS source in hybrid rice breeding) but is itself a source of CMS cytoplasm with a japonica rice cultivar; it should, therefore, have a different source of cytoplasm. Crosses of CMS lines (IR66707 A) possessing O. perennis cytoplasm with nine restorers of CMS-WA showed almost complete pollen sterility (93-100%), indicating that the male sterility source of IR66707 A is different from WA sterility. Research at IRRI (India) and in

other countries have indicated that male sterility-inducing cytoplasmic factors are widely distributed in wild and cultivated rices. Therefore, development of CMS lines possessing diverse cytoplasmic and nuclear background is possible (Virmani 1994)

2.4 Cytoplasmic genetic male sterility and fertility restoration

In rice, primarily, three types of CMS systems are deployed for commercial hybrid seed production.These are Wild Abortive (WA), Bao Tai (BT) and Honglian (HL). Among these three, the WA cytoplasm is the most widely used since it is a more stable system and the pollen sterility is almost nearly complete (Shinjyo and Omura 1966). The role of cytoplasm in causing male sterility in rice was first reported by Sampath and Mohanty (1954). The first cytoplasmic male sterile line in cultivated rice was developed by Shinjyo and O'mura (1966) in Japan from an indica source of cytoplasm (Chinsurah Boro II) in the genetic background of a japonica variety Taichung 65. Later, Erickson (1969) and Carnahan et a1 (1972) developed CMS lines in japonica varieties (Calrose and Caloro) of California, USA, using another indica source of cytoplasm Birco (PI 279120). At IRRI, Athwal and Virmani (1972) developed a CMS line in an indica variety derived from the cytoplasmic source of Taichung Native-1 and nuclear source of variety Pankhari 203. Cheng and Huang (1979) also developed CMS line in the genetic background of variety Taichung 65 with a cytoplasmic source of Oryza rufipogon. Among these CMS lines, the one most stable for complete pollen sterility was developed by Shinjyo and O'mura (1966). This line has been used extensively in China to develop japonica hybrids. Other CMS lines developed outside China have not been used for lack of stability or effective restorer lines. After CMS lines were developed in China from Oryza sativa f. spontanea, designated as WA cytoplasm (Lin and Yuan 1980, Yuan 1972), IRRI and several national rice improvement programmes developed new CMS lines by transferring the cytosterility system of the WA CMS lines such as V20 A, Zhen-Shan 97 A,Er-Jiu-Nan 1 A, and V41 A, into elite lines by backcrossing

(Shinjyo 1969, 1972a,b, 1975; Virmani et a1 1981; Virmani 1986). The frequency of restorer lines among japonica varieties is negligible (Shinjyo 1975). A number of Tongil (indica/japonica derivatives) varieties from Korea are effective restorers (Virmani et a1 1986), perhaps due to gene(s) inherited from the indica parents. Vanaja *et al.*. (2003) reported that Cytoplasm of Vytilla 3, an improved saline tolerant variety of Kerala as a new source for cytoplasmic male sterility in rice (Oryza sativa L.), suitable to warm humid tropical climatic conditions experienced in the rice growing tracts of the world. The varieties IR36 and Hraswa (an extra short duration variety of Kerala) are the proposed maintainer lines and the variety Mattatriveni is the proposed restorer line.

The discovery of cytoplasmic male sterility (CMS) in rice (Athwal and Virmani 1972, Erickson 1969, Shinjyo 1969) suggested that breeders could develop a commercially viable F_1 hybrid, but little serious interest was paid until China reported, in 1977, successful production of F_1 hybrids. Those hybrids yielded 20-30% higher than conventionally bred varieties (Lin and Yuan 1980). This was the first example of hybrid rice which used cytoplasmic-genetic male sterility and fertility restoration systems for seed production being commercially grown in farmers' fields.

2.5 Maintainers and restorers in CGMS system

Successful use of a CMS line in breeding hybrids depends on its stability and adaptability across environments, the possibility of restoration, its genetic diversity from restorer parents, its outcrossing potential and its combining ability. Of hundreds of CMS lines developed in China over the years, fewer than 20 have been used to develop commercial rice hybrids (Yuan and Virmani 1986). Of the 40 CMS lines bred at IRRI during 1980-88, only three (IR58025 A, IR62829 A, and IR64608 A) possessed traits suitable for developing commercial hybrids (Virmani *et al.* 1991).

Frequency of restorer lines among rice cultivars of different origins, IRRI,1982-89.(Virmani 1994)

Origin	Total tested(No.)	Restorer frequency(%)
IRRI	2320	36
India	70	21
Philippines	19	63
Indonesia	61	44
South Korea	53	53

The existence of such a high number of diverse R genes explains the high frequency of restorer lines among elite indica breeding lines. No restorers have yet been identified for CMS line IR66707 A possessing Oryza perennis cytoplasm (Dalmacio *et al.* 1992).

In heterosis breeding programme using cytoplasmic male sterility (CMS) system, identification of maintainers and restorers is fundamental. 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). While a large number of restorers have been identified for the wild abortive (WA) CMS lines (Virmani and Edwards, 1983; Pradhan *et al..*, 1992), effective restorers for Oryza perennis source have not been identified so far. Leenakumari *et al..*(1998) reported new restorers and maintainers for WA cytoplasmic male sterile (CMS) lines in rice (Oryza sativa). 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. Rosamma and Vijaya Kumar (2005) reported restorers and maintainers for seven cytoplasmic-genic male sterile (CMS) lines of rice having wild abortive (WA) cytoplasmic male sterility source and one having Oryza perennis CMS source .They crossed these lines with 34 entries to assess their maintainer/restorer behaviour and reported that most of the genotypes expressed differential fertility reactions when crossed with CMS lines having WA cytoplasm and all test entries produced sterile hybrids when crossed with CMS line having O. perennis source. Among the genotypes tested, 'Annapoorna', 'Kanchana', 'IR 36', 'Mattatriveni' and 'Aiswarya' are 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.

2.6 Classification of restorer genes.

The fertility-restoring ability of CMS-WA cytoplasm is sporophytic and governed by two genes, one of which has a stronger effect than the other. Allelism tests conducted for restorer (R) genes in some restorer lines showed that these lines differed in R gene content and an appropriate combination of two R genes conferred full fertility in crosses with a CMSWA line (Govinda Raj and Virmani 1988).

To classify restorer genes, Zhu *et al.* (l985) tested14 restorers with 18 MS lines of differing cytoplasm and divided the restorers into four groups. Group 1, including IR24, has effective restorers for WA and Gam MS lines and weak restorers for Hong-Lien, BT, and Dian 1 MS lines. Group 2, including Tai-Ying 1, Peta, IR8, Indonesia 6, and Xue-Gu-Zao has restorers for WAand Gam MS lines but maintainers for Hong-Lien, BT, and Dian 1 MS lines. Group3, including Zhen-Shan 97 and Long-Zi 1, are maintainers for WA MS lines but restorers for Hong-Lien, BT, and Dian 1 MS lines. Their restorer genes belong to another type. And Group 4, such

as No. 5350, No. 75 P12, No. 85661, and No. 300 developed by cross breeding are restorers for WA, Gam, Hong-Lien, BT, and Dian 1 MS lines, but are relatively weak restorers (Zhu 1984 and Wang 1983) divided restorer genes for WA MS lines into 3 kinds: strong restorer R s , weak restorer R w , and recessive restorer gene r.

2.7 Geographical distribution of restorer genes

The geographical distribution of restorer genes for BT MS lines was studied by Shinjyo (1972). 150 Japanese varieties and 153 varieties from other

countries were tested for fertility restoration. Of the 150 Japanese varieties, 131 had

complete male sterile F_1 progeny and 19 had partial fertile F_1 progeny, ranging from 25% to 53% in pollen fertility and 1.7% to 11.5% in seed setting rate. These weak restorers were concentrated in southern Japan (Shinjyo 1975). Of the 153 varieties from 15 countries, progeny of 54 varieties had some fertile pollens and seed setting rates of 70% or higher. These varieties were considered to have effective restorer genes. Twenty-eight varieties showed lower seed setting rates. The remaining 71 showed complete male sterility and were considered to lack restorer genes. In the three ecotypic varieties–aman, aus, and boro–of indica, all aman and boro varieties tested possessed effective restorer genes, but aus varieties had weak or no restorer genes. Of the two ecotypic varieties on Java Island, bulu and tjereh, bulu contained no effective gene while tjereh had one.

Virmani (1994) reported that in Asia, effective restorers were mainly in southern countries and South China, while non restorers were concentrated in northern countries. Effective and weak restorers and non restorers were found in European countries. In the U.S., no effective restorer was found in California, where japonica varieties were cultivated exclusively, but three effective restorers were detected in Louisiana and Texas, where indica is the main type. In the three grain types, A, B, and C, the frequency of effective restorer genes was 8.5%,27.6% and

62.9%, respectively. The effective restorer genes were mainly distributed in the tropics while the nonrestorers were concentrated in temperate countries (Shinjyo 1975).

Zhu (1984) studied geographical differences in restorer genes in native varieties by testing them with Zhen-Shan 97 A of WA type and Hua-Ai 15 A of Hong-Lien type. The native varieties were selected from six geographical areas: A (Guangxi, Guangdong, and Fujian), B (Yunnan), C (Sichuan, Guizhou, and Hanzhong district of Shanxi), D (Yangtze Valley, including Hubei, Hunan, Jiangxi, and Anhui), E (Jiangxu, Zhejiang, Shanghai), and F (northeast, northwest, and north China). The results showed genes possess different restoring abilities in japonicas.

2.8 Inheritance of restorer genes

Shinjyo (1969) studied fertility inheritance of BT type and found pollen fertility was a main criterion. In studying inheritance of the sterility of MS lines of WA type, Hu and Li (1985) used three indices: percentage of fertile pollen, percentage of bagged seed set, and percentage of natural seed set. Percentage of fertile pollen was the most reliable criterion of fertility (Hu,1983). These three indices plus morphological characteristics such as degree of panicle exertion, anther shape and anther color were used by Yang et a1 (1984) to appraise fertility. They suggested that the percentage of stained pollen or the percentage of typical aborted pollen should be used as an essential index for determining plant fertility. Most research confirms that pollen fertility could be a main criterion for assessing fertility.

Hu and Li (1985) investigated fertility segregation in F_1, F_2, and F_3 hybrids descended from crosses between WA type Zhen-Shan 97 and IR24, IR26, and WA type V20 A. Results suggested that there were two pairs of major fertility genes in the same linkage group and the average recombination frequency was 34%, They analyzed the pedigree of IR24 and pointed out that IR24 possessed two pairs of major

restorer genes (R 1 R 1 R 2 R 2). One pair (R 1 R 1) came from Cina, a late indica variety in China, while another pair (R 2 R 2) was from SLO 17, inherited to IR127 through CP-SLO, R 1 R 1 was then combined with R 2 R 2 to form the strong restorer line IR24 (R 1 R 1 R 2 R 2) by crossing IR8 with IR127. But Wang (1980) considered that the fertility restoration of WA type Zhen- Shan 97 A was controlled by one pair of genes. Research on the inheritance of fertility restorer genes for WA type MS line has also been done by He (1985), Cai *et al.* (1983), and Huang *et al.* (1986).

2.9 Main factors affecting fertility restoration

2.9.1 Genetic diversity

Genetic diversity includes differences in sterile cytoplasm and backgrounds of maintainers and restorers. Isogenic MS lines with different sterile cytoplasms may belong to different sterile types and have different restorers. For example, the cytoplasms of WA and Hong-Lien types, although both coming from wild rice plants of Hainan Island, belong to different sterile types. WA lines are sporophytic, while Hong-Lien types are gametophytic. When both WA type Hua-Ai 15 A and Hong-Lien type Hua-Ai 15 A were tested with IR24, the F_1 progeny of WA were completely fertile but progeny of Hong-Lien were partially fertile (Zhu 1984). The genetic background of maintainers influences the fertility of F_1 hybrids having the same sterile cytoplasm. For example, fertility restoration of WA type MS line Zhen-Shan 97 A was easier than of WA type Er-Jiu-Nan 1 A. The genetic background of restorers apparently influences fertility restoration. When restorers IR24, IR28, and Gu 154 were crossed with the same MS line, the fertility of F_1 hybrids revealed that the restorers differed in fertility restoring ability (Li and Yiao 1982).

2.9.2 Environmental variation

Hybrid rice is more sensitive to environmental variations than most conventional varieties. Environmental factors, particularly temperature, greatly influence fertility restoration. Seed setting rate may drop when unfavorably high or low temperature occurs during the pollen mother cell meiosis stage or heading stage. Joint IRRI-China research indicated that 15% of Chinese elite breeding materials and 24% of IRRI materials tested were effective restorers , only 6% were effective at both sites. Thus, the frequency of restorer genotypes was higher in the tropics than in the subtropics (Virmani and Edwards 1983). Govinda Raj (1990) tested WA type MS lines V20 A and Zhen-Shan 97 A with restorer ADT33. The F_1 hybrid was cultivated at Delhi in the rainy season and at Aduthurai from July to October. Hybrids at Delhi appeared completely fertile but partially fertile at Aduthurai. Chinese research showed that temperature and moisture affect fertility restoration. The most sensitive stage of hybrid rice to temperature is the flowering stage. If unfavorably high or low temperature occurs during that stage, many florets will not flower, few anthers will dehisce and the germination rate will decrease, with low seed setting. Hybrids derived from different MS lines and restorers differ in their reactions to environmental variations (Li and Yiao 1982).

2.10 Genetics of Fertility Restoration in Rice

Kitamura (1962a) reported that high fertility in the F_1 hybrids of cytosterile TA 820 was controlled by a recessive gene combined with modifiers or polygenes. Shinjyo (1969) identified a single dominant fertility restoring gene (RO) in rice cultivar Chinsurah Boro II; its effect was gametophytic in the male sterility inducing cytoplasm (cms-bo). Shinjyo *et al.*. (1974) discovered two fertility restoring genes in a Japonica variety Fukuyama, and a strain (ms-boro) Rf-Taichung 65 for the male sterility inducing cytoplasms of variety ‘Lead’ rice detected by Watanabe *et al.*. (1968). The effects of the Fukuyama restorer gene, tentatively named as Rfx, and the Rf gene from the strain (ms boro) Rf-Taichung 65 were gametophytic in the Lead rice

cytoplasm. Shinjyo (1975) also introduced the fertility restoring gene of variety Tadukan (Kitamura 1962a, b) into a Japonica variety and implied that weak (Rfx) and effective (Rf) genes were probably allelic in their relation. He further suggested that male sterility inducing Chinsurah Boro IT was more or less identical to that of Lead rice and that Rf and Rfx genes did restore fertility of the CMS system (Oryza sativa f spontanea) identified by Katsuo and Mizushima (1958).

Inheritance of fertility restoration for the cms-WA cytosterility system (most widely used in China and elsewhere to breed F_1 rice hybrids) has been studied by Wang (1980), Gao (1981), Govinda Raj and Siddiq (l983), Zhou *et al.*. (1983), Yang and Lu (1984), Young and Virmani (1984), Li (1985), Huang *et al.*. (1986), Li and Yuan (1986), Virmani *et al.*. (1986), Govinda Raj and Virmani (1988) and Singh and Sinha (1988). All these studies indicated sporophytic effect of the fertility restoring gene(s). Wang (1980) reported a single dominant gene restoring fertility in cytosterile Zhen Shan 97A, whereas all other studies showed that fertility restoration was controlled by two dominant genes. One of the two genes had a stronger effect than the other (Zhou *et al.*. 1983; Yang and Lu 1984; Young and Virmani 1984; Virmani *et al.*. 1986; Govinda Raj and Virmani 1988). Hu and Li (1985) suggested that the two fertility restoring genes for the cms-WA cytosterility system were in the same linkage group with an average recombination frequency of 34%. Virmani *et al.*. (1986) and Govinda Raj and Virmani (1988) found that the mode of action of the two genes varied with the cross and certain crosses showing epistasis with dominance (F_2 ratio 12:3:l), while other crosses showed epistasis with recessive gene action (F_2 ratio 9:3:4). Some crosses also displayed epistasis with incomplete dominance (F_2 ratio 9:6:1).

Govinda Raj and Virmani (1987) provided evidence for the role of inter-varietal hybrid sterility and/or inhibitory genes present in a CMS line of rice, in causing incomplete fertility restoration by some established restorer lines possessing cms-WA cytoplasm.

Pollen abortion in WA-type CMS is sporophytic, forming typical abortive pollen (Huang *et al.*. 2003). Earlier studies indicated that a major dominant gene controls fertility restoration of WA-cytoplasm (Huang *et al.*.1986; Anandakumar and Subramaniam 1992). Later, it was discovered that fertility restoration is controlled by two independent dominant nuclear genes with one stronger in action than the other (Young andVirmani 1984; Virmani *et al.*. 1986). Studies also indicated different types of gene interaction like recessive epistasis (Govinda Raj and Virmani 1988; Pradhan and Jachuck 1999) semi-epistasis (Pradhan and Jachuck 1999), epistasis with incomplete dominance (Govinda Raj and Virmani 1988; Sarkar *et al.*. 2002), epistasis with complete dominance (Sohu and Phul 1995) or no interaction (Li and Yuan1986). Some studies have revealed that the two fertility restorer genes are additive in their inheritance giving rise to an F_2 segregation ratio of 15:1 (fertile:sterile) (Gao 1981; Li and Yuan 1986). Yao *et al.*. (1997) reported two genes, Rf-3 and Rf-4,located on chromosome 1 and 10 respectively, which have a major influence on fertility restoration in many restorer lines for WA-CMS lines. Another gene called Rf-(u1) identified in a Basmati-type restorer line, PRR78 R located on chromosome 10 is also known to restore fertility for WA-CMS system (Mishra *et al.*. 2003).

Inheritance of fertility restoration in WA-CMS system in particular has been extensively investigated (Govinda Raj and Virmani 1988; Virmani 1994; Bharaj *et al.*. 1995; Zhang *et al.*. 1997; Yao *et al.*.1997; Zhuang *et al.*. 2000; Ali *et al.*. 2003). Mishra *et al.*. (2003a) had partially reviewed the literature on inheritance of fertility restorer genes to be monogenic, digenic with different epistasis types, trigenic, and tetragenic. A recent review of Rf genes in Gramene website over different CMS lines shows the complexity of Rf allele/gene location across the different chromosomes. In Gramene website, the different Rf genes that has been reported in the past with or without chromosomal locations have been allotted gene symbols from Rf1 up to Rf 17 (Gramene database release 20-2006). It will be good to have an allelic study carried out before allocation of gene symbols from Rf7 onwards with known linkage groups. However, Rf1 to Rf6 has been reported while Rf 7 to Rf 17 is under curation

(Gramene database release 20-2006). Most of the researchers have reported two genes controlling this trait in a given mapping population. Bharaj *et al.*. (1995) located the two restorer genes viz. Rf4 (Rf-WA-1) and Rf 6 (Rf-WA-2) and on chromosome 7 and 10 respectively, by using a trisomic set analysis.

2.11 Molecular markers for fertility restoration genes

The use of molecular markers had enabled several research groups to determine the chromosomal location of the Rf genes for CMS-WA and other CMS systems (Gramene database release 20-2006). Akagi *et al.*. (1996) developed a new set of primers pRf1 and pRf2 specific for a new locus OSRRf closely linked to nuclear restorer gene Rf-1 at a genetic distance of 3.7 ± 1.1 cM and mapped on chromosome 10 which produced 345 and 329 bp DNA fragments from R and A lines, respectively. Zhang *et al.*. (1997) mapped the Rf3 gene using RFLP markers on chromosome 1 for the CMS-WA system. Yao *et al.*. (1997) identified two Rf genes for the WA CMS system in the rice genome on chromosomes 1 (Rf3) and 10 (Rf6). The fertility restorer genes Rf-3 and Rf-4 for WA-type CMS have been mapped on chromosome 1 and 10 respectively (Yao *et al.*. 1997). Ichikawa *et al.*.(1997) also mapped this Rf gene using RAPD/RFLP markers. Tan *et al.*. (1998) mapped two QTLs on chromosome 10 that controlled the fertility restoration. One was in the middle of the long arm and the second QTL, on the short arm of chromosome 10.These QTLs explained 71.5 and 27.3% of the phenotypic variance, respectively. Huang *et al.*. (1997) identified a microsatellite marker RM258 linked with fertility restorer gene Rf-5, for HL-type CMS at a genetic distance 7.8 cM on chromosome 10. Jing *et al.*. (2003) mapped Rf-4 with microsatellite markers RM171 (OSR 33) and RM228 at distances of 3.7 and 3.4 cM respectively, on the long arm of chromosome 10. Another SSLP marker RM244, on the short arm of chromosome 10 was found to be linked with the fertility restorer locus of IR64, designated as Rf6 (t) at a genetic distance of 17.3 cM. Gyan *et al.*.(2001) mapped fertility restorer gene in Basmati

using SSR markers. He has stated RM 258 as a probable marker for screening restorers.

Komori *et al.* (2003) mapped Rf-1 gene(CMS-BT) between S12564 Tsp5091 and C1361MwoI at genetic distances 0.1 and 0.2 cM, respectively, from the Rf locus. Two STS markers RG140FL/RG140RL and RG532FL/RL located on chromosome 1 were reported to be closely linked with Rf-3 (Nguyen *et al.*. 2003). Two more microsatellite markers, HL01 and MRG4456, which flanked the nuclear fertility restorer gene Rf-5 (CMS-HL) and mapped at a genetic distance of 0.63 and 1.57 cM, respectively, from the Rf gene on the long arm of chromosome 10 (Huang *et al.*. 2003). Two simple sequence repeat (SSR) markers, RM258 and RM216, located on chromosome 10 were found to be linked to the restorer gene, Rf-(u1) in a basmati quality restorer line PRR78 R. RM258 was mapped at a distance of 9.5 cM from the restorer locus while the marker RM216 was at a distance of 30.4 cM from the maker locus RM258 (Mishra *et al.*. 2003). Singh *et al.*. (2005) identified a microsatellite marker, RM6100, linked with Rf gene in restorer lines PRR78 R, IR40750 and MTU9992 at a distance of 6–7 cM on chromosome10.

Each of the Rf mapping studies in the past found Rf genes to be either located at one or two different loci. However, to identify more Rf genes on different loci requires utilization of unique kind of genetic source such as the RMCP bred restorers. RMCP facilitated by genetic male sterility as a breeding approach to improve and develop new restorer and maintainer lines at IRRI Philippines and in India were quite successful (Liu *et al.* 1998; Krishnaiah *et al.* 2001; Virmani 2003; Mishra *et al.* 2003b).

In the WA-CMS system, the applicability of markers associated with Rf loci for routine screening of restorer lines has been used at IRRI since 2001 when Nas *et al.* (2003) established a single-gene MAS system for Rf3 on chromosome 1 with an accuracy rating of 80% indicating a further need for the development of PCR-based markers for the Rf4 gene on chromosome 10. Sattari (2007) reported the

development and application of a MAS procedure for fertility restoration involving the two major Rf genes for selecting fertility restorer loci in three CMS systems. Ahmadikhah *et al.* (2007) studied the inheritance of the fertility restoration and genotyping of rice lines at the restoring fertility loci using molecular markers. They did bulk segragant analysis and showed that fertility in rice WA system is controlled by more than two loci one on the short arm of the chromosome 1 ,one on the short arm of chromosome 10 and an unknown Rf gene in the rice genome . They also found the SSR marker 171 linked to Rf4 gene in lines IR 28 , Amol 1 and Amol 2. Bazrkar *et al.* 2008 tagged four fertility restorer loci for wild abortive cytoplasmic malesterility system in rice using microsatellite markers. They reported the new molecular marker RM6344 linked to Rf4 locus on chm.7.

Despite the knowledge available on genetic locations, the applicability of markers associated with Rf loci for extensive and routine screening of restorer lines from previously uncharacterized rice germplasm has not yet been reported. Perhaps,complex inheritance, inconsistent reports on chromosomal locations of Rf genes, and the use of markers not well suited for routine screening for traits governed by more than one gene discouraged hybrid rice breeders from deploying marker aided selection (MAS) for fertility restoration (Sheeba *et al.* 2009).They validated linkage of these markers in mapping populations involving two fertility restorer lines for WA-cytoplasm, viz., KMR3 and IR10198R and to analyze the allelic status of the closely linked markers in a set of high priority fertility restorer and maintainer lines used for hybrid rice breeding in India. In both the populations studied, the trait of fertility restoration was observed to be under digenic control. Eight SSR 2 markers (RM6100, RM228, RM171, RM216, RM474,RM311, G4456 and pRf1&2) showed polymorphism between the parents of the F2 population, while the SSR markers RM6100 and RM474 showed polymorphism between the parents of both the F2 and BC_1F_1 populations. Only one CAPS marker,RG146FL/RL was polymorphic between the parents of the BC_1F_1 population. 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. Using the marker-trait segregation data derived from analysis of both the mapping populations, a local linkage map of the genomic region around Rf-4, a major fertility restoration locus on Chromosome 10 was constructed and RM6100 was observed to be very close to the gene at a distance of 1.2 cM. The accuracy of the marker RM6100 in predicting fertility restoration was validated in 21 restorers and 18 maintainers. RM6100 amplified the Rf-4 linked allele in a majority of the restorers with a selection accuracy of 94.87%. Through the present study, we have established the usefulness of the marker RM6100 in marker-assisted selection for fertility restoration in segregating populations and identification of restorers while screening rice germplasm for their fertility restoration ability.

Material and methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Plant Breedding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2009-2011 to identify fertility restorers from traditional Kerala varieties using Marker Assisted Selection. The experimental material consisted of 19 rice accessions of Kerala and two known restorers from Tamil Nadu Agricultural University The details of 21 rice varieties are enlisted in Table.1

The present study on identification of good restorers were analysed in two sections morphological analysis and molecular analysis.

3.1 Morphological analysis

The 21 plant varieties were raised in pots in three replications and the morphological observations were taken at desirable dates and stages of crop growth period.

1. Plant height (cm)

Plant height was measured from the soil level to the tip of the longest panicle at the time of maturity.

2. Flag leaf area (cm^2)

The length and maximum width of flag leaf were measured and area calculated as follows.

$$\text{Flag leaf area} = k \times l \times b$$

Where k=0.65 (The constant used at maturity stage), l = the length of the flag leaf along the longitudinal axis, b = the maximum breadth along the flag leaf (Ambili2000).

Table.1. Details of varieties

Sl.No	Varieties released	Source	Station Released	Kernal Colour
1	Aryan (Ptb-1)	PLS from Aryan	Pattambi	Red
2	Ponnaryan (Ptb-2)	PLs from Ponnaryan	Pattambi	Red
3	Velutharikayama (Ptb-5)	PLS from Velutharikayama	Pattambi	Red
4	Parampuvattan (Ptb-7)	PLS from Parampuvattan	Pattambi	Red
5	Chuvannari Thavalakkannan (Ptb-8)	PLS fromChuvannari Thavala kkannan	Pattambi	Red
6	Veluthari (Ptb-9)	PLS from Veluthari	Pattambi	White
7	Thekkancheera (Ptb-10)	PLS from Thekkancheera	Pattambi	Red
8	Kayama(Ptb-13)	PLS from Kayama	Pattambi	Red
9	Muskati(Ptb14)	PLS from Muskathi	Pattambi	White
10	Veluthavattan (Ptb-22)	PLS from Veluthavattan	Pattambi	Red
11	Aishwarya (Ptb-52)	BR 51 × Jyothi	Pattambi	Red
12	Manupriya	PK3355-5-1-4×Bhadra	Moncompu	Red
13	Co-48	Co43×ASD-19	TNAU	White
14	CB-87R	Restorer used in developing CORH-3	TNAU	White
15	Jyothi(Ptb-39)	Ptb 10 × IR 8	Pattambi	Red
16	Matta triveni (Ptb-45)	Reselection from Triveni	Pattambi	Red
17	Swarna Prabha (Ptb-43)	Bhavani × Triveni00	Pattambi	White
18	Uma(MO-16)	MO 6 × Pokkali		Red
19	Neeraja (Ptb-47)	IR 20 × IR 5	Pattambi	White
20	Kanchana (Ptb-50)	IR 36 × Pavizham	Pattambi	Red
21	Varsha(Ptb-56)	M 210 × Ptb-28	Pattambi	Red

3. Number of productive tillers per plant

In each plant, number of ear bearing tillers with uniformly matured grains at the time of physiological maturity was counted and recorded.

4. Days to flowering

The number of days taken from the date of sowing to panicle emergence in three replication.

5. No of filled grains per panicle

The total number of fully developed and well filled grains counted in the primary panicles.

6. Spikelet sterility

The ratio of unfilled grains to total number of spikelets per panicle was calculated and expressed in percentage

The spikelet sterility percentage of individual plants was calculated based on the average spikelet sterility of individual panicles selected from each plant.

7. Pollen fertility per cent

The pollen study was done using acetocarmine staining method. The number of fertile pollen grains to the total number of observed pollen grains in a standard microscopic field. is expressed in per centage.

$$\text{Pollen fertility per cent} = \frac{\text{Number of fertile pollen grains} \times 100}{\text{Total number of pollen grains}}$$

8. Pollen count: The number of pollen grains present in a field of microscope after standard acetocarmine staining and the value is expressed as average of ten fields.

9.100 seed weight: From each plant, one hundred well filled grains selected at random were weighed and expressed as percentage.

10. Orientation of flag leaf: The angle of inclination of flag leaf after anthesis

3.1.1 Statistical analysis:

The mean data of 21 lines for each biometrical trait were subjected to analysis of variance for CRD. For discriminating the desirable genotypes from undesirable

ones on the basis of their phenotypic performance, selection indices were worked out (Smith, 1936).

1. Analysis of Variance

Analysis of variance of the data on restorer lines using standard procedure and discrimnant function analysis to grade the restorers. Evaluation of heterosis in the hybrids as per cent deviation of the F_1 from the best popular variety

The Analysis of Variance was carried out for various characters (Panse and Sukhatme, 1957).

- To test the significance of differences among various genotypes with respect to various characters and
- To estimate the variance components and other genetic parameters like coefficient of variation, heritability and Genetic advance.

Estimation of components of variance

1. Variance (for a trait X)

Environmental variance, $\sigma^2 ex$ = Exx

Genotypic variance, $\sigma^2 g_x$ = $\frac{Gxx-Exx}{r}$

Phenotypic variance, $\sigma^2 px$ = $\sigma^2 g_x + \sigma^2 ex$

Where Exx = Observed mean square for error

Gxx = Observed men square for genotypes

2. Coefficient of variation

Phenotypic and genotypic coefficients of variation (PCV and GCV) for a trait X were estimated as suggested by Singh and Chaudhary(1979).

3. Correlation Analysis

The correlation coefficients (phenotypic,genotypic and environmental) were worked out based on the formulae given by Singh and Chaudhary(1979).

3.2 Molecular Marker Analysis

3.2.1 Isolation of genomic DNA

Isolation of genomic DNA was done from all the 21 samples following the procedure of Regowsky et al. (1991) with required modifications. The procedure is as follows:

- Approximately 0.1 g of tender leaf sample was taken in a clean autoclaved mortor and crushed by freezing in liquid nitrogen.
- The powder was transferred to 2ml eppendorf tube and 1ml of extraction buffer [1.00g SDS (1%), 1.576g Tris Hcl (100mM) 0.584g of Sodium Chloride (100mM), volume made up to 100ml with distilled water]was added.
- The tubes were then placed in a water bath maintained at 60°c for 30 min after homogenization.
- The mixture was then centrifuged at 10000 rpm at 4°c for 10 min.
- The aqueous phase was collected and 400 µl of phenol Chloroform (25:24) was added and again centrifuged at 10000 rpm at 4°c for 10 min.
- The supernatant was collected to which 200µl of chloroform iso-amylalcohol (24:1) was added again and centrifuged at 10000 rpm at 4°c for 5 min.

- The supernatant was collected and 60 µl of 3M sodium acetate and 600 µl of ice cold iso-propanol was added and kept overnight at -20°c for precipitation.
- The solution was centrifuged after about 16hrs at 12000 rpm for 10min and the supernatant was discarded without dislodging the pellet.
- The precipitate was then washed twice using 70% ethanol and dried.
- After drying ,the precipitate was dissolved in 100µl 0.1x TE buffer [Tris buffer 0.12g(10mM) ,EDTA 0.037g(1mM) and stored at -20°c.

3.2.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit. Required amount of agarose was weighed and melted in 1x TAE buffer. For genomic DNA 0.8 per cent gel was used. After cooling the solution to 42-45°c, ethidium bromide was added at the rate of 12µl 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 15-20min.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 60 V was used for the run. The power supply was turned off when the loading dye moved about ¾ th of the gel. The gel was documented using gel documentation system (BIORAD).

3.2.3 Quantification of DNA: After ensuring the quality of DNA in samples by electrophoresis the quality and quantity was measured by using spectrophotometer. 5µl of DNA dissolved in 0.1xTE was added to 3ml of distilled water and absorbance at 260nm and 280nm was read againt distilled water as

blank, using UV spectrophotometer (Spectronic Genesys 5). The concentration of DNA in sample was calculated using the formula.

$$\text{Amount of DNA } (\mu g/ml) = \frac{A260 \times 50 \times \text{Dilution factor}}{1000}$$

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

3.2.4 PCR analysis

Five primers reported to be linked to *Rf* genes were used for the analysis. The details of primers sequences along with their references are given in Table 2. The SSR primers were synthesized according to the base pairs.

Polymerase chain reaction was carried out in a total volume of 15µl with

- 15–20 ng of template DNA,
- 200 µM of dNTPs (Eppendorf, USA),
- 5 pmoles of each F and R primer,
- 1unit of Taq DNA polymerase (Bangalore Genei, India),
- PCR reaction buffer (Bangalore Genie,India)

. The cycling conditions were an

- Initial denaturation at 94°C for 5 minutes.
- followed by 35 cycles of PCR amplification under the following parameters
- 30 sec at 94°C,
- 30 sec at 54°C,
- 1 min at 72°C, followed by a final extension at 72°C for 5 minutes.

Amplified products were separated on 3% gel with Metaphor Agarose and Agarose in 3:1 ratio with 100 bp molecular marker (Bangalore Genie, India) and and visualized IN Gel documentation System.

Table.2. Details of SSR markers

Sl. No	SSR marker	Chromosome	Forward primer	Reverse primer	Annealing Temp (°C)	Linked *Rf* gene
1	RM6344	7	ACACGCCATGGATGATGC	TGGCATCATCACTTCCTCAC	62	***Rf* 4**
2	RM258	10	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	62	***Rf* -5, *Rf* -4 and *Rf* -(U-1)**
3	RM171	10	CATCCCCCTGCTGCTGCTCG	CGCCGGATGTGTGGGACTAG	58	***Rf* -4**
4	RM228	10	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC	60	***Rf* -4**
5	RM6100	10	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC	62	***Rf* -4**

Results

4. RESULTS

In the present investigation 19 rice accessions of Kerala were compared with two known restorers from TNAU based on 10 morphological traits and 5 SSR markers. The results are elaborated in this chapter.

4.1 Morphological markers

4.1.1 Variability studies

Data on 10 morphological traits were recorded on 21 varieties belonging to six genotypes. Mean, standard error and critical difference for the nine biometric variables and observation on one qualitative trait is given in Table 2. Analysis of variance showed significant F values for all the variables studied. F value ranged from 7.68 to 372.41.

1. Plant height (cm)

The plant height ranged from 42.13cm to 152.33cm. Mean plant height was 99.51 cm. Ptb-1 has recorded the highest (152.33cm) and Co-48 (42.13cm) recorded the lowest.

2. Pollen fertility per cent (%)

The pollen fertility per cent ranged from 90.2 to 95.0. Manu Priya has recorded the lowest (90.20) and CB87R (95.00) recorded the highest.

Table.3. Mean values of different characters for the genotypes studied

Varieties	X1	X2	X3	X4	X5
Ptb-1	**152.33**	91.29	**108.00**	2.74	40.24
Ptb-2	149.00	92.06	98.00	2.71	39.56
Ptb-5	131.16	91.83	90.66	2.49	**41.96**
Ptb-7	90.83	90.66	82.33	2.54	25.16
Ptb-8	111.33	90.58	95.00	2.46	26.32
Ptb-9	119.833	93.56	88.33	2.48	30.53
Ptb-10	102.16	93.96	**65.00**	2.58	18.20
Ptb-13	131.16	92.29	105.33	2.4	24.87
Ptb-14	117.00	93.43	95.00	2.37	27.69
Ptb-22	130.66	93.53	82.33	2.86	22.11
Aishwarya	103.00	91.96	95.00	2.51	16.01
Manu Priya	50.70	90.20	80.33	2.71	**12.82**
Co-48	**42.13**	94.40	89.66	**1.92**	17.63
CB-87R	42.30	95.00	94.66	2.24	16.27
Jyothy	82.66	93.20	80.33	**2.87**	15.81
Mattatriveni	84.00	92.36	74.66	2.40	15.93
Swarna Prabha	104.03	90.73	73.33	2.62	24.55
Uma	71.43	93.12	75.33	2.36	17.36
Neeraja	98.50	93.60	100.00	2.30	14.29
Kanchana	83.66	92.76	71.66	2.63	15.95
Varsha	91.86	92.56	91.00	2.32	13.40
SE	2.811	0.69	1.29	3.53	2.54
CD	8.03	1.98	3.69	0.101	7.26

X_1 Plant Height(cm)
X_2 Pollen fertility percent(%)
X_3 Days to 50% flowering
X_4 100 grains weight(g)
X_5 Area of flag leaf(cm^2)

Table.3. continued on next page

Varieties	X1	X2	X3	X4	X5
Ptb-1	25.33	62.33	4.33	8.30	V
Ptb-2	25.00	109.00	3.66	7.03	V
Ptb-5	32.00	61.00	3.66	7.40	V
Ptb-7	28.33	53.33	4.66	6.33	V
Ptb-8	24.33	69.66	2.66	4.90	I
Ptb-9	25.00	84.33	3.66	7.50	h-i
Ptb-10	24.00	57.33	6.66	7.03	I
Ptb-13	25.66	108.66	3.33	8.06	v-i
Ptb-14	28.66	44.00	2.66	7.76	v-i
Ptb-22	28.66	63.00	3.33	7.23	I
Aishwarya	30.33	122.33	8.00	5.66	V
Manu Priya	45.00	66.00	7.66	6.56	v-i
Co-48	36.33	103.00	8.33	4.50	H
CB-87R	36.66	122.33	9.66	4.43	I
Jyothy	20.66	149.66	5.33	5.66	I
Mattatriveni	56.00	146.66	3.33	4.83	h-i
Swarna Prabha	43.00	107.66	3.33	6.66	I
Uma	33.66	69.66	8.33	7.80	V
Neeraja	26.66	121.33	7.33	6.73	H
Kanchana	33.33	109.66	8.33	6.23	I
Varsha	45.00	98.00	8.33	5.86	V
SE	0.94	2.71	0.34	0.25	
CD	2.7	7.74	0.99	0.71	

X_6	Pollen count	v	Vertical
X_7	No of grains per panicle	h	Horizontal
X_8	No of productive tillers	i	Inclined
X_9	Spikelet sterility (%)	v-i	Vertically inclined
X10	Orientation of flag leaf	h-i	Horizontally inclined

3. Days to 50% flowering

Days to 50% flowering ranged from 65- 108. The mean number of this character is 87.42. Ptb-1 recorded 108 and Ptb10 recorded 65.

4. 100 grain weight

The grain weight ranged from 1.92g to 2.87g. Jyothy recorded highest range (2.87g) and CO-48 recorded the lowest (1.92g).

5. Area of flag leaf

The area of flag leaf ranged from 12.82 cm^2 to 41.96 cm^2. The mean value of this character was 22.70 cm^2. It was maximum in Ptb-5 (41.96 cm^2) and minimum in Manu Priya (12.82 cm^2).

6. Pollen count

The pollen count ranged from 20.66 to 56. Mean value of this character was 32.07.Mattathriveni recorded the highest (56.00) and Jyothy recorded the lowest (20.66).

7. Number of grains per panicle

The mean number of grains per panicle ranged from 44.00 to 149.66. The mean value of this character was 91.85.Ptb-14 recorded the lowest (44.00) and Jyothy recorded the highest (149.66)

8. Number of productive tillers

The number of productive tillers ranged from 2.66 to 9.66. The mean number of productive tillers spikelet was 5.55. It has recorded maximum in CB-87R (9.66) and minimum in both Ptb-8 and Ptb-14.

9. Sterility (%)

The sterility percentage ranged from 4.43 to 8.30. The mean value of this character was 6.49. CB-87R has recorded the lowest (4.43) and Ptb-1 the highest (8.3).

10. Orientation of flag leaf

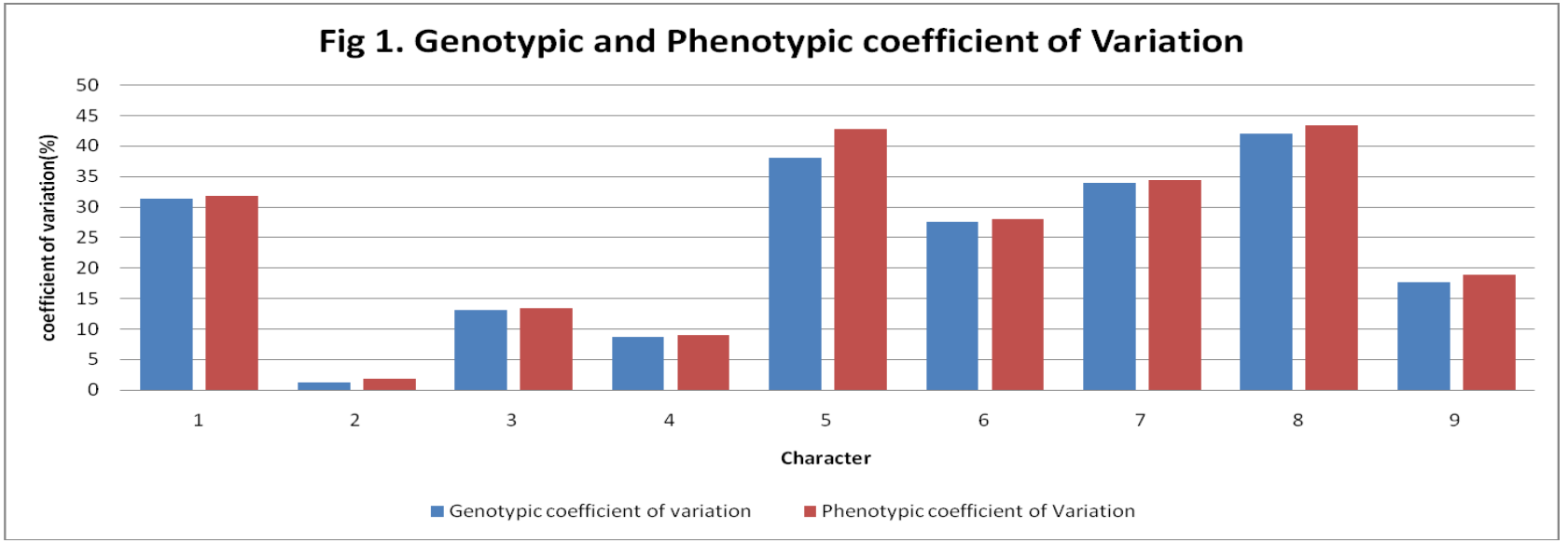
Seven varieties showed vertical inclination of flag leaf (Ptb-1, Ptb-2, Ptb-5, Ptb-7,Aiswarya,Uma,Varsha), seven varieties showed inclined orientation(Ptb-8, Ptb-10, Ptb-22, CB-87, Jyothy, Swarna Prabha, Kanchana),three varieties showed vertically inclined orientation(Ptb-13, Ptb-14,Manu Priya) and two varieties showed horizontally inclined orientation (Mattathiveni and Ptb-9. Two varieties showed horizontal inclination (Co-48, Neeraja).

4.1.2. Variation, Heritability and Genetic advance

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance for different characters are given in Table 3. Coefficients of variation for the 9 variables are compared in the histogram (Fig 1).

Table.4. Heritability, genetic advance, genotypic and phenotypic variances

Sl.No	Character	GCV	PCV	Heritability	GA
1	Plant Height	31.45	31.83	0.976	63.72
2	Pollen fertility percent (%)	1.21	1.78	0.465	1.58
3	Days to 50% flowering	13.15	13.4	0.963	23.25
4	100 grains weight	8.72	9.06	0.927	0.43
5	Area of flag leaf	38.16	42.8	0.794	15.91
6	Pollen count	27.55	28.02	0.966	17.9
7	No of grains per panicle	34.04	34.43	0.977	63.71
8	No of productive tillers	42.14	43.52	0.937	4.67
9	Spikelet sterility (%)	17.61	18.83	0.874	2.20



X1 - Plant height (cm)

X2 - Pollen fertility percent(%)

X3 - Days to 50% flowering

X4 - 100 Grains weight (g)

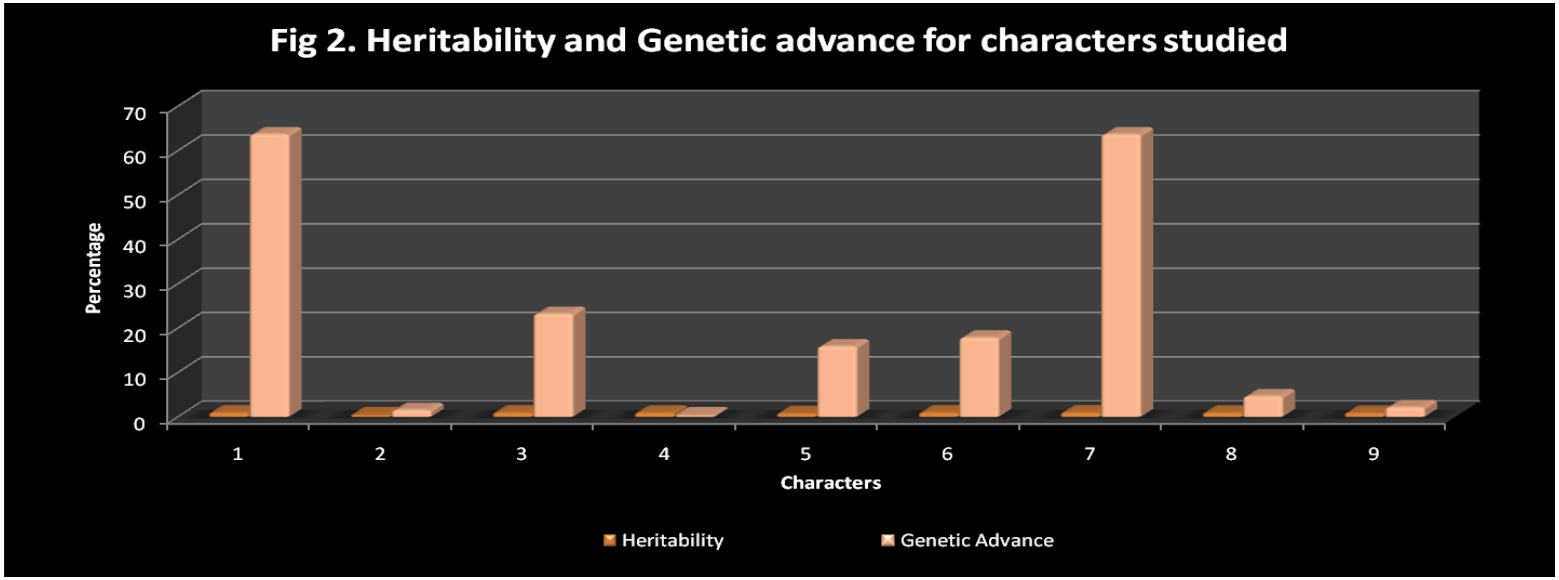
X5 - Area of flag leaf (cm^2)

X6 - Pollen count

X7 - No of grains per panicle

X8 - No of productive tillers

X9 – Spikelet Sterility (%)



X1 - Plant height (cm)

X2 - Pollen fertility percent(%)

X3 - Days to 50% flowering

X4 - 100 Grains weight (g)

X5 - Area of flag leaf (cm²)

X6 - Pollen count

X7 - No of grains per panicle

X8 - No of productive tillers

X9 – Spikelet Sterility (%)

Heritability and genetic advance for the variables are also compared in the histogram (Fig 2).

1. Plant height (cm)

The genotypic coefficient of variation (31.45) and the phenotypic coefficient of variation (31.83) were found to be high for plant height. Heritability was highest (0.976) for this character indicating more genetic influence. Expected GA was highest for this character.

2. Pollen fertility per cent (%)

The genotypic coefficient of variation (1.21) and the phenotypic coefficient of variation (1.78) were found to be the minimum for pollen fertility per cent. The heritability of this character was only 0.465 and GA was also lowest.

3. Days to 50% flowering

Days to 50% flowering showed medium GCV (13.15) and PCV (13.4). High heritability of per cent (96.3) was noted for this character and an expected GA of 23.5.

4. 100 grain weight

Low genotypic and phenotypic coefficient of variation (8.72 and 9.06 respectively) was observed for 100 grain weight. High heritability (93 %) was indicating high genetic influence on the character. But the expected GA was low.

5. Area of flag leaf

The GCV and PCV for area of flag leaf were 38.16 and 42.80 per cent respectively. High heritability (0.794) and GA of 15.91 was showed.

6. Pollen count

Medium GCV (27.55) and PCV (28.02) were recorded for pollen count. Maximum heritability value 0.966 and GA of 17.90 was showed for this character.

7. Number of grains per panicle

The GCV and PCV for number of grains per panicle were 34.04 and 34.43 respectively. High heritability (0.977) and very high expected GA (63.71) was recorded for this trait.

8. Number of productive tillers

High genotypic and phenotypic coefficients of variation were recorded for number of productive tillers (42.14% and 43.52%). This character recorded the highest heritability (0.937) and a GA of 4.67.

9. Sterility (%)

Low GCV and PCV (17.61 and 18.83) were recorded for sterility percentage. High heritability (0.874) was also recorded in this character.

Table .5 Selection indices of genotypes based on morphological traits in descending order

Sl.No	Varieties	Selection Indices
1	Mattathriveni	974.8362
2	CB-87	927.9025
3	Jyothy	906.5571
4	Neeraja	906.4861
5	Aiswarya	903.2479
6	Ptb-13	871.0661
7	Ptb-56	864.5131
8	Co-48	850.4646
9	Ptb-2	847.7329
10	Swarna prabha	819.1713
11	Ptb-50	808.6113
12	Ptb-9	749.401
13	Ptb-1	744.1164
14	Manupriya	734.9015
15	Ptb-8	713.0817
16	Uma	705.6938
17	Ptb-5	705.4233
18	Ptb-22	678.6787
19	Ptb-14	658.8041
20	Ptb-7	647.0726
21	Ptb-10	606.4876

4.1.3. Discriminant function analysis

Selection indices obtained for the twenty one genotypes based on the morphological traits studied in descending order is given in Table.4. The varieties are arranged in the descending order of selection values. The table shows that Mattathriveni is selected as the best based on the characters studied with a value of 974.831 closely followed by CB 87 with the value 927.90. The least value was shown by Ptb-10 (606.48).

4.1.4. Cluster analysis

Euclidean cluster analysis was conducted in the twenty one genotypes with the five characters, number of productive tillers, pollen fertility, spikelet fertility, pollen count and hundred grain weight. Dissimilarity matrix from the Euclidean cluster analysis is given in Table 5. Co-48 showed the least dissimilarity with Varsha (31.07) followed by Ptb-13 (62.48) and Aiswarya (63.35). CB 87 showed the least dissimilarity with Aiswarya (22.00) followed by Neeraja (37.77) and Kanchana (58.76).

4.2 Molecular marker analysis

The genomic DNA from the 21 accessions was isolated following the procedure of Regowsky *et al*. (1991) with required modifications. The procedure yielded good DNA whose quality and quantity was checked by gel electrophoresis using 0.8 per cent agarose gel (Plate 1) and by spectrophotometer, respectively. The quantity of DNA obtained was ranged from 60 to 690 ng/µl (Table 6).

Table 6. Dissimilarity matrix from cluster analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0.00																				
2	143.26	0.00																			
3	55.99	147.18	0.00																		
4	82.33	173.87	36.15	0.00																	
5	46.35	118.66	38.12	63.59	0.00																
6	88.85	79.62	73.60	95.76	49.90	0.00															
7	130.42	184.25	82.15	56.17	98.78	107.49	0.00														
8	139.30	22.38	150.84	180.13	121.60	89.18	196.26	0.00													
9	68.67	195.60	53.88	48.50	79.02	123.17	100.24	196.71	0.00												
10	78.08	146.27	28.10	30.66	46.32	67.41	57.37	153.73	68.57	0.00											
11	185.29	46.08	185.06	210.83	159.89	117.79	215.77	55.56	235.72	182.74	0.00										
12	103.10	152.41	53.68	63.75	78.44	86.34	83.07	160.01	95.01	52.83	180.19	0.00									
13	139.16	49.13	128.02	153.43	109.43	67.78	160.32	62.48	180.30	125.38	63.35	118.27	0.00								
14	188.87	58.22	186.25	212.77	164.17	122.51	218.04	65.88	237.41	184.62	22.00	176.98	60.08	0.00							
15	275.38	133.85	270.08	290.09	244.52	198.04	281.04	145.18	321.10	261.29	97.88	261.66	150.69	105.32	0.00						
16	287.40	162.37	271.29	293.05	257.18	213.02	286.44	172.73	324.65	265.18	123.22	245.32	151.43	112.77	107.97	0.00					
17	179.32	91.79	153.00	171.03	142.79	99.60	163.92	109.40	206.49	143.55	88.45	127.57	58.22	83.45	144.59	123.62	0.00				
18	104.36	139.41	55.04	57.38	68.47	65.82	56.64	150.32	99.62	36.02	169.11	39.97	109.67	168.99	243.87	241.17	118.76	0.00			
19	179.09	39.68	184.27	211.17	156.87	117.04	219.00	43.41	233.00	183.36	19.84	184.85	69.91	35.77	105.26	139.59	103.45	173.10	0.00		
20	181.17	84.12	157.47	173.12	142.75	95.49	160.86	104.87	210.29	145.12	80.22	138.30	58.76	79.98	128.86	131.42	34.15	120.61	94.17	0.00	
21	133.21	73.09	118.60	145.90	107.45	74.66	158.09	80.66	170.64	119.81	86.10	101.49	31.07	78.50	174.54	158.24	62.83	103.08	93.16	76.27	0.00

Table.7. Quality& quantity of DNA of the 21 rice accessions

Sl No.	Accession name	A 260 nm	A 280 nm	A260/A280	DNA Yield (ng/µl)
1	Ptb-1	0.013	0.008	1.62	390
2	Ptb-2	0.013	0.009	1.44	390
3	Ptb-5	0.004	0.004	1.00	120
4	Ptb-7	0.013	0.008	1.62	390
5	Ptb-8	0.014	0.010	1.40	420
6	Ptb-9	0.020	0.012	1.66	600
7	Ptb-10	0.009	0.008	1.125	270
8	Ptb-13	0.014	0.009	1.75	420
9	Ptb-14	0.015	0.013	1.15	450
10	Ptb-22	0.008	0.005	1.60	240
11	Aiswarya	0.025	0.015	1.66	750
12	Manupriya	0.005	0.003	1.66	150
13	Co-48	0.012	0.006	1.71	360
14	CB-87	0.008	0.005	1.60	240
15	Jyothy	0.009	0.007	1.28	270
16	Mattathriveni	0.029	0.013	2.23	870
17	Swarna prabha	0.003	0.002	1.50	90
18	Uma	0.012	0.008	1.259	360
19	Neeraja	0.015	0.006	2.50	450
20	Ptb-50	0.037	0.016	2.31	1110
21	Ptb-56	0.007	0.004	1.75	210

Table.8.Details of the Amplified Products of primers

Sl.No.	Primer	Alleles	Penetrance	Size
1	RM228	1 2 3	52.38 28.00 47.61	300bp
2	RM258	1 2 3	57.14 57.14 38.09	100-200bp
3	RM171	1 2	95.23 14.28	300-400bp
4	RM6100	1 2 3	57.14 80.95 33.33	100-200bp
5	RM6344	1 2 3	23.80 85.71 47.61	<100bp

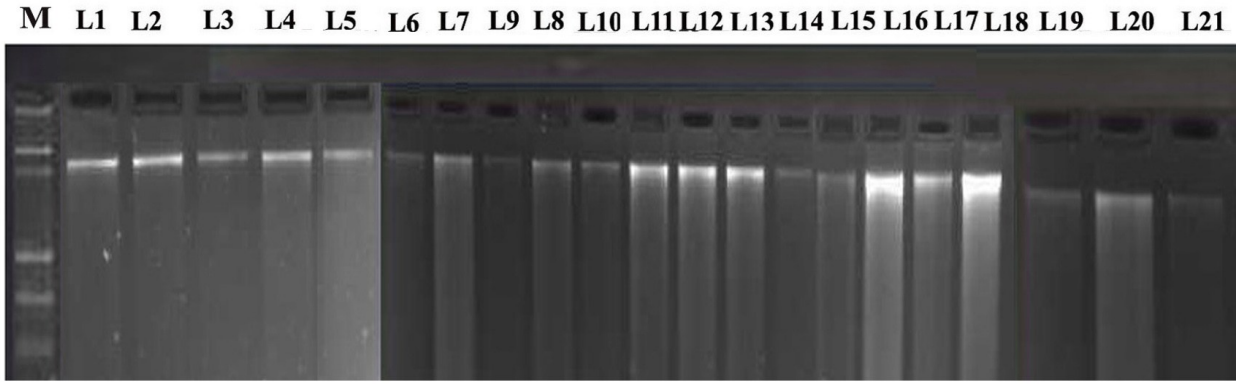


Plate 1. Genomic DNA of 21 rice accesions

Five SSR markers were used for the search of restorer genes in the 21 lines. The forward and reverse primers for the respective SSR primers were synthesized by Biovision. Different concentrations of the primer dntps and DNA were tested and the amplification was standardized with different annealing temperatures. The standardized protocol along with the annealing temperatures of the primers is given in materials and methods. All the five primers studied produced amplification and the details of the alleles, its penetrance and molecular weight of the alleles given in Table.7. All the SSR primers amplified products below 400 bps. SSR marker RM171 alone produced alleles above 300 bp. Most of the alleles were between 100-200bp. A total of 14 alleles were obtained and they are numbered from 'a' to 'n'. The barcode for the genotypes with respect to *Rf* loci is given in Fig.3.

4.2.1 SSR marker RM 228

The amplification profile of this marker is given in Plate.2. This marker produced 3 polymorphic products in the loci at 100-200 bp Allele 'a' was present in, Ptb10, Aiswarya, Jyothy and Uma. The second allele 'b' was present in all except Kanchana. The third allele was present in 8 accessions Ptb-2, Ptb-7, Ptb-8, Ptb-9, Ptb-10, Ptb-22, Jyothy, Mattathriveni and Swarna Prabha)

4.2.2 SSR marker RM 258

The amplification profile of this marker is given in Plate.3. This marker also produced 3 polymorphic products in the loci at 100-200 bp Allele 'd' was present in,Ptb1Ptb 7,CB87R,Jyothy,Swarna Prabha, Neeraja, Kanchana, and Varsha. The second allele 'e' was present in Ptb 8,Ptb 9,Ptb10,Ptb 13,Ptb14,Ptb22,Aiswarya,Manu Priya,CO48,CB87R,Jyothy and Neeraja.The third allele' f' was present in all except Ptb1,Ptb5,Ptb9,Ptb13,Mattathriveni,Swarna Prabha and Neeraja.

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	
Ptb-1															
Ptb-2															
Ptb-5															
Ptb-7															
Ptb-8															
Ptb-9															
Ptb-1															
Ptb-13															
Ptb-14															
Ptb-22															
Aiswarya															
Manupriya															
CO48															
CB87R															
Jyothy															
Matta thriveni															
Swarna Prabha															
Uma															
Neeraja															
Kanchana															
Varsha															

Fig.3 Barcode of rice genotypes at R*f* loci

Plate 2: Amplification profile of rice genotypes with SSR marker RM 228

1.Ptb-1, 2.Ptb-2, 3.Ptb-5, 4.Ptb-7, 5.Ptb-8, 6.Ptb-9, 7.Ptb-10, 8.Ptb-13, 9. Ptb-14, 10.Ptb-22, 11.Aishwarya, 12.Manupriya, 13.Co-48, 14.CB-87R, 15.Jyothy, 16.Mattatriveni, 17.Swarnaprapha, 18.Uma, 19.Neeraja, 20.Kanchana, 21.Varsha

m- Molecular weight marker

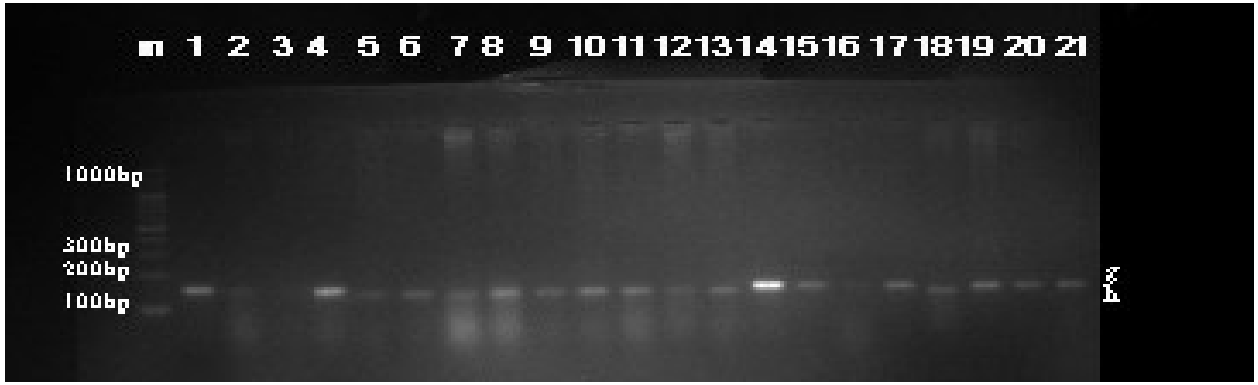
a,b,c - alleles

Plate 3: Amplification profile of rice genotypes with SSR marker RM 258

1.Ptb-1, 2.Ptb-2, 3.Ptb-5, 4.Ptb-7, 5.Ptb-8, 6.Ptb-9, 7.Ptb-10, 8.Ptb-13, 9. Ptb-14, 10.Ptb-22, 11.Aishwarya, 12.Manupriya, 13.Co-48, 14.CB-87R, 15.Jyothy, 16.Mattatriveni, 17.Swarnaprapha, 18.Uma, 19.Neeraja, 20.Kanchana, 21.Varsha

m- Molecular weight marker

d,e,f- alleles



Pate 4: Amplification profile of rice genotypes with SSR marker RM 171

1. Ptb-1, 2.Ptb-2, 3.Ptb-5, 4.Ptb-7, 5.Ptb-8, 6.Ptb-9, 7.Ptb-10, 8.Ptb-13, 9. Ptb-14, 10.Ptb-22,11.Aishwarya, 12.Manupriya, 13.Co-48, 14.CB-87R, 15.Jyothy, 16.Mattatriveni, 17.Swarnaprapha, 18.Uma, 19.Neeraja, 20.Kanchana, 21.Varsha

m- Molecular weight marker

g,h- allele

4.2.3 SSR marker RM 171

The amplification profile of this marker is given in Plate.4. This marker produced 2 polymorphic products in the loci at 300- bp. The two products were very close and differed only with 10bp. Allele ‘g’ was present in all except CB87R, Mattathriveni, Swarna Prabha, Neeraja, Kanchana and Varsha.The second allele ‘h’ was present in eight accessions only.(Ptb-2,Ptb- 8,Ptb-10,Ptb-22, Aiswarya, Manupriya ,Co 48, Jyothy and Varsha).

4.2.4 SSR marker RM 6100

The amplification profile of this marker is given in Plate.5. This marker produced 3 alleles in the loci between 100-200bp.The allele ’I’ was present in Ptb7, Ptb8, Ptb10, Co48, Jyothy, Mattathriveni, Uma, Neeraja and Kanchana. The second allele ‘j’ was abscent only in Ptb5, Ptb8, Ptb14, CB87R, Swarna prabha, Neeraja and Varsha. The third allele ’k’ was present in 9 accessions (Ptb 2, Ptb8, Ptb13, Ptb22, Aiswarya, Manupriya, Co48, Mattathriveni and Uma).

4.2.5 SSR marker RM 6344

The amplification profile of this marker is given in Plate.6. This marker produced 3 polymorphic products in the loci at 100- 200- bp. The two products were very close and differed only with 10bp. Allele ‘l’ was present in Ptb-8, Ptb-10, Ptb-22, Manu Priya,Co48, Jyothy, Uma and Neeraja. The second product ‘m’ was seen in Ptb-10, Manupriya, Jyothy and Uma. The third product ‘n’ was present only in Ptb-5, Ptb-7, Ptb-8, Ptb-10, Ptb-22, Co48, Jyothy and Mattathriveni.

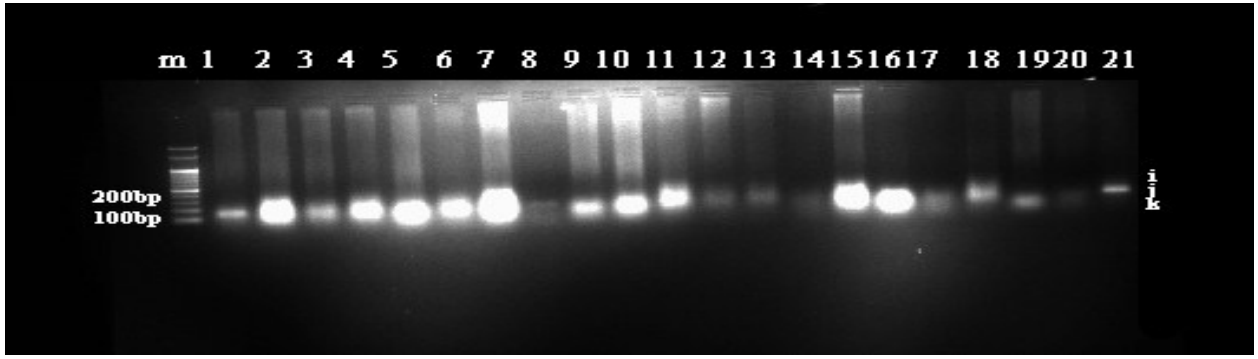


Plate 5: Amplification profile of rice genotypes with SSR marker RM 6100

1. Ptb-1, 2.Ptb-2, 3.Ptb-5, 4.Ptb-7, 5.Ptb-8, 6.Ptb-9, 7.Ptb-10, 8.Ptb-13, 9. Ptb-14, 10.Ptb-22, 11.Aishwarya, 12.Manupriya, 13.Co-48, 14.CB-87R, 15.Jyothy, 16.Mattatriveni, 17.Swarnaprapha, 18.Uma, 19.Neeraja, 20.Kanchana, 21.Varsha

m- Molecular weight marker

i,j,k- alleles

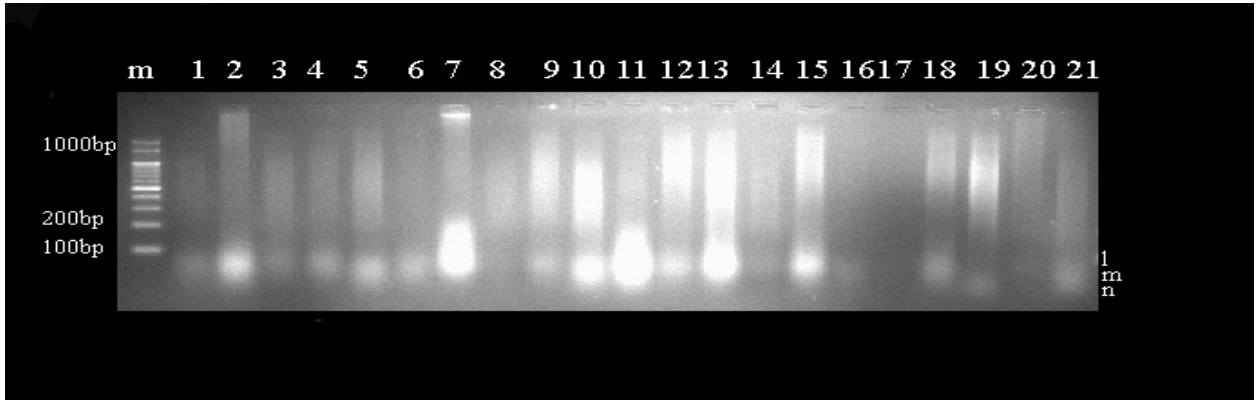


Plate 6: Amplification profile of rice genotypes with SSR marker RM 6344

1.Ptb-1, 2.Ptb-2, 3.Ptb-5, 4.Ptb-7, 5.Ptb-8, 6.Ptb-9, 7.Ptb-10, 8.Ptb-13, 9. Ptb-14, 10.Ptb-22, 11.Aishwarya, 12.Manupriya, 13.Co-48, 14.CB-87R, 15.Jyothy, 16.Mattatriveni, 17.Swarnaprapha, 18.Uma, 19.Neeraja, 20.Kanchana, 21.Varsha

m- Molecular weight marker

l,m,n- alleles

Discussion

5. DISCUSSION

Hybrid rice technology is considered as one of the promising, practical, sustainable and eco-friendly options to break the yield ceiling witnessed in rice. Following the success of hybrid rice in China the technology is being adopted now in India, Vietnam, Philippines, Indonesia, Myanmar and Bangladesh. The area under hybrid rice is increasing and many hybrids have been released both under private and public sector.

The major constraint in the hybrid cultivation is a sound system for hybrid seed production .Three-line system involving a cytoplasmic male sterile (CMS) line, a maintainer line and restorer line is the most popular method worldwide in almost all the crops including rice in which hybrids have been developed and commercialized. Cytoplasmic male sterility (CMS) is a maternally inherited trait that results in the inability of the plant to produce fertile pollen (Virmani et al.1994).

For developing high yielding heterotic hybrids, the first step is to identify restorers that can efficiently restore the fertility of F_1. The process of screening for the trait of fertility restoration is laborious and time consuming as it involves test crossing with a set of CMS lines and evaluation of F_1 for pollen and spikelet fertility. 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 (Barzkar 2008). The genes controlling fertility restoration do not behave identically under different genetic backgrounds because of which different segregation ratios are obtained in different combinations of CMS and restorer lines (Zhou et al. 1986

The nature of genetic control and mode of action of *Rf* genes have not been completely deciphered. The markers linked to the *Rf* genes could be of significant help in understanding the inheritance of the trait and targeted identification and introgression of *Rf* genes in breeding programmes. However, the markers which have been reported to be linked to the *Rf* genes have not been validated in alternate populations and the different restorer lines used in India have not been

characterized for their allelic status with respect to these markers. The above study was undertaken to screen the local varieties of Kerala for locating good restorers using morphological traits linked to restoration and with SSR markers linked to *Rf* loci.

The results are discussed below.

5.1. Morphological analysis

Among the morphological traits studied, pollen fertility percentage which contribute maximum to restorer capacity (Hu and Li 1985) was found to be above 90% for all the genotypes studied.CB87R the known restorer had the maximum value followed by Neeraja and Ptb-10. Euclidean cluster analysis conducted with the restorer characters gave a clustering which is depicted in the dendrogram (Fig.4) .This produced two major clusters. The first cluster included Aiswarya,Neeraja, CB87R, Ptb-2, Ptb-13, Co48, Varsha, S.Prabha and Kanchana. The varieties Aiswarya, Neeraja, Ptb-2, Ptb-13, Varsha, S.Prabha and Kanchana can be considered as having characters similar to the fertility characters of the known restorers Co48 and CB87R .Of these Neeraja had been reported as restorer for WA cytoplasm by Leenakumary et al.(1998) and Rosamma and Vijayakumar (2005). With flag leaf orientation also this variety is similar to CB87R both having horizontal flag leaf orientation.With respect to selection indices Mattathriveni is the best with maximum scores followed by CB87R. Vanaja et al. (2003) had reported Mattathriveni as a probable restorer for male sterile source Vyttila 3. Considering the morphological analysis Neeraja and Mattathriveni are showing good restorer qualities.

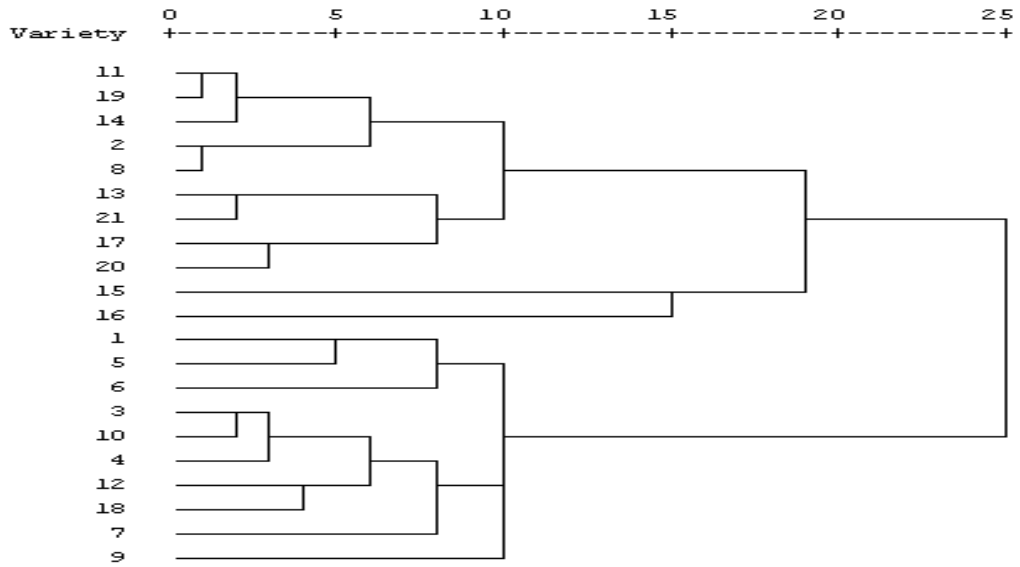


Fig.4.Dendrogram constructed with Eucledian cluster analysis

5.2. Molecular marker analysis

All the SSR markers selected in the study showed amplification in all the genotypes. The SSR markers RM 258, RM228, RM6100, RM6344 and RM171 showed polymorphism in the profile. The difference between the polymorphic loci was less than 100bp and could be made visible in the gel with Metaphor agarose.

RM 228 is an SSR marker mapped on chromosome 10 and reported to be linked to *Rf -4* gene (Mishra et al.2003). Gramene site (www.gramene.org) have given the position of the marker as in Fig.5. RM 228 produced three alleles at the loci between 100-200 bp. The same was reported by Sheeba et al. (2009). Two of the alleles were abscent in the two known restorers included in the study. The allele "b" was present in all except Kanchana. But this allele cannot be considered as linked to *Rf* loci as Jyothy and Ptb 10 have been reported as maintainers by Rosamma and Vijayakumar 2005. Leenakumari et al. 1998 also have reported Jyothi to be a good maintainer.

RM 258 is an SSR marker mapped on chromosome 10 and reported to be linked to *Rf -4* ,*Rf 5* and *Rf* (U1)genes (Haung et al.2003 and Mishra et al.2003. Gramene site(www.gramene.org) have given the position of the marker as in Fig.6. RM258 produced three alleles but only one of them was present in both the known restorers. Nine other lines, Ptb8, Ptb9, Ptb10, Ptb13, Ptb14, Ptb22, Aiswarya, Manupriya and Uma had this allele. But of these Ptb10 is reported to be a maintainer and Ptb9 a partial maintainer by Rosamma and Vijayakumar (2005). They have also reported Aiswarya as a restorer for WA cytoplasm. Leenakumari et al.(1998) in their screening of lines for restoring ability have reported complete absence of pollen fertility in the F1s of crosses with Aiswarya and IR 58025A.

RM6100 is an SSR marker mapped on chromosome 10 and linked to *Rf 4* gene (Singh et al.2005).The map position of this marker is given in site

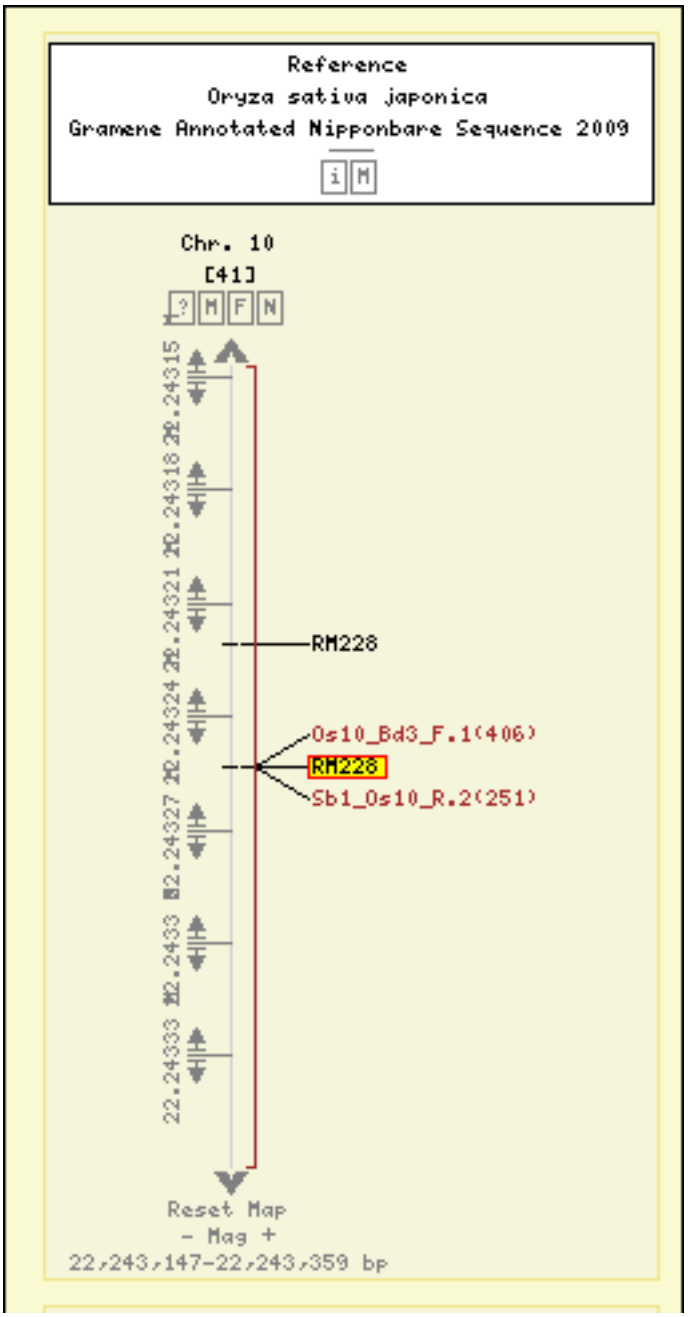


Fig.5.Map position of RM228 marker as given by graminae

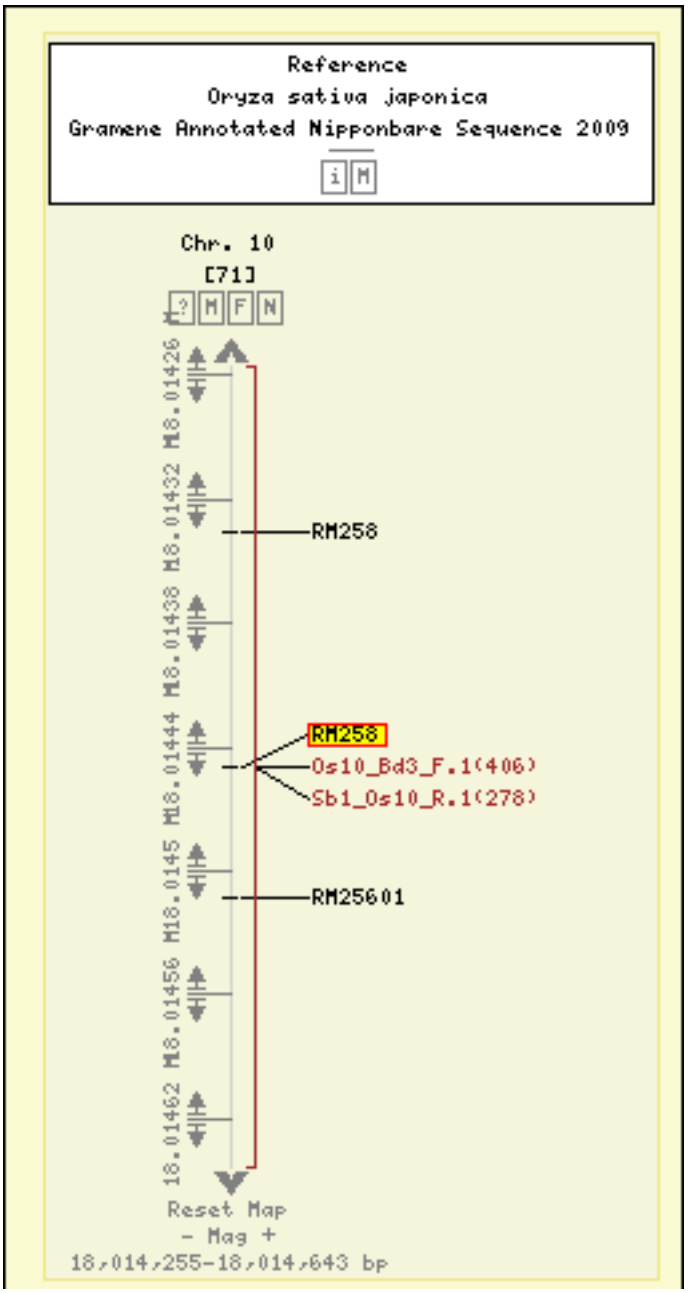


Fig.6.Map position of RM258 marker as given by gramin

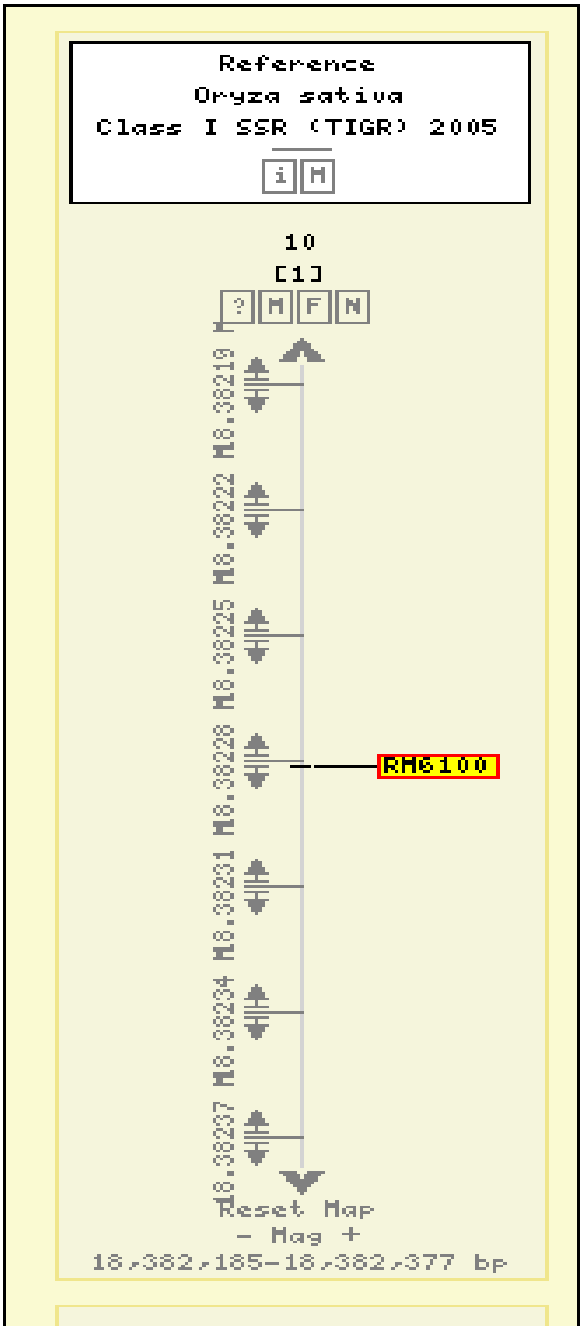


Fig.7 Map position of RM6100 marker as given by graminae

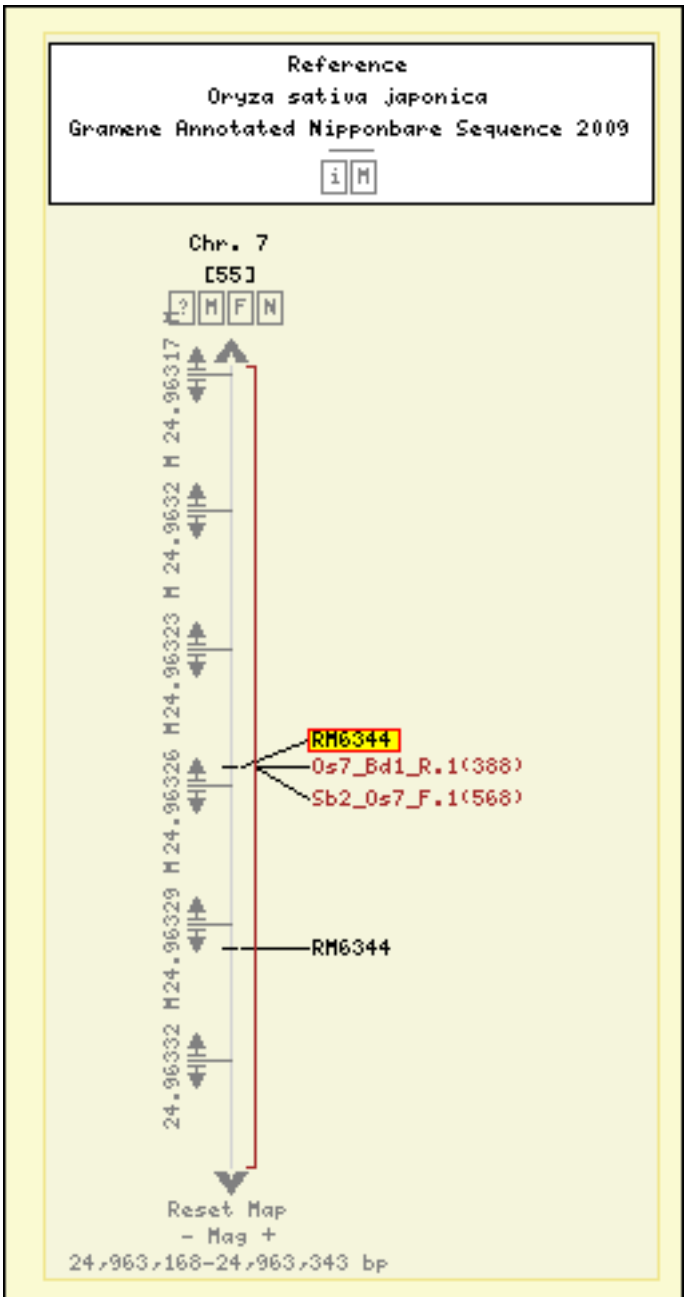


Fig.8 Map position of RM6344 marker as given by graminae

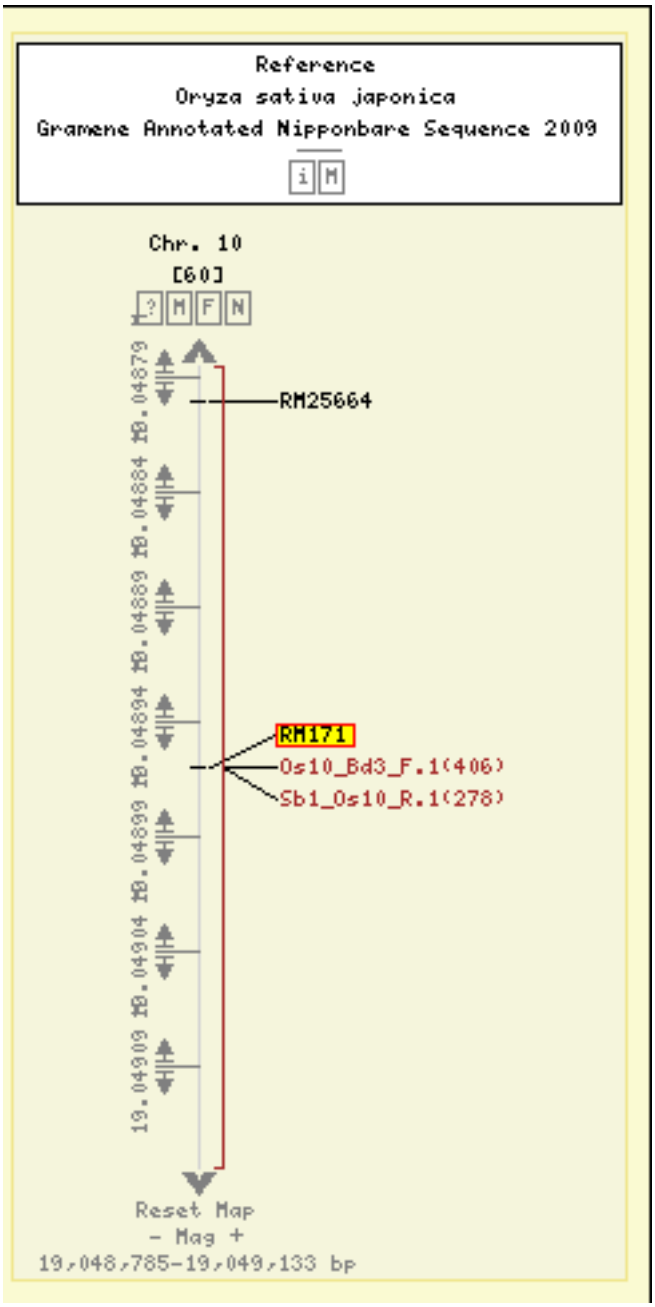


Fig.9.Map position of RM171 marker as given by graminae

the existence of four or more *Rf* loci partly responsible for the many partial restorers that we identify regularly through test crosses using the source nursery of hybrid rice breeding in many countries. Therefore, an ideal restorer should carry homozygous dominant *Rf* genes on all such loci to give a high degree of restoration (Bazrkar et al 2008). In this study RM171 and RM 6100 gave easily detectable polymorphism and could distinguish the restorers in a comparatively better way. These two markers can be recommended for screening of restorers through marker aided selection.

Restoration of fertility in rice is mainly controlled by at least three major genes (*Rf -3* ,*Rf -4* and *Rf* (U1) along with many QTLs control the trait of fertility restoration for WA cytoplasm in different restorer lines and each restorer lines may have a different combination of alleles of three different genes and QTLs which ultimately decide the degree of fertility restoration ability of that particular line. The genes controlling fertility restoration do not behave identically under different genetic background because of which different segregation ratios are obtained in different combinations of CMS and restorer lines (Zohu et al 1986). The nature of genetic control and mode of action of *Rf* genes have not completely been deciphered .The markers linked to through *Rf* genes could be of significant help in understanding the inheritance of the trait and argeted gene identification and introgression of *Rf* genes in the breeding programmes (Sheeba et al 2009). However the markers which have been linked to the *Rf* genes have not been validated in alternate populations and the different restorer lines used in India have not been characterized for their allelic status with respect to these markers. In this context this study has standardized the amplification of rice genomic DNA of Kerala with SSR markers for the *Rf* loci. Screening of lines with more markers linked to other genes of restorers such as *Rf 3* and *Rf* (U1) might give a clear idea. Poly Acrilamide Gel Electrophoresis with silver staining might help in locating alleles even in 10bp difference and this may give a better assessment of the markers.

To get a better idea of the nature of *Rf* loci in the genotypes a dendrogram was constructed with data from the amplification profile of different primer pairs, to know the similarity of the genotypes with respect to *Rf* locus.(Fig.10) The dendrogram shows that the varieties Varsha and Neeraja with close similarity with Co48 and CB87R with respect to *Rf* locus. The dendrogram based on the fertility traits also clustered Varsha and Neeraja along with Co48 and CB87R. Ptb-10 and Aiswarya are on par with each other, both were reported as maintainers by Leenakumari et al (1998). At around 70% similarity they got grouped with Ptb22 and Jyothy, these varieties are also reported maintainers (Rosamma and Vijayakumar 2005 and Leenakumary et al 1998). Manupriya clustered with CB87R and Neeraja at around 60% similarity. Members of these two clusters can be used to develop maintainers(Cluster 1) and restorers (Cluster 2) respectively.

Most of the restorers so far reported are white kernelled and a probable linkage between white kernel colour and Rf loci was reported by Leenakumary et al 1998.In this study also Neeraja which showed tight linkage with Co48, the known restorer is a white kernelled variety. However, Manupriya and Varsha which also showed close similarity with the known restorers is red kernelled varieties.Keralites are very specific in the quality of rice they consume. A red kernelled bold grain with nonstickiness after cooking is preferred mostly. So the development of restorers and maintainers in this quality back ground is a must for the commercial success of hybrid rice in Kerala. This is one of the reasons for the nonpreference of hybrids released in the neighbouring states in Kerala. In this context the red varieties Varsha and Manupriya can be tested for their restoring ability and can be used for the development of restorers with specific quality requirements of Kerala and thereby superior hybrids which can break the yield plateau of the high yielding varieties can be released.

Summary

6. SUMMARY

The present study on "Locating restorers from Kerala rice for hybrid rice technology using marker assisted selection" was conducted at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during the year 2009-2011. The study was under taken in 19 rice varieties released by Kerala Agricultural University and two restorers used in hybrid rice technology in Tamil Nadu Agricultural University. The objectives of this study was to compare the morphological characters of the genotypes with the known restorers and to study the allelic status of the Kerala varieties with respect to SSR markers linked to *Rf* loci. It is also intended to assess the possibility of using Marker Assisted Selection for screening for restorers.

Biometrical analysis showed significant variability for all the characters chosen for the study.Euclidean cluster analysis conducted with the restorer characters produced two major clusters. The first cluster included Aiswarya,Neeraja,CB87R,Ptb2,Ptb13,co48,Varsha,Swarnaprabha and Kanchana The varieties Aiswarya,Neeraja,Ptb2,Ptb13,Varsha,Swarnaprabha and Kanchana can be considered as having characters similar to the fertility characters of the known restorers . All the SSR markers selected in the study showed amplification in all the genotypes. The SSR markers, RM258, RM228, RM6100, RM6344 and RM171 showed polymorphism in the profile. SSR marker RM228 mapped on chromosome 10 and linked to *Rf4* gene produced three alleles at the loci between 100-200 bp.SSR marker RM 258 mapped on chromosome 10 and linked to *Rf -4* ,*Rf 5* and *Rf (u1)*genes produced three alleles but only one of them was present in both the known restorers.SSR marker RM6100 mapped on chromosome 10 and linked to Rf4 gene produced three alleles.SSR marker RM171 on chromosome 10 linked to

Rf 4 gene produced two alleles between 100-200bp. SSR marker RM6344 located on chromosome 7 and linked to *Rf 4* locus produced three alleles between 100-200bp.

Many workers have reported close association of the SSR markers chosen for the study with restorer genes. Among the five SSR markers tested in this study RM171 and RM 6100 showed a comparatively better performance in screening restorers. To get a better assessment of the *Rf* loci in these lines a dendrogram was constructed with data from the amplification profile of different primer pairs to know the similarity of the genotypes with respect to *Rf* locus. The dendrogram shows that the varieties Varsha and Neeraja with close similarity with Co48 and CB87R with respect to *Rf* locus. The dendrogram based on the fertility traits also clustered Varsha and Neeraja along with Co48 and CB87R. Ptb-10 and Aiswarya are on par with each other both were reported as maintainers. Members of these two clusters can be used to develop maintainers and restorers respectively.

Most of the restorers so far reported are white kernelled and a probable linkage between white kernel colour and *Rf* loci was reported in this study also. Neeraja which showed tight linkage with Co 48, the known restorer is a white kernelled variety. However, Manupriya and Varsha which also showed close similarity with the known restorers are red kernelled varieties. Keralites are very specific in the quality of rice they consume. A red kernelled bold grain with nonstickiness after cooking is preferred mostly. The red varieties Varsha and Manupriya can be tested for their restoring ability and can be used for the development of restorers with specific quality requirements of Kerala and there by superior hybrids which can break the yield plateau of the high yielding varieties can be released

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IDENTIFICATION OF FERTILITY RESTORER GENE IN HYBRID RICE TECHNOLOGY THROUGH MARKER ASSISTED SELECTION

By

KOSANA ABHISHIKTH

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Abstract of the Thesis

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ABSTRACT

The research project entitled "Identification of restorers for hybrid rice technology through molecular marker aided selection" was carried out in the department of Plant Breeding and Genetics ,College of Agriculture,Vellayani during the year 20010-11. The major objectives of this study was to Compare the morphological characters of the genotypes with the known restorers and to study the allelic status of the Kerala varieties with respect to SSR markers linked to *Rf* loci. This study also intended to assess the possibility for using Marker Assisted Selection for screening for restorers. The study was under taken in 19 rice varieties released by KAU and two restorers used in hybrid rice technology in Tamil Nadu Agricultural University.

Biometrical analysis showed the varieties Aiswarya,Neeraja,Ptb2,Ptb13,Varsha,Swarna Prabha and Kanchana are having characters similar to the fertility characters of the known restorers . All the SSR markers selected in the study showed amplification in all the genotypes .The SSR markers, RM258, RM228, RM6100, RM6344 and RM171 showed polymorphism in the profile. SSR marker RM228 mapped on chromosome 10 and linked to *Rf4* gene produced three alleles at the loci between 100-200 bp. SSR marker RM 258 mapped on chromosome 10 and linked to *Rf -4* ,*Rf 5* and *Rf (u1)*genes produced three alleles but only one of them was present in both the known restorers. SSR marker RM6100 mapped on chromosome 10 and linked to *Rf4* gene produced three alleles.SSR marker RM171 on chromosome 10 linked to *Rf 4* gene produced two alleles between 100-200bp. SSR marker RM6344 located on chromosome 7 and linked to *Rf 4* locus produced three alleles between 100-200bp

Among the five SSR markers tested in this study RM171 and RM 6100 showed a comparatively better performance in screening restorers. The dendrogram constructed with data from the amplification profile of different primer pairs show that the varieties Varsha and Neeraja with close similarity with Co48 and CB87R with respect to *Rf* locus. The dendrogram based on the fertility traits also clustered Varsha and Neeraja along with Co48 and CB87R. Ptb-10 and Aiswarya are on par with each other both were reported as maintainers. Members of these two clusters can be used to develop maintainers and restorers respectively.

Most of the restorers so far reported are white kernelled and a probable linkage between white kernel colour and *Rf* loci was reported in this study also Neeraja which showed tight linkage with Co 48 the known restorer is a white kernelled variety. However Manupriya and Varsha which also showed close similarity with the known restorers are red kernelled varieties. Keralites are very specific in the quality of rice they consume. A red kernelled bold grain with non stickiness after cooking is preferred mostly. The red varieties Varsha and Manupriya can be tested for their restoring ability and can be used for the development of restorers with specific quality requirements of Kerala and there by superior hybrid which can break the yield plateau of the high yielding varieties can be released