GENETICS OF CYTOPLASMIC-NUCLEAR MALE STERILITY AND IDENTIFICATION OF MOLECULAR MARKERS OF FERTILITY RESTORER GENES IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)

BY

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Thesis submitted to the Chaudhary Charan Singh Haryana Agricultural University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

PLANT BREEDING

COLLEGE OF AGRICULTURE

CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY, HISAR-125 004

2005

Dedicated

to

My Late Grand-Parents

Who Were Pearl Millet Farmers

&

With Gratitude

to

My Parents

CERTIFICATE I

This is to certify that this thesis entitled, **"Genetics of cytoplasmic-nuclear male sterility and identification of molecular markers of fertility restorer genes in Pearl millet (*Pennisetum glaucum* (L.) R. Br.)"** submitted for the degree of Doctor of Philosophy in the subject of **Plant Breeding** of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Dev vart Yadav** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(R. K. Behl)

Major Advisor

CERTIFICATE II

This is to certify that this thesis entitled, **"Genetics of cytoplasmic-nuclear male sterility and identification of molecular markers of fertility restorer genes in pearl millet (*Pennisetum glaucum* (L.) R. Br.)"** submitted by **Dev vart Yadav** to the Chaudhary Charan Singh Haryana Agricultural University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the subject of **Plant Breeding** has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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Acknowledgement

It gives me immense pleasure to express my esteemed and profound sense of gratitude to the Chairman of my Advisory committee, Dr. R.K. Behl, Professor & Head (Teaching section), Department of Plant Breeding, CCS HAU, Hisar, for his kind encouragement, guidance and unstinted support during my study at HAU and during the course of achieving the final shape of this thesis.

I am sincerely obliged and grateful to the Co-chairman of my Advisory committee, Dr. K.N. Rai, Special Project Scientist, Pearl millet Breeding, ICRISAT, Patancheru, for providing excellent research facilities, meticulous guidance and constructive criticism throughout the period of this investigation and bringing out the manuscript.

I am also very thankful to members of my advisory committee, Dr. M.S. Punia, Professor, Plant Breeding, Dr. R.K. Jain, Professor, Biotechnology & Molecular Biology, Dr. Lajpat Rai, Professor, Mathematics & Statistics and Dr. O.P.S. Rana, Professor, Genetics and Dean, PGS nominee for constructive suggestions and guidance for completion of this endeavor.

I owe my sincere thanks to Dr C.T. Hash, Principal Scientist, Cereal Molecular Breeding, ICRISAT, for his invaluable guidance and pertinent suggestions and steadfast help in formulating and successfully carrying out the molecular lab work of the study.

I am sincerely thankful to Dr. R.S. Waldia, Professor & Head, Drs M.L. Saini and B.P.S. Lather, ex-Head, Department of Plant Breeding, CCS HAU Hisar for their constant encouragement and support throughout the course of study. I would also like to thank the Dean PGS, CCS HAU for his kind authorization and full support for carrying out my research work at ICRISAT.

My special thanks to Dr V.N. Kulkarni, Visiting Scientist, pearl millet breeding, ICRISAT for his critical comments and suggestions and constant encouragement and support during the crucial phase of writing of the manuscript.

All possible logistic support and technical assistance rendered by Mr. A.K. Singh, Scientific Officer and the technical staff namely Mr Chandra Reddy, Mr Ishwar Rao, Mr Krishniah, Mr Anantha Kishan, Mr Ahmeddudin, Mr. Bhaskar Raj and team, Mr. S.B. Stanley, Ms. S. Devi, and the RWF staff of pearl millet breeding team for field work and AGL technical staff especially Mr. Narsi Reddy and Ms. Seetha Kanan, ICRISAT, who helped me in completing my lab work efficiently, expediently and smoothly, is duly acknowledged. I also express my sincere thanks to former Scientific Officers, pearl millet breeding, Mr A.S. Rao and Mr. Satish Pareek for their support and help.

I would like to express my heartfelt and profound gratitude to Dr. J.H. Crouch, former Head, Applied Genomics Laboratory, for providing help and facilities for carrying out lab work successfully.

I wish to express my sincere and wholehearted gratitude to Dr. I.S. Khairwal, Co-ordinater, All India Co-ordinated Pearl Millet Improvement Program

(AICPMIP) for his continuous encouragement and great support to carry out my doctoral research at ICRISAT. I also express my sincere gratitude to Dr. C.R. Bainiwal, former Head Bajra section and Dr A.K. Chhabra, Associate Professor (Plant Breeding), CCS HAU, Hisar.

With earnest regards and immense gratitude, I wish to acknowledge and express my thanks to Dr. S. Chandra (Principal Scientist & Head, Biometrics & Bioinformatics), Ms. Rupa Devi and Mr. Prashant Kumar (Scientific Officers, Biometrics unit), ICRISAT, for their valuable advice and help rendered during analyses of field and molecular experiments.

I am also grateful to Dr. Rattan Yadav, Molecular Geneticist, (IGER, Aberystwyth, UK), Dr. K.L. Saharawat, Consultant, ICRISAT, Dr. O.P. Yadav, Senior Scientist, CAZRI, Jodhpur, Dr. H.C. Sharma, Principal Scientist, Entomology, Dr R.P. Thakur, Senior Scientist, Plant Pathology, Dr Ramesh, Visiting Scientist, Sorghum breeding, ICRISAT and Dr. A.K. Sarial, Associate Professor, University of Addis Abbaba, Ethiopia for being supportive and encouraging throughout this research project, and for giving me time for scientific discussions.

I take this opportunity to thank Dr. O.P Rupela, Head, Learning Systems Unit and Dr. C.L.L. Gowda, Program Leader, Global Theme-Crop Improvement, ICRISAT for their kind cooperation to work in collaboration with PMB division of ICRISAT on Memorandum of Understanding.

Special thanks are extended to Library staff, Learning Systems Unit staff especially Mr. Prasad Rao and Mr. Thayag Raj and the field staff of FME unit for their excellent assistance during my research work at ICRISAT. I am also thankful to Housing and Food services staff for making my stay at ICRISAT comfortable and enjoyable.

From the core of my heart, I owe this achievement to the highest blessings and good wishes of my parents, sisters and brother-in-laws, and affection of my nieces and nephews that I could attain academic heights by undergoing doctoral studies successfully.

I express my cordial thanks to all my senior colleagues and friends, Arun, Ranjana, Azhaguvel, Raghu, Surender, Lava Kumar, Gauri, Masood Rizvi, Senthil, S.P. Mehtre, Santosh, Pranjan, Mukesh, Mohan, Pradeep, Nepolean, Satish, Velu, Rupesh & Sonia, Manoj & Shivani, Sukhbir, Anil, Virender and Gaurav for giving a nice company and full assistance during my stay, both at ICRISAT and CCS HAU Hisar.

I gratefully acknowledge HAU and ICRISAT for providing research scholarships and funds and excellent facilities to carry out my doctoral research.

Date :

Place:

(Dev Vart Yadav)

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1. INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a multi-purpose cereal grown for grain, stover and green fodder on about 27 million hectares, primarily in the arid and semi-arid regions of Africa and Asia (FAO, 2000). Although its productivity in these environments is low, its ability to tolerate drought, heat and low soil fertility makes pearl millet an especially attractive crop species. It responds well to improved moisture and soil fertility conditions. In terms of annual production, pearl millet is the seventh most important cereal crop in the world. following wheat, rice, maize, barley, oat and sorghum (Qi *et al.*, 2004). India is a major pearl millet producing country with an area of around 9.5 million hectares and production of about 7 million tones, where five states (Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana) account for nearly 95% of the pearl millet cultivated area (CMIE, 2004).

The reproductive biology of pearl millet plays an important role in the application of a wide range of breeding procedures. Protogynous flowering leads to high outcrossing rate ranging between 70 to 80% (Burton, 1974), making the population structure of this crop highly heterozygous and heterogeneous. With the added advantage of commercially exploitable cytoplasmic-nuclear male-sterility systems, both open-pollinated cultivars (synthetics and composites) and hybrid cultivars (single cross, three-way cross, top cross and inter-population hybrids) are feasible. However, single crosses are the most common hybrid cultivar type used commercially in pearl millet. Seed of all of the pearl millet hybrid types can be produced with or without the aid of cytoplasmic male-sterile seed parents, but use of cytoplasmic male-sterile seed parents greatly facilitates commercial hybrid seed production.

A high degree of heterosis for grain yield has been reported in pearl millet, with grain yield of F_1 hybrids exceeding those of the higher-yielding parental lines by up to 425% (Virk, 1988). The discovery of a stable cytoplasmic-nuclear male-sterility (CMS) system (Burton, 1965) opened up the possibility of commercial exploitation of heterosis through hybrid cultivars. Since then, most of the commercial hybrids developed so far have the A_1 cytoplasm in the female parents. Several other CMS systems, like A_2 and A_3 (Burton and Athwal, 1967), A_4 (Hanna, 1989) and A_5 (Rai, 1995) differing from each other and from the A_1, have been documented; and a few others have also been reported such as PT 732A (Appadurai *et al.*, 1982), ex-Bornu (Aken'Ova, 1985), A_v (Marchais

and Pernes, 1985), Ghana and Botswana sources (Appa Rao *et al.*, 1989) and A_{egp} (Sujata *et al.*, 1994). Utilization of these CMS systems in male-sterile line development will have significant effect in overcoming the potential vulnerability of the commercial hybrids to disease and insect-pest epidemics due to cytoplasmic uniformity, as witnessed in case of southern leaf blight epidemic on Texas cytoplasm-based maize hybrids in the USA (Scheifele *et al.*, 1970).

Although a knowledge of the genetics of male-sterility and fertility restoration behaviour of the CMS systems would have considerable impact on enhancing hybrid parents breeding efficiency, the current knowledge regarding the genetics of cytoplasmic-nuclear male-sterility, the effect of different nuclear backgrounds on the inheritance pattern of male-fertility restoration, the linkage between fertility restorer genes, if any, and whether the fertility restorer gene(s) in different restorer lines of a CMS system are allelic or non-allelic, is not well understood in pearl millet. There are a few reports on the genetics of CMS systems in pearl millet involving A_1, A_2 and A_3 CMS systems (Burton and Athwal, 1967; Siebert, 1982) and the A_4 CMS system (Du *et al.*, 1996). These studies lacked a comprehensive approach with respect to the genetic material used, segregating populations studied and seasons in which these populations were evaluated. Therefore, results of these studies have remained only preliminary in nature. Reports on the effect of nuclear genetic background on fertility restoration of hybrids in pearl millet (Rai and Hash, 1990) and maize (Beckett, 1971) further emphasized the need for using isonuclear A-lines for the fertility restoration pattern analysis for CMS classification and for genetics of male-sterility. Development of isonuclear A-lines of five diverse cytoplasms (A_1, A_4, A_v, A_{egp} and A_5) in three nuclear genetic backgrounds of 81B, 5054B and ICMB 88004 (Rai, 1995; Rai *et al.*, 1996; Rai *et al.*, 2001; K.N. Rai, unpubl.) and their single-, dual- and triple-fertility restorers that restore fertility to either one or two or three sterile cytoplasms, respectively, laid the foundation for carrying out a comprehensive study in pearl millet with the aim to resolve the genetics of the above-mentioned five CMS systems.

The identification of molecular markers tightly linked to fertility restoration loci would further enhance the breeding efficiency by enabling for the classification of lines as either maintainer (B-line) or restorer (R-line) without the need for field evaluation of test crosses; and it would also permit their rapid backcross transfer of fertility restorer genes in elite inbred lines.

Resolving the genetics of CMS systems and identification of molecular markers that are closely linked to fertility restorer genes will represent an important step towards increasing the efficiency of breeding cytoplasmically diverse and stable male-sterile lines as well as their restorer lines for the eventual development of hybrid cultivars. The present study was designed to investigate the genetics of five CMS systems (A_1, A_4, A_v, A_{egp} and A_5) with greater emphasis on the A_1 and A_4 systems because the A_1 CMS is already an established system for commercial hybrid exploitation, while the A_4 CMS has high frequency of maintainers (60%) as compared to less than 30% for the A_1, but there are also about 30-40% restorers of the A_4 CMS in the germplasm (K.N. Rai, pers. comm.). Therefore, the proposed study was planned with the following objectives:

I. To investigate the genetics of five diverse CMS systems in pearl millet
II. To establish linkage relationships among fertility restorer genes of the A_1 and A_4 CMS systems
III. To test the allelism of fertility restorer genes of the A_1 and A_4 CMS systems
IV. To identify molecular markers linked to fertility restorer gene(s) of the A_1 and A_4 CMS systems

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The objective of this review is to present in brief the published research done on the subject in pearl millet and other crops. Cytoplasmic-nuclear male-sterility (CMS) has been the subject of many reviews in the past and has been dealt with in detail by Kaul (1988). Various aspects of cytoplasmic-nuclear male-sterility have been covered in some of the major crops by Edwardson (1970) and by Hanson and Conde (1985), and more recently by McVetty (1998) and Budar and Pelletier (2001). Schnable and Wise (1998) presented an excellent review on the molecular mechanisms underlying cytoplasmic male-sterility maintenance and fertility restoration systems in various crops. This review deals with CMS, its origin, characterization of CMS sources, various mechanisms responsible for male-sterility and male-fertility restoration and genetics of fertility restoration/sterility maintenance in various crops, with an emphasis on pearl millet.

2.1 Cytoplasmic-nuclear male-sterility

Cytoplasmic male-sterility is a maternally inherited trait characterized by the inability of plants to produce viable and/or functional pollen grains. It was discovered in 1921 in two strains of flax (*Linum usitatissimum* L.), which produced nearly 25% male-sterile F_2 progeny when crossed in one direction but not the other (Bateson and Gairdner, 1921). Cytoplasmic male-sterility has been observed in more than 150 plant species (Kaul, 1988). Cytoplasmic-nuclear male-sterility results from interaction of a sterile (S) cytoplasm and homozygous recessive alleles (*rf*/*rf*) at one or more nuclear fertility restorer genes. When a dominant nuclear fertility restorer allele (*Rf*) is present, fertility is restored irrespective of whether a sterile (S) or normal/fertile (N/F) cytoplasm is present. Thus, plant species exhibiting CMS possess both *rf* (male-sterility maintainer) nuclear genes as well as N/F- and S-cytoplasm types. CMS provides, at least in theory, a mechanism of pollination control in plants permitting easy and economical production of commercial hybrid seed. Dominant nuclear fertility restorer genes, *Rf*, restore the self-fertilization ability of hybrid plants having the male-sterility-inducing S-cytoplasm and are indispensable in CMS-based hybrid cultivars of crops in which fruit or seeds are harvested, such as cotton, pearl millet, sorghum, and rice. Furthermore, the phenomenon of CMS and fertility restoration make excellent models for the study of nuclear-cytoplasmic interactions, because fertility restoration relies on nuclear genes that suppress cytoplasmic gene dysfunction.

The morphological changes associated with CMS occur at different developmental stages and in different tissues depending upon the crop and CMS system observed. In several systems, one of the first visible effects of CMS is the premature degeneration of tapetum layer of the anthers, which plays an important role in microspore development.

2.1.1 Origin

CMS has several origins, both spontaneous and induced: inter-generic crosses, inter-specific crosses, intra-specific crosses, and mutations or antibiotic effects on cytoplasmic genes (Kaul, 1988). CMS occasionally arises from inter-generic distant crosses and has often been reported from *Aegilops* × *Triticum*, *Raphanus* × *Brassica*, *Triticum* × *Secale* crosses (Kaul, 1988). These crosses have the potential to maximize the difference between nucleus and cytoplasm resulting in incompatible nuclear-cytoplasmic interactions, thus increasing the chances of obtaining a new CMS system/source (Hanson and Conde, 1985). However, such crosses are also more likely to result in negative pleiotropic effects of the incompatible nuclear-cytoplasmic interactions on agronomic adaptedness, so successful application of this type of CMS is very limited. CMS frequently arises from interspecific crosses. There are a few successful examples of CMS created by such crosses such as in wheat, sunflower, tobacco, and cotton. CMS arising from intraspecific crosses is the most frequently reported and can be discovered in the wild, among progeny crosses of wild and cultivated forms, or among progeny from crosses of cultivars. CMS systems derived from such crosses can be cited in onion, maize, sorghum, *Brassica*, pearl millet and rice. CMS plants originating spontaneously in populations of male-fertile plants are quite common in crops such as onion, *Brassica*, maize, sorghum, *Raphanus* and pearl millet. CMS may be induced by the use of mutagens (e.g. pearl millet and sorghum) but none of the sources developed by this method have been found to be commercially usable till date. Induction of male-sterility and its restoration in plants by tissue-specific expression of a ribonuclease gene (*barnase*) and its corresponding inhibitor (*barstar*) from the bacterium *Bacillus amyloliquefaciens* have been reported by Mariani *et al.* (1990, 1992) in tobacco and Bisht *et al.* (2004) in *Brassica*.

2.1.2 Characterization of CMS sources

Because of their value in hybrid seed production, CMS systems have been identified and characterized in many crop species, including maize, sorghum, rice, rapeseed, wheat,

sunflower, rye, common bean, beat, carrot, onion, tobacco, petunia and pearl millet. CMS systems have traditionally been characterized by various ways:

2.1.2.1 Differential fertility restoration pattern

Fertility restoration patterns of F_1 hybrids developed from crosses between male-sterile lines possessing different CMS sources and a genetically diverse set of inbreds have been conventionally used for the classification of CMS sources in pearl millet (Burton and Athwal, 1967; Hanna, 1989; Rai *et al.*, 1996; Rai *et al.*, 2001), maize (Duvick, 1965; Gracen and Grogan, 1974; Beckett, 1971; Laughnan and Gabay-Laughnan, 1983), sorghum (Schertz and Ritchey, 1978; Worstell *et al.*, 1984; Schertz *et al.*, 1989), sunflower (Miller, 1996), barley (Ahokas, 1979, 1982), and many other crops. In light of the fact that significant effects of nuclear genetic background on fertility restoration of F_1 hybrids can reduce the power of fertility restoration pattern analysis in the classification of CMS sources, studies in pearl millet (Rai and Hash, 1990) and maize (Beckett, 1971) have emphasized the need for using hybrids made on isonuclear A-lines for fertility restoration pattern analysis and CMS classification. Based on this approach, Rai *et al.* (1996) characterized five CMS systems (A_1, A_2, A_3, A_v and A_4) in pearl millet. Differential male-fertility restoration patterns of hybrids made on isonuclear A-lines $81A_v$ and $81A_4$ indicated that the A_v and A_4 represent CMS systems that are different from each other and from A_1, A_2 and A_3. In another study, Rai *et al.* (2001) characterized and evaluated the stability and potential of various CMS sources in pearl millet using isonuclear A-lines. Virk and Brar (1993) and Virk *et al.* (1993) assessed the cytoplasmic effects on several pearl millet agronomic traits, including GCA and SCA effects, using near-isonuclear polycytoplasmic versions of 81A and two Pb 402A male-sterile lines. Significant differences among near-isonuclear polycytoplasmic lines were observed for traits such as plant height, leaf length and peduncle length but the differences for combining ability were more pronounced. Differential behavior of cytoplasms, both in combinations with a common pollinator and across pollinators, was observed for several traits. The results provided evidence for the distinctiveness of the cytoplasms in these CMS systems and their influence on phenotypic expression of nuclear genes in pearl millet. Chhabra (1995) characterized five CMS systems in pearl millet on the basis of microsporogenesis pattern, using isonuclear lines. Morphological and cytological characterization of the CMS-D_8 system of cotton has been reported by several workers (Black and Stewart, 1995; Stewart, 1995; Stewart and Zhang, 1996; Stewart *et al.*, 1996).

2.1.2.2 Mitochondrial DNA pattern analysis

Mitochondrial DNA (mtDNA) restriction endonuclease fragment patterns revealed by maize mitochondrial gene probe hybridization have been used to distinguish the cytoplasms in pearl millet and various other crops. Male-sterile cytoplasms have also been characterized through mtDNA restriction endonuclease profiles in maize (Levings and Pring, 1976; Pring and Levings, 1978; Sisco *et al.*, 1985 for CMS-S) and sorghum (Pring *et al.*, 1982) and many other crops. Smith *et al.* (1987) compared mtDNAs of pearl millet CMS-A_1 male-sterile lines and their male-fertile revertants, with the normal cytoplasm of their male-fertile maintainer lines. Their results revealed the presence of a unique 4.7-kb *Pst*I fragment in the male-sterile lines that was not detected in the fertile-revertant lines. A 9.7-kb fragment in the fertile-revertant line appeared to have replaced the 13.6-kb fragment. Smith and Chowdhury (1989) were able to differentiate various CMS cytoplasms in pearl millet on the basis of mitochondrial DNA restriction and hybridization patterns using maize mitochondrial gene probes. *Bam* HI restriction patterns differentiated the male-sterile cytoplasms, CMS-A_m (=A_4), CMS-A_1, CMS-A_3, but grouped together CMS-A_2, and five male-sterile mutants from the fertile maintainer of CMS-A_2. Smith and Chowdhury (1991) found that 4.7-kb, 10.9-kb, and 13.6-kb mtDNA fragments were associated with CMS in pearl millet. The cloned maize mitochondrial genes *rrn18*, *rrn5*, and *cox1* hybridized with these fragments. Rajeshwari *et al.* (1994) characterized diverse pearl millet cytoplasms by Southern blot hybridization using maize mtDNA probes.

2.1.2.3 Specific polypeptides synthesized by isolated mitochondria

Characteristic polypeptide patterns resulting from ^{35}S-methionine incorporation products by isolated mitochondria have been used to differentiate cytoplasms in maize (Forde *et al.*, 1978; Forde and Leaver, 1980) and sorghum (Dixon and Leaver, 1982). Reorganization of the mitochondrial genomes in the cytoplasms of CMS plants has been reported in numerous species, and in many cases it results in the formation of chimeric genes that are responsible for the CMS trait (Schnable and Wise, 1998). CMS-associated genes are often chimeric, derived from portions of known genes fused with previously unknown sequences. In most instances, the sequences of CMS-associated open reading frames are unrelated, except for parts of common mitochondrial genes such as *atp6*, *atp8* (*orfB*), *atp9*, or *cox2* (Schnable and Wise, 1998). Transcripts produced from such chimeric genes may inhibit expression of the normal gene, impair the formation of the normal gene products, and impair the formation of fertile pollen. In

a number of plant species, such malformed transcripts in the CMS mitochondria are altered in the presence of *Rf* genes, leading to the production of fertile pollen. These observations suggest that at least some *Rf* genes are involved in the processing of mitochondrial mRNA associated with CMS.

2.1.2.4 Polymerase Chain Reaction assays for CMS systems and their fertility restoration/sterility maintainer genes

Liu *et al.* (2002) developed a polymerase chain reaction (PCR) assay for discrimination of male-sterile cytoplasms in maize by designing PCR primers specific to the mitochondrial DNA sequences of three major classes of maize CMS cytoplasms: T, C and S. By following a single-seed multiplex PCR procedure, they categorized 73 maize cytoplasmic male-sterile inbred lines into one of these three major CMS cytoplasms. PCR-based markers have also been used in onion (Sato, 1998) and carrot (Nakajima *et al.*, 1999) for screening and identifying unknown cytoplasms using mitochondrial DNA as template. Ichikawa *et al.* (1997) developed a PCR-based method to screen rice lines containing nuclear gene *Rf1* involved in male-fertility restoration in the CMS-bo system.

2.1.3 Cytoplasmic-nuclear male-sterility in pearl millet

As early as 1940, Kadam *et al.* observed cases of complete sterility (rudimentary anthers without any pollen and complete absence of stigmas) and partial sterility (characterized by partial grain set) in pearl millet. Cytoplasmic male-sterility was discovered and confirmed almost simultaneously in India (Kajjari and Patil, 1956; Madhava Menon, 1958, 1959) and the United States of America (Burton, 1958). Burton (1958) described a male-sterile plant occurring naturally within inbred line 556, which provided the cytoplasmic source of what is now referred to as the A_1 CMS system in this crop. A stable A_1 cytoplasm male-sterile line, Tift 23A, and its maintainer Tift 23B, were released in 1965 (Burton, 1965) and formed the basis of worldwide pearl millet hybrid cultivar development efforts (Dave, 1987). Cytoplasmic male-sterility in pearl millet has been reviewed by Burton and Powell (1968), Hanson and Conde (1985) and Anand Kumar and Andrews (1993).

Pearl millet grain hybrids produced using A_1 cytoplasm CMS line Tift 23A from USA, were first produced in India in the mid-1960s. Since then, most of the commercial pearl millet grain and forage hybrids bred so far in India and the USA have the A_1 cytoplasm. Several other promising cytoplasms, like A_2 and A_3 (Burton and Athwal, 1967), A_4 (Hanna, 1989) and A_5 (Rai, 1995) differing from each other and from the A_1, have been documented and a few others have also been reported such as A_v (Marchais

and Pernes, 1985), A_{egp} (Sujata *et al.*, 1994), A_{β} (PT 732A) (Madhava Menon, 1958, 1959; Appadurai *et al.*, 1982), and ex-Bornu (Aken'Ova, 1985). Hanna (1989) identified a new male-sterility-inducing cytoplasm, A_4, from an accession of *Pennisetum glaucum* ssp. *monodii* (=*violaceum*) from Senegal and showed it to be different from the A_1, A_2 and A_3 cytoplasms. Marchais and Pernes (1985) identified a cytoplasm from an accession of *Pennisetum glaucum* ssp. *violaceum*, different from Hanna's A_4 *violaceum* accession, and showed that it too is different from the A_1, A_2 and A_3 cytoplasms. Sujata *et al.* (1994) used RFLP hybridization pattern analyses of mtDNA to characterize a new source of CMS derived from the ICRISAT Early Gene Pool (now ICRISAT Early Composite II), and designated it as A_{egp}. Differential fertility restoration patterns of hybrids on a male-sterile source from the ICRISAT Large-Seeded Gene Pool (LSGP) revealed a new CMS system that was designated as A_5 (Rai, 1995). Even though there are reports in pearl millet suggesting no association of downy mildew susceptibility with the sterile A_1 cytoplasm (Yadav, 1994), still there is a need for continuous and sustained cytoplasmic as well as nuclear diversification of the seed as well as pollen parents to avoid potential problems associated with cytoplasmic uniformity that were so clearly demonstrated in case of Southern leaf blight on maize in the USA (Scheifele *et al.*, 1970). Conflicting reports on smut susceptibility due to A_1 cytoplasm (Yadav *et al.*, 1992; Thakur *et al.*, 1992) and indications of higher ergot severity in male-sterile cytoplasm-based hybrids (Thakur *et al.* 1989) also suggest the need for diversification in the CMS systems to permit commercial exploitation of a wider range of both pollen and seed parents in pearl millet hybrid breeding.

Genetic diversification of hybrid seed parental lines in pearl millet is now achieved by using more than one CMS system and by using several diverse nuclear genotype combinations within each system. Several CMS systems other than A_1 are currently available in pearl millet and a few of them such as A_4 and A_5 are being utilized in CMS line development (K.N. Rai, pers. comm.). As a result, large numbers of male-sterile lines with diverse nuclear as well as cytoplasmic genotypes have been made available in pearl millet. Also, sets of isonuclear lines are now available in several diverse nuclear backgrounds in which the five best-documented and well-characterized CMS cytoplasms have been incorporated through repeated backcrossing for more than 6-7 generations (Rai *et al.*, 1996; Rai *et al.*, 2001). The isonuclear lines are best experimental systems for Mendelian genetic studies, as well as molecular genetics and biochemical, histological, ultra structural, and physiological comparisons, as differences

between members of a pair are likely coded by organeller DNA or result from interactions between organeller and nuclear DNA rather than being solely by nuclear DNA.

2.1.4 Genetics of fertility restoration

There is a great deal of diversity in the inheritance of fertility restoration in various CMS systems, both within and among species. Fertility restoration systems are classified as being either sporophytic or gametophytic: sporophytic restoration systems act prior to meiosis in sporophytic tissues; gametophytic restoration systems act post-meiosis in microspores or pollen grains. These differences lead to very different transmission patterns. A diploid plant that carries a male-sterile cytoplasm and is heterozygous for a restorer gene will produce two classes of pollen grains: those that carry the restorer allele for that gene and those that do not. In the case of a sporophytic restorer, both genotypic classes of gametes will be functional. By contrast, in the case of a plant heterozygous for a gametophytic restorer gene, only those gametes that carry the restorer allele will be functional. S-cytoplasm maize is an example of a well-characterized CMS system that is restored gametophytically (Kamps *et al.*, 1996).

2.1.4.1 Population genetics of restorers

Information regarding the allelic frequencies of restorer genes can prove useful in trying to understand their evolutionary origins and to search for new ones. For example, although the *Rf1* restorer allele, which confers pollen fertility in T-cytoplasm maize, is quite rare among maize inbred lines, the restorer allele of the *Rf2* gene for this cytoplasm is present in almost all maize inbred lines, even though most of these lines have never been exposed to the T-cytoplasm. This suggests that restorer alleles of the *Rf2* gene have been maintained during evolution by selection and must therefore have a significant function independent of pollen fertility restoration (Schnable and Wise, 1994). The *ogu*, *pol* and *nap* cytoplasms of *B. napus* induce male-sterility in all, some, and only a few cultivars, respectively (Jean *et al.*, 1997). Hence, it can be inferred that the *ogu* restorer (*Rfo*) is absent from *B. napus* germplasm, *pol* restorers are rare, and *nap* restorers are more common.

In pearl millet, Appa Rao *et al.* (1989) reported that out of 428 diverse accessions from 12 countries, 20.3% were maintainers, 7.5% were restorers and 65.9% segregated for male-fertility restoration when crossed onto an A_1 cytoplasm male-sterile line. The A_4 CMS system can act as a potential alternative to the already established and commercially exploited A_1 system due to high frequency of maintainers of A_4 (60%)

system as compared to less than 30% for A_1 and availability of about 30-40% restorers for A_4 (K.N. Rai, pers. comm.).

2.1.4.2 Mechanisms of fertility restoration

The mechanisms by which male-fertility restoration occurs are probably as diverse as the mechanisms by which mitochondrial mutations cause CMS. Although restorer alleles are known to affect all the well-characterized CMS-associated genes, the mechanism of action has not been determined definitively for any restorer allele. The possible mechanisms of male-sterility maintenance and male-fertility restoration have been dealt in several recent reviews (Schnable and Wise, 1998; Budar *et al.*, 2003; Hanson and Bentolila, 2004). With the exception of maize T-cytoplasm fertility restoration locus *Rf2*, all restorer genes are known to affect either the transcript profile or the protein accumulation of the mitochondrial CMS-associated locus, and some have been observed to affect both RNA and protein products. Fertility restorer genes could overcome male-sterility through the following mechanisms:

a) Physical loss of CMS-associated gene from mitochondria as observed in *Phaseolus* where in the presence of nuclear gene *Fr*, the mitochondrial sequence responsible for CMS-*pvs* is lost (He *et al.*, 1995).

b) Processing of CMS-associated transcripts is observed in a number of systems. Mostly, alterations are observed in accumulation of specific transcripts via northern blot analyses. Hence, it is not possible to distinguish between transcriptional and post-transcriptional mechanisms *e.g.* maize CMS-T *urf*13 gene processing has been correlated with *Rf1*, *Rf8* and *Rf** (Wise *et al.*, 1999).

c) Post-transcriptional RNA editing plays a role in some systems. For example, editing might change the length of predicted CMS-associated ORFs by creating new start (AUG) and/or stop (i.e. UAA, UAG, or UGA) codons, because the most common editing in plant mitochondria is C-to-U. Tissue-specific editing might allow a CMS-associated sequence to become deleterious only at microsporogenesis or microgametogenesis, *e.g.* editing of the *atp6* mitochondrial gene in CMS sorghum is strongly reduced relative to fertile sorghum in anthers but not in seedlings. RNA editing of this gene increases following fertility restoration.

Sequence analysis of restorer genes should provide significant clues about their functions. Till date, four restorer genes, *Rf2* of maize (Cui *et al.*, 1996), *Rf* of Petunia (Bentolila *et al.*, 2002), *Rfk1* (*Rfo*) of radish (Brown *et al.*, 2003; Desloire *et al.*, 2003;

Koizuka *et al.*, 2003) and *Rf1* of rice (Komori *et al.*, 2004) have been cloned. Proteins encoded by these cloned *Rf* genes have been identified also. For example, maize *Rf2* encodes an aldehyde dehydrogenase (Liu *et al.*, 2001). On the other hand, petunia *Rf*, radish *Rfk1* and rice *Rf1* were demonstrated to encode a protein composed of 14 and 16 repeats of the 35-aa pentatricopeptide repeat unit (PPR) motif, respectively.

2.2 Number of genes controlling fertility restoration

The diversity in restoration systems extends to the number of restorer genes. In a majority of CMS systems described to date, one or two major restorer loci confer complete male-fertility restoration. In a few CMS systems, full male-fertility restoration requires the concerted action of a number of genes, many of which provide only small incremental effects. CMS system male-fertility restoration is by dominant nuclear genes in commercially exploitable systems for cotton and cereals, in many cases few in number (*i.e.* one to four dominant genes, sometimes with minor male-fertility restoration modifiers required for complete fertility restoration in some environments). Precise determination of genetic control of sterility maintenance and fertility restoration in CMS systems is confounded due to complicated nuclear-cytoplasmic interactions with the effects of minor genes and environmental factors such as temperature and humidity. There are few reports on the inheritance of fertility restoration in pearl millet and therefore examples have been taken from other crops. Studies of the inheritance of fertility restoration using a set of isonuclear materials has not previously been reported in crops except for one study in sorghum (Schertz *et al.*, 1989).

2.2.1 Monogenic control

Monogenic modes of inheritance have been reported in pearl millet for the A_1, A_2 and A_3 (Burton, 1966; Burton and Athwal, 1967) and A_4 (Du *et al.*, 1996) CMS systems; in sorghum for the A_1 (milo) CMS system (Maunder and Pickett, 1959; Kidd, 1961; Schertz *et al.*, 1989; Murty and Gangadhar 1990), and for the 9E and A_4 CMS systems (Elkonin *et al.*, 1998); in maize for CMS-T (Blickenstaff, 1958), CMS-S (Kheyr-Pour *et al.*, 1981), and CMS-C (Laughnan and Gabay, 1978); in wheat for CMS-*timopheevi* (Tahir and Tsunewaki, 1967); in rice for CMS-bo (Shinjyo, 1969; Hu and Li, 1985; Teng and Shen, 1994), CMS-BT (Komori *et al.*, 2003), CMS-HL (Huang *et al.*, 2000), and CMS-Dian Type-1 (Tan *et al.*, 2004); in cotton for CMS-D_8 by its own restorer D8-R (Zhang and Stewart, 2001a) or by the D2-R restorer (Stewart, 1995; Zhang and Stewart, 2001b), CMS-$D_{2\text{-}2}$ (Kohel *et al.*, 1984; Weaver and Weaver, 1977; Liu *et al.*, 2003, Zhang and Stewart, 2001b), CMS-*hir* (Wang *et al.*, 1996a), and CMS-C1 (Zhang

and Stewart, 1999); in *Brassica* for CMS-*pol* (Fang and McVetty, 1989; Yang and Fu, 1990), CMS-*ogu* (Ogura, 1968; Pelletier *et al.*, 1987; Yamagishi and Terachi, 1997), CMS-*nap* (Fan *et al.*, 1986; Thompson, 1972), CMS-*tour* (Sodhi *et al.*, 1994), CMS-*lyr* (Janeja *et al.*, 2003a), and CMS-*Diplotaxis catholica* (Pathania *et al.*, 2003); in sunflower for CMS-GIG1 (Kural and Miller, 1992), CMS-PET1 (Kural and Miller, 1992; Seiler and Jan, 1994; Horn *et al.*, 2003), and for CMS-ANL2, CMS-PEF1 and CMS-PET2 (Horn and Friedt, 1997); in *Phaseolus* for CMS-*sprite* but with incomplete dominance (Mackenzie and Besset, 1987; Jia *et al.*, 1997); in *Petunia* (Edwardson and Warmke, 1967); in barley for CMS-*msm1* (Ahokas, 1979); and in rye for CMS-pampa (Miedaner *et al.*, 2000; Stracke *et al.*, 2003).

2.2.2 Digenic control

Several CMS systems display a digenic mode of inheritance such as the T-cytoplasm of maize, PET-cytoplasm of sunflower and T-cytoplasm of onion, for which two unlinked restorers are required for full male-fertility restoration. Duplicate restorer loci exist in a number of systems. For example, in maize, *Rf8* can at least partially substitute for *Rf1* to restore CMS-T (Dill *et al.*, 1997). Similar cases exist in, for example, the PET1 cytoplasm of sunflower, the T-cytoplasm of onion and in *Phaseolus* CMS. Such overlapping functions could be a consequence of duplication of gene functions or an indication that multiple mechanisms can induce fertility restoration.

Digenic control of fertility restoration has been reported in pearl millet by Yadav (1974a) based on observations of F_1 hybrids. Siebert (1982) suggested two major dominant complementary genes with at least one modifier for control of pollen fertility restoration in A_1 cytoplasm whereas for the A_2 system, two major genes with duplicate action were responsible for fertility restoration.

In sorghum digenic control of fertility restoration with complimentary gene action has been reported for A_1 CMS (Erichsen and Ross, 1963; Miller and Pickett, 1964; Appadurai and Ponnaiya, 1967; Schertz *et al.*, 1989; Lonkar and Borikar, 1994), A_2 CMS (Murty and Gangadhar, 1990), A_3 (IS1112C) CMS (Tang *et al.*, 1998; Pring *et al.*, 1999), and for 9E and A_4 CMS (Elkonin *et al.*, 1998). Digenic control with inhibitory gene action (F_2 ratio 13:3) was observed for fertility restoration in the A_2 CMS system (Lonkar and Borikar, 1994). Based on a similar F_2 ratio of 13:3, Wang *et al.* (1996a,b) concluded that one completely dominant gene (*Rf1*) and another partially dominant gene (*Rf2*) govern fertility restoration in upland cotton. Meyer (1975) reported two genes for fertility restoration in the cotton CMS-$D_{2\text{-}2}$ system. In maize, Duvick (1956) reported at

least two dominant complementary genes, plus one or more dominant gene that modify the action of one of the dominant complementary genes, for full pollen fertility restoration in the CMS-T system. Another report for CMS-T indicated the presence of two dominant restorer genes *Rf1* and *Rf2*, (Laughnan and Gabay-Laughnan, 1983).

In wheat, digenic control of fertility restoration has been reported involving one major and one minor gene (Miller and Schmidt, 1970; Bahl and Maan, 1973; Miller *et al.*, 1974). In CMS-*timopheevi* the two dominant fertility restoration genes are complimentary to each other (Livers, 1964). Nettevich and Naumov (1970) observed that complete fertility restoration required two dominant genes in conjunction with one recessive epistatic gene. In rye, two dominant complimentary genes are required for fertility restoration in the CMS-pampa system (Madej, 1976; Miedaner *et al.*, 2000). Two dominant genes have been reported for restoration of male-fertility in the CMS-WA system in rice (Yao *et al.*, 1997; Zhang *et al.*, 1997; Zhang *et al.*, 2002) with one gene appearing stronger than other (Young and Virmani, 1984; Virmani *et al.*, 1986; Govinda Raj and Virmani, 1988). Similar reports in *Brassica* for CMS-*tour* indicate digenic epistatic gene action for male-fertility restoration (Banga *et al.*, 1994; Janeja *et al.*, 2003b) with one of the genes stronger than the other (Pahwa *et al.*, 2004). Reports for control of male-fertility restoration in sunflower indicate two dominant genes for the CMS-PET2 system (Kural and Miller, 1992), two dominant genes with complimentary gene action for the CMS-MAX1 (Kural and Miller, 1992), CMS-RMX and CMS-RIG1 (Jan *et al.*, 2002), and CMS-ANN4 systems (Horn and Friedt, 1997); two dominant genes with cumulative gene action (F_2 ratio 9:6:1) for the CMS-PEF1 system (Miller, 1996); and two dominant duplicate genes for the CMS-ANL1 and CMS-MAX1 systems (Horn and Friedt, 1997). Leclercq (1984) also reported two dominant nuclear genes *Rf1* and *Rf2* for fertility restoration in most of the cultivated sunflower lines. In sugarbeet, two independent dominant genes for fertility restoration have been reported (Owen, 1942 and 1945; Bliss and Gabelman, 1965; Theurer and Ryser, 1969).

2.2.3 Trigenic or higher order gene control

In sorghum, trigenic and tetragenic interactions have been reported in various A_1 and A_2 CMS crosses by Lonkar and Borikar (1994); but based on testcross ratios these authors concluded that a trigenic model (F_2 ratio 54F:10S) best explained male-fertility restoration for the A_2 CMS system. Tripathi *et al.* (1985) postulated a four-gene model with a possibility of a fifth gene, controlling male-fertility restoration for sorghum male-sterile cytoplasms VZM2A and G1A. In the CMS-$D_{2\text{-}2}$ system of cotton, three major

dominant genes along with presence of three modifiers were suggested by da Silva (1981) and Maranhao *et al.* (1984) to control male-fertility restoration. Bett and Lydiate (2004) identified three loci/genes controlling the *ogu* CMS cytoplasm in *Raphanus*. Results in barley suggest that there are one to four dominant restorer genes, and that three of these are alleles of the *Rfm1a* locus (Ahokas, 1980). In rye, Scoles and Evans (1979) found three dominant fertility restorer genes with different levels of dominance and no epistasis. Fertility restoration in the G-type CMS system is also governed by at least three genes. One major gene, *ms1* is located on chromosome 4R, whereas two modifying genes, *ms2* and *ms3*, were found to be located on chromosomes 3R and 6R, respectively (Melz and Adolf, 1991). In *Brassica*, Shiga (1976) and Shiga *et al.* (1983) indicated that male-fertility restoration was controlled by dominant genes at two to four loci, depending upon the restorer line used. Fertility restoration in the PET1 CMS system of sunflower (Serieys, 1996) has been reported to be governed by one to four dominant restorer genes depending on parental cross combination. Fertility restoration in a cross of CMS-PET1 (HA-89) with wild species was reported to be under the control of three genes (F_2 ratio 54F:10S and BC ratio 1F:1S) with dominant fertility restoration alleles required to be present at least at two of the three loci (Seiler and Jan, 1994).

2.2.4 Modifiers

A single major gene and one or more modifiers with additive effect control fertility restoration of the milo (A_1) CMS system in sorghum. Three dominant modifier genes can induce fertility in the absence of major restorer genes (Kidd, 1961). For the Pampa CMS system of rye, Miedaner *et al.* (2000) reported major dominant male-fertility restoration genes on chromosomes 1RS and 4RL and three minor genes on chromosomes 3RL, 4RL and 5R in. In one rye population (Pico Gentario), a dominant modifier gene contributed by the female parent was found on chromosome 6R. Wang *et al.* (1996a,b) in cotton also reported a fertility enhancer gene (E) from the male-sterile female parent having a positive contribution to male-fertility restoration in the *harknessii* CMS system. Duvick (1956) indicated one or more dominant genes modify the action of one of the dominant complementary genes required for full pollen fertility in maize lines restoring fertility in hybrids of CMS-T lines. In case of digenic control of male-fertility restoration, several reports indicate a major or strong effect of one gene and a minor or weak effect of the second gene such as in rice (Young and Virmani, 1984; Govinda Raj and Virmani, 1988), wheat (Miller and Schmidt, 1970; Bahl and Maan, 1973; Miller *et al.*, 1974), and rapeseed (Pahwa *et al.*, 2004). In cotton, da Silva

et al. (1981) and Maranhao *et al.* (1984) presented evidence for the presence of modifying genes for pollen fertility restoration on chromosomes 16D, 25D and telosomic 15L based on monosomic analysis. In rye, two modifying genes, *ms2* and *ms3*, were found to be located on chromosomes 3R and 6R, respectively (Melz and Adolf, 1991) along with a major gene for fertility restoration, *ms1*, on chromosome 4R.

The observed differences in types of gene interactions could presumably be due to the influence of male and female parent genotypes and/or variable expression of the weaker gene in different genetic backgrounds. Certain modifier genes could also be responsible for changing the observed segregation pattern in the F_2 and BC generations. Penetrance and expressivity of the restorer genes in wheat (Maan, 1985) and rice (Govinda Raj and Virmani, 1988) are known to be affected by parental genotypes of a cross as well as by genotypes of individual plants among the segregating progenies. In pearl millet, Rai and Hash (1990) concluded that there are significant effects of nuclear backgrounds of the parents on male-fertility restoration. Schertz *et al.* (1989) conducted a detailed investigation of fertility restoration in CMS sorghum and determined that the inheritance of fertility restoration in hybrids with A_1 cytoplasm varied, depending on the nuclear backgrounds of the female and male parents. Some parents differed by a single gene for fertility restoration while others differed by many genes as evidenced by a complete range in fertility within a backcross progeny. Backcross progenies, from isonuclear females possessing different cytoplasms crossed with a certain male parent, exhibited different segregation ratios for fertility restoration. Depending on the cytoplasm of the female, however, when these same females were backcrossed with a different male parent the segregations did not differ. Murty and Gangadhar (1990) investigating the genetics of male-fertility restoration in milo (A_1) and non-milo (A_2) male-sterility-inducing cytoplasms observed that on the same cytoplasm, both monogenic and digenic ratios were observed with different restorers and also a restorer gave different ratios when crossed on two different cytoplasms indicating the influence of the nuclear background as well as nuclear-cytoplasmic interactions. Elkonin *et al.* (1998) studied the genetics of male-fertility restoration in sorghum in the 9E and A_4 CMS systems and concluded that one or two dominant genes control male-fertility restoration depending on the nuclear background of the parents. Pahwa *et al.* (2004) inferred that influence of female parent nuclear genotypes or modified expression of the restorer gene(s) in different backgrounds could be probable reasons for observed differences in gene interactions in the rapeseed *tour* CMS system.

2.2.5 Environmental factors influencing CMS

CMS expression depends on the interaction of cytoplasmic factors and alleles of nuclear fertility restorer genes that may be further confounded by various environmental factors. Stability of CMS Tift 23A cytoplasm in pearl millet was examined by Burton (1972, 1977), who reported reversion of sterility to fertility as is also observed in maize CMS-S cytoplasm. Fertile revertants may be of two types, either nuclear mutations that render a recessive maintainer allele of the restorer gene into a dominant restorer allele, or an alteration of the cytoplasmic genome. Rates of reversion of the cytoplasmic type appear to be under the control of nuclear genes in pearl millet (Clement, 1975).

Initial studies in pearl millet observed higher frequency of pollen shedders in Tift $23A_1$ (A_1 cytoplasm) during hot dry conditions than in the cooler, moisture rainy season and suggested that high temperatures might be involved in male-sterility/maintenance breakdown (Balarami Reddy and Reddi, 1970; Thakre, 1977; Saxena and Chaudhary, 1977). These studies, however, neither provided information on the temperature regime nor on the seed source. Results reported by Rai *et al.* (2001) are contrary to these earlier observations. During the flowering period of the late-summer season crop, with mean daily maximum temperatures of 38.0 to 40.0°C and low RH (45-64%), there were no pollen shedders in $81A_1$, while low frequency (<1%) of pollen shedders in this line were observed in all four screening environments with lower temperatures and higher RH values. Lower temperature and higher relative humidity environment have been found to be more favorable for male-fertility restoration in maize (Duvick, 1959). These results support earlier findings of Rai *et al.* (1996) that high temperatures and/or low RH lead to greater expression of male-sterility in pearl millet. In $81A_v$, no such relationships were observed between temperature regimes and frequency of pollen shedders. With respect to selfed seed set (SSS), however, there were clear indication that high temperatures and low RH in the late-summer dry season reduced SSS, especially in the 1-5% and 6-10% SSS classes. The most consistent patterns across seasons, both for pollen shedding and SSS, were obtained for hybrids of $81A_4$, followed by those of $81A_1$ and $81A_v$. Rai *et al.* (1996) also concluded that irrespective of the evaluation criteria, identification of a maintainer line for breeding a stable male-sterile line will be more effective in the rainy season as the level of fertility was higher in the rainy season than the dry summer season. In contrast, identification of a restorer line for breeding hybrids with good male-fertility will be more effective in the dry season. This is in agreement with the observations of Rai and Hash (1990).

In wheat, pollen fertility restoration is influenced by location (Lucken and Maan, 1967), photoperiod (Welsch and Klatt, 1971) and day temperature (Johnson and Patterson, 1973). Long photoperiods or high temperature enhance pollen fertility in many restored lines.

In cotton, expression of male-sterility in the partially male-sterile alloplasmic lines conditioned by A_2 and B_1 cytoplasms was strongly affected by temperature (Meyer, 1969; Marshall *et al.*, 1974). Genetic studies on male-fertility restoration factors can potentially be confounded significantly by the direction of cross and environmental conditions.

Izhar (1978) found that male-fertility restoration conditioned by a major dominant gene was not affected by temperature or genetic background in *Petunia* whereas in the multigenic system of pollen fertility restoration, different degrees of fertility restoration were reported depending on temperature (Izhar, 1977). Expression of male-sterility maintenance and pollen fertility restoration in *msm1* cytoplasm of barley appeared to be unaffected by environmental conditions and latitude differences (Ahokas and Hockett, 1981). The effectiveness of the restorer genes in rye depended upon the parental genotype and the environment, with temperature being the most important environmental variable (Scoles and Evans, 1979). At lower temperatures, male-fertility restoration was completely inhibited whereas at higher temperatures, fertility restoration was complete. Some deviations in the segregation ratios were observed, which could be due to modifier genes, inter-allelic interactions or segregation of additional restorer genes. Heterosis and inter-allelic interactions at the restorer gene loci are known in sorghum also (Miller and Pickett, 1964).

The expression of fertility restorer genes can be affected by other nuclear genes and be sensitive to environmental conditions (Duvick, 1965). Blickenstaff *et al.* (1958) reported an inhibiting effect of long photoperiod and high temperature on pollen fertility restoration of some inbreds in maize CMS-T cytoplasm. High temperatures are known to influence the expression of male-sterility in the *Brassica* CMS-*nap* cytoplasm (Thompson, 1972; Shiga, 1976; Fan and Stefansson, 1986). The precise stage of temperature sensitivity is also very important with the pre-meiotic stage (*e.g.* wheat, sorghum, rye), post-meiotic stage, or when microspores are released (*e.g.* pearl millet, rice, radish) being especially sensitive in different CMS systems.

2.3 Linkage between fertility restorer genes

The present investigation is handicapped due to non-availability of reports on genetic linkage among fertility restorer genes of two or more different CMS systems. A few reports are available on the linkage between fertility restorer gene(s) and one or more qualitative or quantitative genes and are discussed here.

Linkage of male-fertility restoration genes to other factors that influence mtDNA gene expression was observed for the dominant gene *Rfp1*, which confers fertility restoration to the *pol* male-sterile cytoplasm of *Brassica* (Singh *et al.*, 1996). Huang *et al.* (1986) reported linkage between the fertility restorer gene and the color of the lemma and palea in rice with recombination value ranging from 9.7% to 27.1% in the two crosses studied. Weaver and Weaver (1977, 1979) reported linkage between an incompletely dominant restorer gene for CMS-$D_{2\text{-}2}$ system from *G. harknessii* and a mutant phenotype known as cracked root in cotton; however, Kohel *et al.* (1984) found no linkage between this *Rf* gene and 13 morphological markers distributed on at least nine chromosomes. Fick and Zimmer (1975) reported monogenic inheritance of male-fertility restoration in sunflower and observed no linkage with genes controlling rust resistance, downy mildew resistance, *Verticillium* wilt resistance, and branching.

2.4 Test of allelism

The test of allelism is based on the assumption that if two or more restorer lines possess alleles for the same gene restoring fertility to a sterile cytoplasm, no sterile or partially sterile plants will be obtained among testcrosses made from the F_1 of those restorer lines, whereas the presence of sterile or partially sterile plants will indicate different loci controlling male-fertility restoration in these restorer lines. In most published studies, allelic relationships were assessed in testcrosses made on CMS lines using pollen from the F_1 hybrids derived from restorer × restorer crosses.

Duvick (1956) carried out allelism tests on five maize restorer lines restoring fertility to CMS-T and showed them to be having alleles of the same single dominant gene required for pollen fertility restoration in this CMS system. Duvick (1956) also demonstrated that just because two or more maize inbreds are found equally male-sterile in T cytoplasm doesn't mean that they have the same genetic composition at all restorer gene loci. Therefore, any conclusions on the number and linkage map location of the restorer genes in a given restorer line is valid to the specific inbred line used as a tester

and to the specific plants tested unless until cross comparisons involving multiple plants of the restorer line and several genetically dissimilar tester lines are made. Moreover, the environment in which segregating populations are evaluated can influence the number of restorer genes that are detected. Kheyr-Pour *et al.* (1981) reported that the same restorer alleles were carried across all of maize CMS-S restorer lines. In rice, Govinda Raj and Virmani (1988) studied allelic relationships among six restorer lines and grouped them into four clusters with different pairs of restorer genes. Hu and Li (1985) concluded that inheritance of male-fertility restoration in the CMS-bo and CMS-D systems in rice was monogenic and the two restorer genes were allelic. Based on allelism tests, Ramalingam *et al.* (1995) grouped five rice restorer lines into two groups possessing a different pairs of restorer genes.

Zhang and Stewart (2001b) studied the genetic relationship of restorer genes for the D_8 and $D_{2\text{-}2}$ CMS systems in cotton by undertaking allelism tests and revealed that restorer genes for these two CMS systems were not allelic, but were tightly linked with an average genetic distance of 0.93 cM. Tests for allelism of the restorer genes for the *pol* CMS system in summer rape (*Brassica napus* L.) were conducted by Fang and McVetty (1989) using F:S segregation ratios observed in F_3 families derived from crosses between F_1 plants containing genes for male- fertility restoration from two restorer gene sources. The male-fertility restoration genes of these two restorer lines were found to be non-allelic and designated *Rfp1* and *Rfp2*. These results were also confirmed in another study by Jean *et al.* (1997). However, in a later study done for the same CMS system, Yang and Fu (1990) obtained no male-sterile or partially male-sterile plants in testcrosses of (R × R) F_1 hybrids made on CMS lines, indicating that the restorer genes of the five restorers were allelic to each other or very tightly linked. Pahwa *et al.* (2004) conducted test of allelism for four restorers of *tour* CMS and observed that the restorer genes present in the four restorers were allelic.

2.5 Morphological marker studies in pearl millet

Morphological variants with distinct phenotypic expression were often used to establish linkage studies prior to the availability of molecular markers. In pearl millet, such variations have been observed and studied for plant height (both quantitative and qualitative), panicle bristling, leaf pubescence, anthocyanin pigmentation and many other morphological traits (Koduru and Krishna Rao, 1983; Anand Kumar and Andrews, 1993).

2.5.1 d_2/non-d_2 plant type

In breeding improved high yielding, lodging resistant crop cultivars, the role played by dwarfing genes in crops such as wheat, rice, barley, sorghum and pearl millet is well established. Dwarf plants in pearl millet were discovered almost simultaneously in India and USA. Burton and Fortson (1966) reported the inheritance of reduced plant height in pearl millet from five different sources, named D_1 to D_5. Dwarfness in source lines D_1 and D_2 was controlled by one or two recessive genes but was controlled by single independently segregating recessive genes, d_1 and d_2 when transferred to near-isogenic backgrounds. The d_2 dwarfing gene has several pleiotropic effects on plant phenotype. Mainly, it reduces plant height by 50% through a reduction in the lengths of all stem internodes, except the peduncle (Burton and Fortson, 1966), leading to a higher proportion of leaves (Rai and Hanna, 1990a). Comparison of tall and dwarf near-isogenic lines (Rai and Hanna, 1990a; Bidinger and Raju, 1990) lead to the conclusion that the d_2 dwarfing gene could be used to advantage by incorporating it into diverse genetic backgrounds.

Minocha *et al.* (1978) reported that the genes for bristled panicle, dwarfism and purple glume are linked and present on one linkage group and the genes for hairy node, hairy leaf and purple node on another linkage group. Minocha and Sidhu (1979) assigned the hairy leaf gene on chromosome 1, bristled ear to chromosome 2. Krishna Rao and Uma Devi (1981) reported independent assortment of the male-sterile gene (ms_1) from the genes for white virescent seedling (*wv*), hairy leaf blade (*hl*), hairy leaf margin (*Hm*), hairy node (*Hn*), and purple seedling base (Pb_1 Pb_2).

Azhaguvel *et al.* (2003) mapped the d_1 and d_2 dwarfing genes on pearl millet linkage group 1 and 4 (bottom part), respectively. Poncet *et al.* (2000, 2002) from a cultivated × wild cross, mapped and identified major QTL linked to bristle length (length of involucre bristles) on linkage group (LG) 6 and LG7. Two unlinked genes controlled the presence (wild) or absence (cultivated) of a long awn (existence of a longer bristle) and are located on LG1 and LG7, with wild alleles being dominant for both of these genes (9F:7S F_2 segregation). Two QTL for plant height were identified on LG6 and LG7.

2.5.2 Leaf pubescence

From the seedling stage onward, pearl millet has hairiness (pubescence) on several plant parts. Hairiness in leaves, especially in seedling leaves, can be easily recognized and is useful as a genetic marker. Singh *et al.* (1967) reported that smooth leaf character was

dominant over hairy leaf and controlled by a single gene. Identical results, indicating that leaf hairiness is controlled by a single recessive gene, *hl*, were reported by several workers (Burton and Powell, 1968; Singh *et al.*, 1968; Khan and Bakshi, 1976; Krishna Rao and Koduru, 1979).

2.5.3 Long panicle bristling

Rangaswami and Hariharan (1936) mentioned that an African pearl millet race, *Pennisetum echinurus*, which has bristled panicles, when crossed with *P. leonis* without bristles, showed an F_2 segregation with a wide range of bristled and non-bristled forms. Grouping all the bristled forms together, they obtained a ratio of 3 bristled : 1 non-bristled types. Ahluwalia and Shankar (1964) reported that panicle bristling (*Br*) is governed by a single dominant gene and variation in the density of bristling is possibly through the influence of modifying factors. Several other authors reported identical results (Athwal and Gill, 1966; Lal and Singh, 1971; Singh and Pandey, 1973; Khan and Bakshi, 1976; Singh *et al.*, 1967; Gill and Athwal, 1970; Gill *et al.*, 1971). A conflicting report by Yadav (1974b) noted monogenic incomplete dominance for bristling. In crosses between long- and short-bristled plants, however, the bristle length was intermediate in the F_1 and continuous variation was observed in the F_2, indicating the additive action of more than one gene (Appa Rao *et al.*, 1988).

2.6 Molecular markers in pearl millet

Over the past ten years, resources have been established for the genetic analysis of pearl millet, *Pennisetum glaucum* (L.) R. Br., an important staple crop of the semi-arid regions of India and Africa. Among these resources are detailed genetic maps containing both homologous and heterologous restriction fragment length polymorphism (RFLP) markers, and simple sequence repeats (SSRs). The first molecular marker-based genetic linkage map of pearl millet was reported by Liu *et al.* (1994), who used 181 RFLP markers covering the seven pearl millet chromosomes to generate a map spanning a genetic distance of 303 cM (Kosambi map distance). A subset of these markers has subsequently been transferred to a series of crosses that segregate for agronomically important traits. The integration of markers previously mapped in other grass species has provided the anchor points to align the pearl millet linkage groups to other cereal genetic maps, including the model species, rice. Although the pearl millet genome appears to be relatively highly rearranged relative to rice, regions of co linearity between the two species can be clearly identified (Devos *et al.*, 2000). These regions

form a framework for exploitation of the rice genomic sequence as a source of new markers and candidate genes underlying traits in pearl millet.

Genetic maps produced in four different crosses have recently been integrated to develop a consensus map of 353 RFLP and 65 SSR markers (Qi *et al.*, 2004). Some 85% of the markers are clustered and occupy less than a third of the total map length. This phenomenon is independent of the cross. The data suggest that extreme localization of recombination towards the chromosome ends, resulting in gaps on the genetic map of 30 cM or more in the distal regions of each linkage group, is typical for pearl millet. The unequal distribution of recombination has consequences for the transfer of genes controlling important agronomic traits from donor to elite pearl millet germplasm.

The RFLP technique is among the most widely used DNA marker assays in plants. The technique is relatively robust, and readily transferable between different labs. RFLP markers segregate in a manner that is Mendelian and co-dominant, phenotype-neutral and free of epistatic interactions. Although it remains widely used, a major limitation of the RFLP technique is the large quantity of DNA required to generate a DNA fingerprint of the entire genome.

Simple sequence repeats (SSRs), also known as microsatellites, remain the markers of choice for practical breeding applications. Although RFLP markers have been used in the transfer of downy mildew resistance QTL to elite seed parents, these markers are too labor-intensive for large-scale genotyping when reliable PCR-compatible markers like SSRs and AFLPs are available. SSRs are abundant in eukaryotic genomes. They provide a co-dominant and usually highly polymorphic marker system (Akkaya *et al.* 1992; Morgante and Olivieri 1993; McCouch *et al.*, 1997). The development of 50 SSRs from pearl millet BAC clones has been described by Qi *et al.* (2001) and Allouis *et al.* (2001). A further 44 SSRs from a $(CA)_n$-enriched small insert library have been developed by Qi *et al.* (2004). Budak *et al.* (2003) reported 18 SSRs from a $(CT)_n$-enriched small insert library and Senthilvel *et al.* (2004) have reported development of 13 polymorphic SSRs detected using primers from pearl millet expressed sequence tag (EST) sequences. These maps and markers provide the base for future genomic and comparative analyses of pearl millet and for use of marker-assisted selection (MAS) in applied breeding programs

2.6.1 Molecular mapping in pearl millet

Molecular markers have been used in pearl millet to study genetic diversity within and among landraces (Busso *et al.*, 2000; Bhattacharjee *et al.*, 2002), genetic diversity in

germplasm (Budak *et al.*, 2003), genotype identification and genetic relationships (Chowdari *et al.*, 1998a), hybrid performance and genetic distance (Chowdari *et al.*, 1998b), cytoplasmic male-sterility through RFLP and transcript analysis (Delorme *et al.*, 1997), characterization of CMS sources (Chhabra, 1995; Sujata *et al.*, 1994; Smith and Chowdhury, 1989; Smith *et al.*, 1987), domestication syndrome (Poncet *et al.*, 1998; 2000), QTL affecting domestication traits between domesticated × wild pearl millet crosses (Poncet *et al.*, 2002), tracking the introgression of genomic segments from the wild progenitors (Lamy *et al.*, 1994), QTL associated with traits determining grain and stover yield under terminal drought-stress conditions (Yadav *et al.*, 2002, 2004), mapping and characterization of QTL × environment interactions for traits determining grain and stover yield (Yadav *et al.*, 2003), comparative mapping with the genomes of foxtail millet and rice (Devos *et al.*, 2000), in mapping QTL for downy mildew resistance (Jones *et al.*, 1995; Azhaguvel, 2001; Kolesnikova, 2001; Jones *et al.*, 2002; Nepolean, 2003; Gulia, 2004), rust and pyricularia leaf spot disease resistance (Morgan *et al.*, 1998), recombination rates in female and male gametogenesis (Busso *et al.*, 1995; Liu *et al.*, 1996), genetic analysis of adaptive traits (Padi, 2002), and marker-assisted backcrossing of QTL for downy mildew resistance and drought tolerance (Sharma, 2001; Satish Kumar, 2004).

2.6.2 Quantitative trait loci (QTL) mapping

Recent and continuing advances in molecular genetics and statistical techniques make it possible to identify the chromosomal regions where gene (blocks) contributing substantially to the control of a particular trait are located. Such genomic regions are often referred to as quantitiative trait loci (QTL). QTL mapping involves finding an association between a genetic marker and a phenotype that one can measure. The statistical tools at the foundation of QTL mapping have been used for many years. Sax (1923) mapped a QTL for seed size in the common bean, *Phaseolus vulgaris*, by statistically associating it with a Mendelian locus for seed pigmentation. Thoday (1961) developed methods for detecting linkage of polygenes with marker loci. Such earlier studies provided a background of theory and observation for more recent work with molecular markers (Dudley, 1993). Recently, the study of number and effect of major QTL has been greatly facilitated by the advent of molecular markers and the development of saturated linkage maps. Paterson *et al.* (1988) reported the development of a genetic linkage map based on RFLP markers in an inter-specific backcross of tomato, mapping at least six QTL controlling fruit mass and four QTL for soluble solids.

Once DNA markers linked with genomic regions controlling such target traits are available, molecular marker-based screening for the trait can be quickly but accurately achieved. At least for some traits such marker-assisted selection will offer advantages compared with conventional screening procedures.

2.7 Molecular mapping of fertility restoration

Molecular markers are a reliable diagnostic system for various plant breeding applications making it possible to analyze thousands of genotypes during a breeding season rapidly and effectively. Molecular marker techniques provide powerful tools to identify and map target genes efficiently.

Molecular markers tightly linked to fertility restoration (*Rf*) loci will have several applications in breeding programs. In many situations, breeders do not know whether a new breeding line (or germplasm accession) should be classified as a maintainer (B-line) or restorer (R-line). Currently, the only method to determine the status of these lines is to test cross the lines to a male-sterile line and score the resulting F_1 hybrid progeny for its male-sterility/fertility reaction. This approach is time-consuming and its results are often affected by environmental conditions. The identification of molecular markers tightly linked to *Rf* loci would permit the classification of lines as either B- or R-line without the need for testcrosses. Molecular markers that are tightly linked to fertility restorer genes have been identified in several crops like maize (Sisco, 1991; Wise and Schnable, 1994), sorghum (Klein *et al.*, 2001; Wen *et al.*, 2002), rice (Zhang *et al.*, 1997; Zhu *et al.*, 1996; Tan *et al.*, 1998; Komori *et al.*, 2003; Akagi *et al.*, 2004), *Brassica* (Delourme *et al.*, 1994, 1998), cotton (Zhang & Stewart, 2004), *Petunia* (Bentolila *et al.*, 1998), and wheat (Ma and Sorrells, 1995); however similar linkage analyses have not been reported in pearl millet so far.

Fertility restoration mapping studies (Table 1) in different crops have been done using different types of mapping populations like F_2 and/or F_2-derived generations, BC and/or BC-derived generations, NILs and RILs developed from either A × R crosses or B × R crosses. In the later case, the phenotyping has been done with testcrosses. In a few studies information from the mapping of more than one population was obtained to reach a more broadly valid conclusion. The marker systems employed were mostly RAPD, RFLP, AFLP, STS markers and in some cases the identified RFLP or RAPD markers were converted to PCR-based markers for further use. Most of the studies involved a Bulk Segregant Analysis (BSA) approach (Michelmore *et al.*, 1991) based on making male-sterile or male-fertile bulks for identifying linked markers and then

performing the genotyping on a sub-set of the whole population with the identified linked markers with the aim to create a localized linkage map of the *Rf* loci. A few studies reported a QTL mapping approach for identifying QTL linked to fertility restoration (Zhu *et al.*, 1996, Tan *et al.*, 1998, Hjerdin-Panagopoulos *et al.*, 2002; Xie *et al.*, 2002; Coulibaly *et al.*, 2003). *Rf*-linked molecular markers have been used in marker-assisted selection in crops like *Brassica* (Hansen *et al.*, 1997) and to identify restorer lines having the *Rf1* gene in rice (Ichikawa *et al.*, 1997). A few crops have seen much advanced work in order to understand the mechanisms underlying fertility restoration as evidenced by cloning of four restorer genes, *Rf2* of maize (Cui *et al.*, 1996), *Rf* of *Petunia* (Bentolila *et al.*, 2002), *Rfk1* (*Rfo*) of radish (Brown *et al.*, 2003; Desloire *et al.*, 2003; Koizuka *et al.*, 2003), and *Rf1* of rice (Komori *et al.*, 2004). Proteins encoded by these cloned *Rf* genes have also been identified. For example, maize *Rf2* encodes an aldehyde dehydrogenase (Liu *et al.*, 2001) while *Petunia Rf*, radish *Rfk1* and rice *Rf1* were demonstrated to encode a protein composed of 14 to 16 repeats of the 35-amino acid pentatricopeptide repeat unit (PPR) motif. The work reported here in pearl millet is a first step towards the goal of cloning the male-fertility restoration genes for CMS systems in this crop. Till date about sixty research papers have been published related to mapping of fertility restorer genes in various crops. A summary of relevant information is tabulated in Table 1.

Table 1. Molecular markers identified with fertility restoration in different crops

Crop/ Species	CMS system	R gene	Genetics	Generation/ Population/ Cross	Technique/ Approach	Marker type	Linked markers/ QTL	Chromos-ome or LG	Distance (cM)/ variation (%)	Reference
Allium cepa	S	Ms	Single dominant	F_2-TCs	-	RFLP	AOB210, AP165	(B)	14, 15	King *et al.*, 1998
Beta vulgaris	Owen	*X*	-	F_2	-	RFLP	pKP1238	3	9.6	Pillen *et al.*, 1993
	H	*R1H*	-	A x R haplotypes	BSA	RAPD, RFLP	K11-1000, pKP753	4	5.2, 1.7	Laporte *et al.*, 1998
	Owen	X, Z	Three QTLs	Three (B × R) F_2s, TCs	QTL mapping	RFLP	2 QTLs 1 QTL	3	15; 79% 5; 72%	Hjerdin-Panagopoulos *et al.*, 2002
B. vulgaris ssp. *Maritime*	cms-G	RfG1	Two loci; epistatic interaction	-	BSA	AFLP SSR	E41M59-H183 E38M48-F200 G 029	8	3 2	Touzet *et al.*, 2004
Brassica napus	*ogura*	*Rfo*	-	2 F_2 pop. 1 seg DH	BSA	RAPD, RFLP	OPC02-1150, OPD02-1000	DY-15	-	Delourme *et al.*, 1994, 1998
	ogura	*R*	-	F_2	BSA	RAPD	OPK12-750, F04-500	-	1.2, 7.7	Hansen *et al.*, 1997
	pol	*Rfp1*	Single dominant	-	-	RAPD, RFLP	4ND7b, 5NE12b CRF 1b	18	10.8, 5.4 0.0	Jean *et al.*, 1997
	-		-	-	-	Iso-zymes	Pgi-2	-	0.25	Delourme & Eber, 1992
	lyr	*Rf1*	Monogenic	F_2	BSA	RAPD	OPK15-700 OPZ06-1300	1	8.2 2.5	Janeja *et al.*, 2003a
	tour	*Rft1* *Rft2*	Digenic epistatic	NIL- F_2	-	AFLP	EACC/MCTT$_{105}$ EAAG/MCTC$_{80}$	-	18.1, 33.2, 18.1	Janeja *et al.*, 2003b
	ogura	*Rfo*	Monogenic	F_2, F_4	BSA	AFLP	4 markers 10 markers	-	0.0 < 3.4	Giancola *et al.*, 2003
	tour		Single dominant	BC_3	BSA	AFLP	Elevan markers	-	3.4	Trendelkamp *et al.*, 1999

Contd.....

Crop/ Species	CMS system	R gene	Genetics	Generation/ Population/ Cross	Technique/ Approach	Marker type	Linked markers/ QTL	Chromos-ome or LG	Distance (cM)/ variation (%)	Reference
Capsicum annuum	S	*Rf*	-	F_2	BSA	RAPD	$OP13_{1400}$ $OW19_{800}$	-	0.37 8.12	Zhang *et al.*, 2000
Coffea	-	*pv*	Two major loci	Inter specific cross BCs	QTL analysis	AFLP	Pv-1, Pv-2, Pv-3	2, 13	LOD 3.9, 3.6, 4.9	Coulibaly, 2003
Gossypium	*harknessii*	*Rf*	-	(A × R)- F_2	BSA	RAPD	R 6592	20	6	Lan *et al.*, 1999
	cms-D8	*Rf1, Rf2*	Single dominant	3 TCs, 2 TCs	BSA	RAPD	$UBC169_{700}$, UBC 659_{1500} $UBC111_{3000}$ $UBC188_{500}$	 -	0.9 2.8	Zhang and Stewart, 2004
	cms-D2 *harknessii* cytoplasm	*Rf1*	Single dominant	3 (A × R) F_2	-	RAPD, SSR	RAPD-3_{1480}, 5_{710}, SSR-2_{135}, 1_{170}, 4_{215}	4L	0.3-1.2	Liu *et al.*, 2003
Helianthus annuus	PET1	*Rf1*	-	-	-	RAPD	OPC07-900, OPD10-750	-	-	Ji *et al.*, 1996
	PET1	*Rf1*	-	F_2	-	RFLP	SUN 069 E1 SUN 094 E3	6	2	Gentzbittel *et al.*, 1995
	PEF1	-	-	Inter-specific BC	-	RAPD	3 markers	1	-	Quillet *et al.*, 1995
	PET1	*Rf1*	Single dominant	(B × R)- F_2	BSA	RAPD/ SCAR AFLP	OPK13-454 OPY10-740 E33M61-136 E41M48-113	6	0.8 2 0.3 1.6	Horn *et al.*, 2003
Hordeum vulgare	msm1	*Rfm1*	monogenic	F_2, BC_1F_1	-	RAPD/STS	OPI-18/900, MWG2218	6H	5.2, 5.6	Matsui *et al.*, 2001
Oryza sativa	BT	*Rf1*	-	-	-	RFLP	G2155, C1361	10L	3.5, 3.9	Kurata *et al.*, 1994
	BT	*Rf1*	-	NILs/BC_1	-	SSR	OSR *Rf*	10	3.7	Akagi *et al.*, 1996

Contd.....

Crop/ Species	CMS system	R gene	Genetics	Generation/ Population/ Cross	Technique/ Approach	Marker type	Linked markers/ QTL	Chromos-ome or LG	Distance (cM)/ variation (%)	Reference
	HL	-	Single dominant	BC_1	BSA	SSR	RM258	10	7.8	Huang *et al.*, 2000
	WA		-	-		RFLP	RG69a, RG413	3	-	Li *et al.*, 1996
	WA		-	-		RFLP	C22, RG4449d	4	-	Li *et al.*, 1996
	WA	-	4 QTLs	-	QTL analysis	RFLP	RZ404c-RG241B	2	-	Zhu *et al.*, 1996
	-	-	-	-	-	RFLP	RG69A-RG413	3	-	Zhu *et al.*, 1996
	-	-	-	-	-	RFLP	C22-RG4449D	4	-	Zhu *et al.*, 1996
	-	-	-	-	-	RFLP	RG435-RG172A	5	-	Zhu *et al.*, 1996
	WA	*Rf3*	Two duplicate dominant loci	2 F_2s- (A x R); 1 BC_1	BSA	RAPD, RFLP	OPK05-800, OPU10-1100, OPW01-350, RG532, RG140, RG458	1	1.4, 1.9	Zhang *et al.*, 1997
	WA	*Rf3*, *Rf(u)*	Two duplicate loci	(A x R)- F_2; A x (B x R)- F_2 TCs	BSA/ QTL analysis	RFLP	RG532, R173 G4003, C234 G4003-C677	1 10	6.0, 18.4 3.3, 19.1 1 QTL	Yao *et al.*, 1997
	WA	*Rf*	-	BC_1F_1	QTL analysis	RFLP	C1361-S11148 R2309-RG257 (additive effect)	10L	1 QTL; 71.5% 1 QTL; 27.3%	Tan *et al.*, 1998
	WA	*Rf4*	-	-	-	SSR	RM171, RM228	10L	3.7, 3.4	Jing *et al.*, 2001
	WA	*Rf6(t)*	-	-	-	SSR	RM244	10S	-	Jing *et al.*, 2001
	WA	*Rf5*	Single dominant	F_2	BSA	RAPD	RG374, RG394	1	10.8, 8.8	Shen *et al.*, 1998
	BT	*Rf1*	-	NIL-derived BC1F1	-	RFLP, CAPS	C1361, fL 601	10	1.00 1.50	Akagi *et al.*, 2004

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Crop/ Species	CMS system	R gene	Genetics	Generation/ Population/ Cross	Technique/ Approach	Marker type	Linked markers/ QTL	Chromos-ome or LG	Distance (cM)/ variation (%)	Reference
	BT	*Rf1*	-	3-way cross	-	PCR markers	S12564Tsp5091 C1361 Mwo1	10	0.3	Komori *et al.*, 2003
	cms-D1	*Rf-D1(t)*	Single dominant	F_2	BSA	SSR	OSR 33 RM228	10	3.4, 5.0	Tan *et al.*, 2004
	HL	*Rf5, Rf6(t)*	Single dominant	3 A x (B x R) BC_1	BSA	SSR	RM1108, 3150, 5373 RM6737, 5373, SBD 07	 10	0.9, 0.0, 1.3 0.4, 0.0	Liu *et al.*, 2004
	cms-DA	-	Digenic interaction	RIL (B x R)-TCs	QTL analysis	RFLP SSLP	qRf-10-2 (RM258-RZ 811) qRf-1 (RG532)	10L 1	Major QTL Minor	Xie *et al.*, 2002
	BT	*Rf1*	-	F_2	-	RFLP	XNpb291-fL601-G2155	10	4	Ichikawa *et al.*, 1997
	WA		Single dominant	(A × R)-F_2	BSA	STMS	RM 258	10	9.5	Mishra *et al.*, 2003
Petunia hybrida	*pcf*	*Rf*	-	BC_2F_2	BSA	RAPD, RFLP	OP704, ECCA/MACT	4	0.8 0.8	Bentolila *et al.*, 1998
Phaseolus vulgaris	*pvs*	*Fr*	Single dominant	Three BC population.	BSA	RAPD, RFLP	Bng228, R335F/UBC487	(K)	4.5, 0.0	He *et al.*, 1995
	pvs	*Fr, Fr2*	Single dominant	Multiple population.	BSA	RAPD RFLP	R335E/ UBC487, Bng 228 Bng228/ UBC190/ UBC487, Bng 102	(K)	0.0 7.5 0.0 0.7	Jia *et al.*, 1997
Raphanus sativus	*ogura*	*Rf*	-	F_2	BSA	RAPD, SCAR	OPH11-410	-	1.2	Murayama *et al.*, 1999
	ogura	*Rfo*	-	F_2	BSA	AFLP	M-T12P18.9 M-F2K11.19	-	0.7	Desloire *et al.*, 2003

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Crop/ Species	CMS system	R gene	Genetics	Generation/ Population/ Cross	Technique/ Approach	Marker type	Linked markers/ QTL	Chromos-ome or LG	Distance (cM)/ variation (%)	Reference
	ogura	*Rfk-1*	-	BC_1F_2 (eight populations)	BSA	RAPD/ STS AFLP/ STS	A06/N763 B32 E90, E280/P200, P220/P180	-	2.3 7.1 0.1 0.2	Imai *et al.*, 2003
	ogura	*Rf1* *Rf2* *Rf3*	Single dominant	BC_1, F_2, R_s F_2, R_s R_s	-	RFLP	pN23×-pN199× pN107z-W179× pO159b-pN168y	Rs1 Rs2 Rs7	1.9 3.4 2.2	Bett and Lydiate, 2004
Secale cereale	CMS-C	Rf_c	Two dominant	Two F_2	BSA, QTL	RAPD	Four QTLs	4RL	4 -14	Stojalowski *et al.*, 2004
	Gulzow (CMS-G)	*Rfg1*	Single dominant	(A x R)-F_2, F_3	BSA	RAPD, RFLP	3 RAPD (XR11) Four RFLP	4RL	9.2 10-20	Borner *et al.*, 1998
	Pampa	Rfp1 Rfp2	Monogenic dominant	(B x R)-F_2s	BSA	AFLP RAPD RFLP	P15M55a/ P39M51a, SCXX04/ P16M60a, Xmwg 59	4RL	-	Stracke *et al.*, 2003
	Pampa	-	MonogenicDominant; complimentary	F_2	-	RFLP	PSR596-SR634 PSR899-MWG57	1RS 4RL	4.6 5.0	Miedaner *et al.*, 2000
Sorghum bicolor	A1	*Rf1*	Single dominant	(A x R) F_2	-	AFLP/ SSR	Xtxa2582, Xtxp18, Xtxp250	(H)	2.4, 12, 10.8	Klein *et al.*, 2001
	A3	*Rf4*	Two complimentary genes	BC_3F_1	BSA	ST/CAPS	LW7, LW8	(E)	5.3, 3.2	Wen *et al.*, 2002
	-	*Rf1*	-	F_2/F_3	BSA	RAPD	2 markers		1.6, 11.2	Pammi *et al.*, 1994

Contd.....

Crop/ Species	CMS system	R gene	Genetics	Generation/ Population/ Cross	Technique/ Approach	Marker type	Linked markers/ QTL	Chromos-ome or LG	Distance (cM)/ variation (%)	Reference
Triticum aestivum	T	*Rf3, Rf4*	-	BCF_1s	-	RFLP	Xbcd 249, Xcdo 442 (Rf3); Xksug 48 (Rf4); Xcdo 786 (Rf)	1BS 6BS 5D	-	Ma and Sorrells, 1995
	T	*Rf3*	-	-	-	RFLP	Xbcd156, Xcdo388	1BS	-	Kojima *et al.*, 1997
	T	*Rf6*	-		-	RFLP	Xksug48	6BS	-	Ma *et al.*, 1995
Zea mays	T	*Rf1*	-	4 popn.	-	RFLP	umc97, umc92	3	1.2, 9.5	Wise and Schnable, 1994
	T	*Rf2*	-	2 popn.	-	RFLP	umc153, sus1 (Rf2)	9L	3.8, 5.8	Wise and Schnable, 1994
	T	*Rf8*	-		-	AFLP	Arf-8	-	4.5	Wise *et al.*, 1999
	S	*Rf3*	Single dominant	4 BC_1 popns.	-	RFLP	whp, bnl17.14	2L	4.3, 6.4	Kamps and Chase, 1997
	C	*Rf4*	Single dominant	3 BC popns	-	RFLP	NP1114A	8S	1.5	Sisco, 1991

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was conducted during the period between January 2001 and August 2004 at the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru (17° N), Andhra Pradesh, India. The material development and evaluation was done in the Alfisol (red soil) fields at ICRISAT, Patancheru. All recommended practices were followed for raising a good pearl millet crop. This chapter describes details of the plant materials and the experimental methods to study the genetics of fertility restoration and sterility maintenance for several cytoplasmic-nuclear male-sterility (CMS) systems and mapping of the fertility restorer gene(s).

3.1 Plant Materials

The description and source of the plant material used in the study is given as under:

3.1.1 Pollen parents (R-lines)

All seven pollen parents used in the study (Table 2) were developed at ICRISAT-Patancheru and have been described earlier by Rai *et al.* (1996, 2001). Three of these seven inbred lines are dual restorer (i.e. restorers of two different CMS systems) and three are triple restorer (i.e. restorers of three different CMS systems). The LSGP-A_5 R-line restores fertility to the LSGP-A_5 CMS system only.

Table 2. Parentage/origin of pearl millet inbred lines used as restorer parents in fertility restoration genetics study*

Pollen parent	Parentage/origin	Restoration of cytoplasm
IPC 382	(B 282 × 3/4 ExB-100-11)-9-2-1 (ICMP 501)	A_1/A_v
IPC 492	(B 282 × J 804-1-3-9)-7-2-2	$A_1/A_4/A_v$
IPC 511	[(J 934-7 × 700544-7-2-1) × EC 298-2-1] -1-5	A_1/A_4
IPC 804	(S 10LB-30 × LCSN 1225-6-3-1)-1-2-1-1	$A_1/A_4/A_{egp}$
IPC 1518	ICRC-F4-146-3	A_1/A_4
L 67B	Inbred line from P.A.U., Ludhiana, India	$A_1/A_v/A_{egp}$
LSGP-A_5 R-line	(81A_5 × LSGP)-OP10-OP5-3-4-8-1	A_5

*B 282 is a breeding line introduced from Malawai; ExB refers to Ex-Bornu Composite from Nigeria; J-series inbreds are from Jamnagar Experimental Station of Gujarat Agricultural University, India; 700544 is a line from Nigerian breeding program; EC 298 is an Early Composite developed at ICRISAT, S 10LB is an inbred line developed at Punjab Agricultural University, Ludhiana, India from a Serere Composite; LCSN refers to a progeny identified at Kamboinse, Burkina Faso from ICRISAT's Late Composite; ICRC is an ICRISAT restorer composite developed at ICRISAT, Patancheru; L 67B is a maintainer of A_3 CMS system; LSGP-A_5 R-line is a selection from the large seeded gene pool (LSGP) at ICRISAT, Patancheru

3.1.2 Seed parents (Isonuclear A-lines along with B-lines)

The five isonuclear A-lines in three diverse nuclear backgrounds were developed by more than seven generations of backcrossing of the nuclear genomes of 81B (ICMB 1), 5054B and ICMB 88004 into cytoplasms of five different CMS systems viz.; A_1 (Burton, 1958), A_4 (Hanna, 1989), A_{egp} (Sujata *et al.*, 1994), A_v (Marchais and Pernes, 1985) and A_5 (Rai, 1995). The isonuclear CMS (A-lines) along with their maintainer (B-lines) are given in Table 3.

Table 3. Male-sterile (A) lines and maintainer (B) lines used in the study

A/B Line	Origin/Description	Reference
81B	ICMB 1: Gamma radiation-induced downy mildew resistant selection from Tift $23D_2B_1$	Anand Kumar *et al.* (1984)
$81A_1$	ICMA 1: Tift $23D_2$ A_1 cytoplasm source backcrossed to 81B	Anand Kumar *et al.* (1984)
$81A_4$	Hanna's *monodii* cytoplasm source (A_4) backcrossed to 81B	Rai *et al.* (1996)
$81A_{egp}$	EGP 261 cytoplasm source (A_{egp}) backcrossed to 81B	Sujata *et al.* (1994)
$81A_v$	ICMA 88001: *violaceum* cytoplasm source (A_v) of Marchais and Pernes backcrossed to 81B	Rai *et al.* (1996)
$81A_5$	ICMA 5: LSGP cytoplasm source (A_5) backcrossed to 81B	Rai and Rao (1998)
5054B	B-line from I.A.R.I., New Delhi, India	Pokhriyal *et al.* (1976)
$5054A_1$	Tift $23A_1$ cytoplasm source (A_1) backcrossed to 5054B	Pokhriyal *et al.* (1976)

contd.

5054A_4	Hanna's *monodii* cytoplasm source (A_4) backcrossed to 5054B	K.N. Rai, pers. comm.
5054A_{egp}	EGP 261 cytoplasm source (A_{egp}) backcrossed to 5054B	K.N. Rai, pers. comm.
5054A_v	*violaceum* cytoplasm source (A_v) backcrossed to 5054B	K.N. Rai, pers. comm.
5054A_5	LSGP cytoplasm source (A_5) backcrossed to 5054B	K.N. Rai, pers. comm.
ICMB 88004	Togo-11-5-2 selection	Rai *et al.* (1995)
ICMA 88004	81A_1 cytoplasm source (A_1) backcrossed to ICMB 88004	Rai *et al.* (1995)
ICMA_4 88004	Hanna's *monodii* cytoplasm source (A_4) backcrossed to ICMB 88004	K.N. Rai, pers. comm.
ICMA_{egp} 88004	EGP 261 cytoplasm source (A_{egp}) backcrossed to ICMB 88004	K.N. Rai, pers. comm.
ICMA_v 88004	*violaceum* cytoplasm source (A_v) backcrossed to ICMB 88004	K.N. Rai, pers. comm.
ICMA_5 88004	LSGP cytoplasm source (A_5) backcrossed to ICMB 88004	K.N. Rai, pers. comm.

3.2 Weather conditions

The main weather parameters during the seasons in which the various populations were evaluated viz., *summer* 2003 and *rainy* 2003, have been provided in the Appendix 1. The temperature (°C) and relative humidity (%) were recorded from the 35th day to the 70th day of crop growth, which refers to one week before the time of first flowering entry to one week after the time of the last flowering entry in each environment.

3.3 Development of segregating populations

Crossing schemes (Figures 1-3) were designed to produce the segregating populations for the various objectives of the study. Details of the crossing schemes along with the specific plant material used for each objective, are described below:

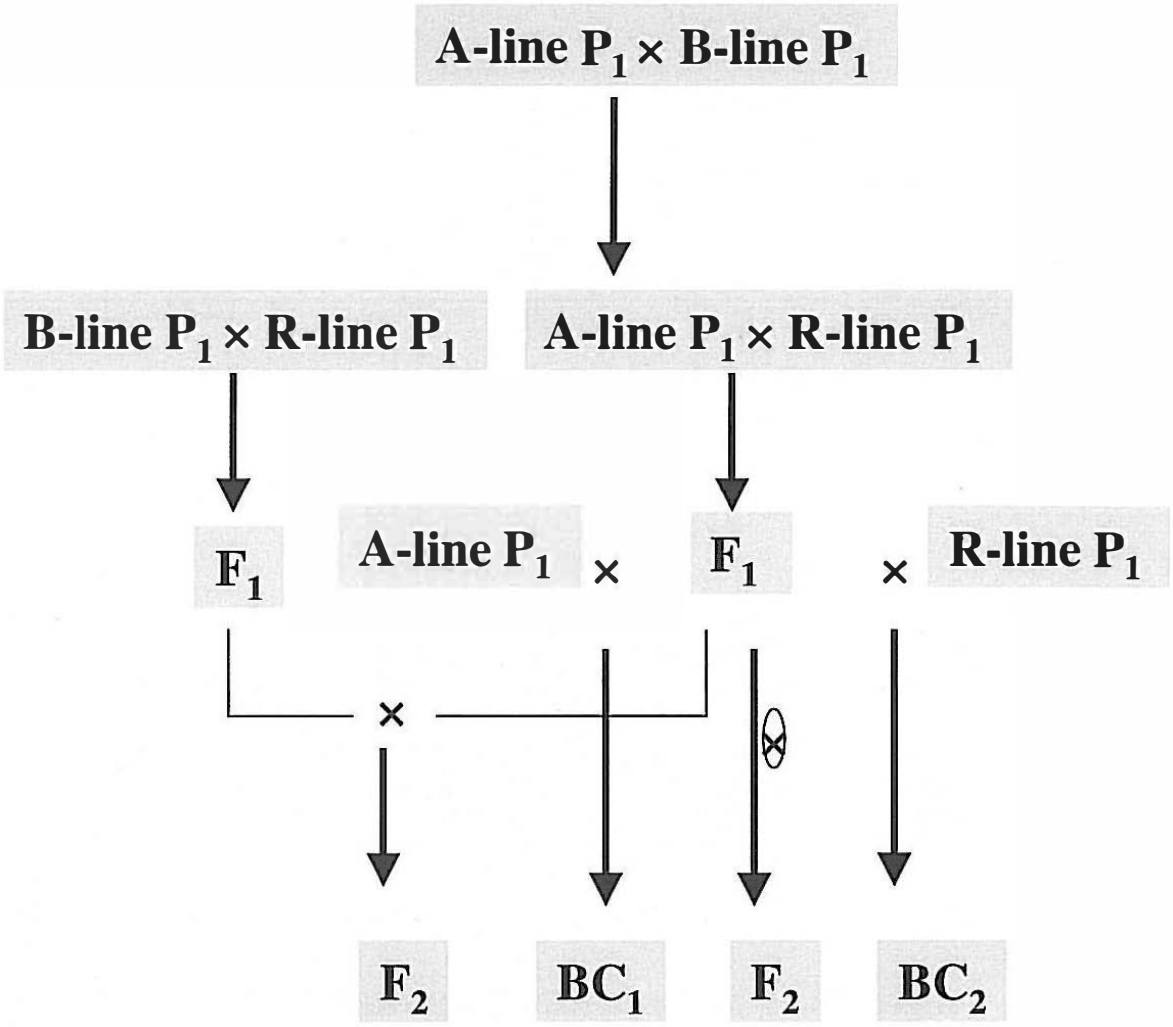

Figure 1. Crossing scheme for inheritance studies

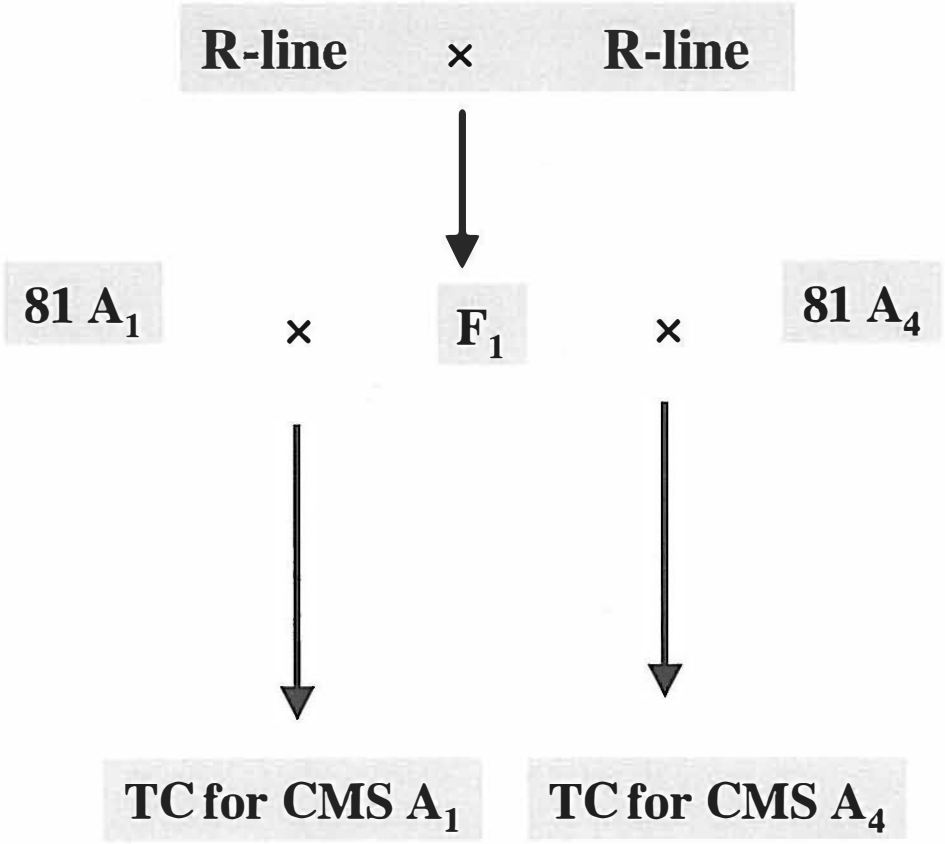

Figure 2. Crossing scheme for test of allelism

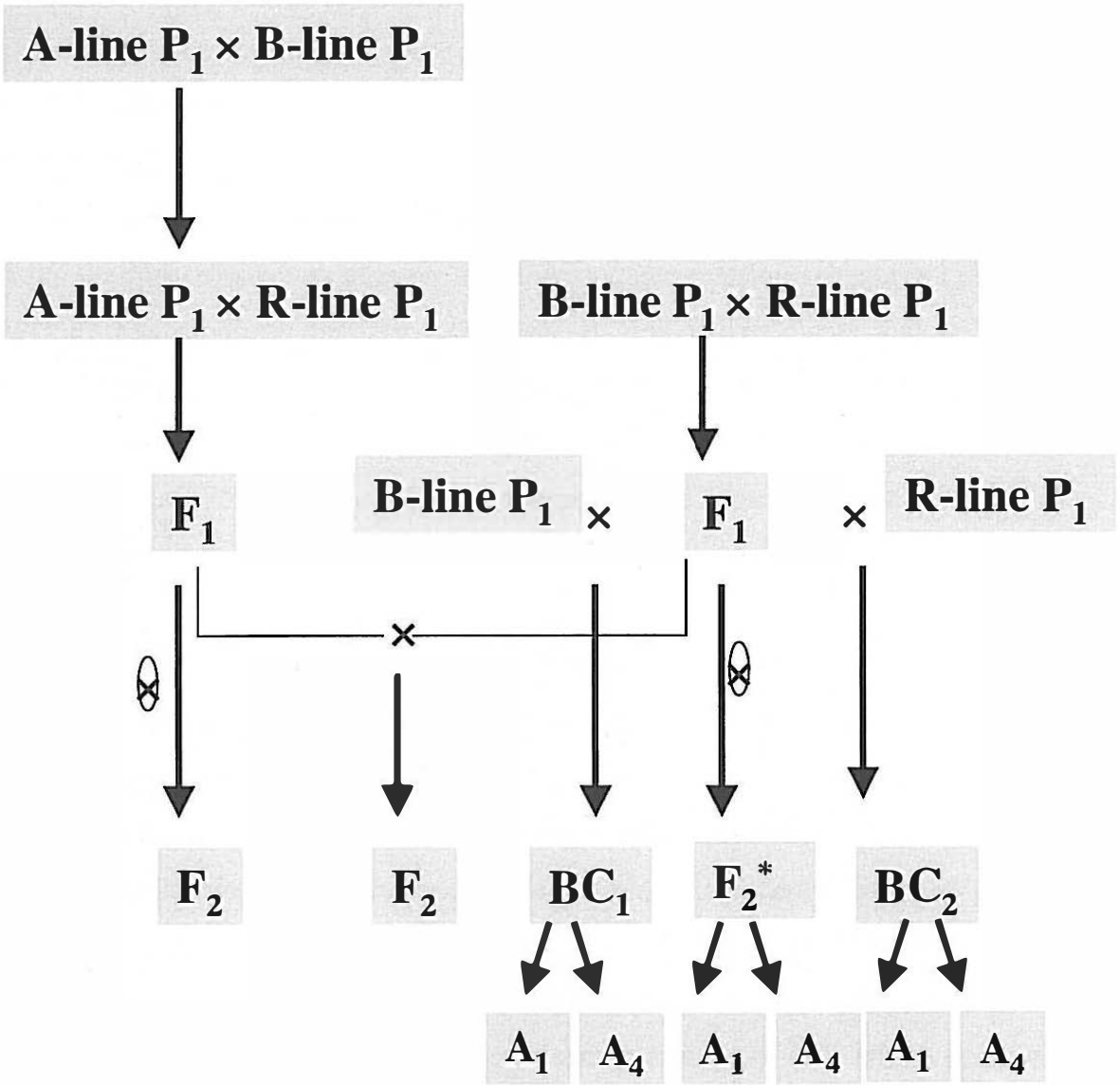

Figure 3. Crossing scheme for linkage and mapping experiment

***(81B x IPC 804) F_2 mapping population**

3.3.1 Inheritance of fertility restoration

Isonuclear A-lines of the five CMS systems (A_1, A_4, A_{egp}, A_v and A_5) in three different nuclear genetic backgrounds of 81B, 5054B and ICMB 88004 were crossed to their respective single- (i.e. restorer of one cytoplasm), dual- (i.e. restorers of two different cytoplasms) and triple-restorers (i.e. restorers of three different cytoplasms) to obtain (A × R) F_1 progenies to investigate the inheritance of fertility restoration (Figure 1). The A-lines were maintained by crossing with their respective B-lines. Selfed seed of B- and R-line plants used in crosses was also produced. In another crossing scheme, a (5054B × IPC 511) F_1 was produced involving the same B- and R-line plants that were also involved in producing the $5054A_1$ × IPC 511 and $5054A_4$ × IPC 511 crosses. All the crosses were produced by making plant × plant crosses during the post-rainy season (October 2001-April 2002). In one nuclear background, the same plant of a restorer line was used to make (A × R) cross on all the CMS systems restored by that particular restorer.

During the following rainy season (July-October 2002), the (A × R) F_1 progenies were selfed to produce the F_2 generations and the corresponding parental A- and R-line plants were used in crossing to produce BC_1 progenies [A × (A × R)] and BC_2 progenies [R × (A × R)] in all three nuclear genetic backgrounds viz., 81B, 5054B and ICMB 88004. Bulk pollen from 5-10 representative F_1 plants was collected and put on receptive stigmas of the corresponding parental A- and R-line plants to produce the backcross seed. A complete set comprising of all six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) was produced for each of the 46 crosses except in case of BC_2 where seed was produced for 43 crosses (Table 4). The (5054B × IPC 511) F_1 hybrid progeny was crossed on to the ($5054A_1$ × IPC511) F_1 and ($5054A_4$ × IPC 511) F_1 progenies to produce two (F_1 × F_1) F_2 populations.

Table 4. Number of segregating populations produced/evaluated in the three nuclear backgrounds for inheritance study

Gen/ CMS	Nuclear background															Total
	81B					5054B					ICMB 88004					
	A_1	A_4	A_v	A_{egp}	A_5	A_1	A_4	A_v	A_{egp}	A_5	A_1	A_4	A_v	A_{egp}	A_5	
F_2	6	4	3	1	1	6	4	3	1	1	6	4	3	2	1	46
BC_1	6	4	3	1	1	6	4	3	1	1	6	4	3	2	1	46
BC_2	4	4	3	1	1	6	4	3	1	1	6	3	3	2	1	43

The 46 sets of (A × R) F_2 populations, BC_1 progenies, BC_2 progenies along with their corresponding parental lines and F_1 progenies and the two [(A × R) F_1 × (B × R) F_1] F_2 populations were evaluated for fertility/sterility (F/S) reaction on the basis of pollen shedding (PS) and selfed seed-set (SSS) data collected at ICRISAT- Patancheru in the summer 2003 season and the rainy 2003 season. Pollen shedding data were recorded on individual plant basis for all the crosses, whereas selfed seed-set data was taken for selected crosses in 81B and 5054B nuclear backgrounds (four crosses each on A_1 and A_4 systems involving restorers IPC 804 and IPC 511; one cross each in A_{egp}, A_v and A_5 systems involving restorers L 67B, IPC 492 and LSGP-A_5 R-line), on an individual plant basis following a rating scale (0-100%) of Thakur and Williams (1980) initially developed for scoring ergot (*Claviceps fusiformis* Loveless) in pearl millet. Selfed seed-set data were also recorded for the two [(A × R) F_1 × (B × R) F_1] F_2 populations involving IPC 511 and 5054B. The parents, F_1 hybrids and BC_2 progenies were each evaluated in single row plots of 4 m length with approximately 30–35 plants per plot. Each F_2 population was evaluated in eight-row plots of 4 m length with approximately 250-350 plants per plot, and each BC_1 progeny was evaluated in four rows of 4 m length with about 125-150 plants per plot. For crosses where less seed was available, seed was re-produced especially for the F_1 and BC_1 progenies.

3.3.2 Allelism among fertility restorer gene(s) of A_1 and A_4 CMS systems

Four dual-restorer lines (IPC 1518, IPC 511, IPC 804 and IPC 492) restoring the A_1 and A_4 CMS systems were crossed in a diallel fashion to generate six (R × R) F_1 progenies during October 2001-April 2002 post-rainy season for studying the allelism among restorer genes of A_1 and A_4 CMS systems (Figure 2). All crosses were made using bulk pollen collected from 8-10 plants of the pollen parent line.

During July-October 2002 rainy season, the six (R × R) F_1 hybrids and the four parental R-lines were test crossed on to 81A_1 and 81A_4 CMS lines to generate 12 and 8 testcrosses, respectively, using bulk pollen (Table 5)

The F_1 and parental testcrosses were evaluated for F/S reaction on an individual plant basis (PS and SSS) in summer 2003 and re-evaluated in rainy season 2003. The parental testcrosses were sown in two-row plots of 4 m length (150-200 plants) in both the seasons whereas the F_1 testcrosses were grown in 6 row plots with approximately 200-250 plants during the summer 2003 season and 8-10 row plots of 4 m length with about 300-400 plants during the rainy season 2003.

3.3.3 Linkage between fertility restorer gene(s) of A_1 and A_4 CMS systems

Two (B × R) F_1 progenies were produced, plant × plant, between 81B and IPC 804 and between 5054B and IPC 511 (both R-lines are dual-restorers of A_1 and A_4 cytoplasms) during the post-rainy season (October 2001-March 2002). In this case, the same B-line plant was used to produce the B × R cross and to maintain the A-line used in the corresponding A × R cross, and the same R-line plant was used to produce both the B × R and A × R cross involving a particular restorer (Figure 3). The selfed B- and R-line parental plants were also harvested along with the crosses. The (5054B × IPC 511) F_1 progenies produced here were also used to produce the [(A × R) F_1 × (B × R) F_1] F_2 populations during the rainy season 2002 (described earlier in section 3.3.1).

Individual plants of the two (B × R) F_1 progenies were selfed to produce the F_2 populations and crossed plant × plant to their corresponding B-line parental plants to produce BC_1 [B × (B × R)] progenies in the greenhouse at ICRISAT-Patancheru during March-June 2002. The F_2 population developed from the cross 81B × IPC 804 served as the mapping population for molecular mapping of fertility restoration genes for the A_1 and A_4 CMS systems and mapping population development has been described in the next section 3.4

The two (B × R) F_2 populations, their BC_1 progenies (harvested from the greenhouse) and their parental plants were sown in the field during the rainy season 2002 along with $81A_1$, $81A_4$, $5054A_1$ and $5054A_4$. Pollen from individual plants of the (81B × IPC 804) F_2 and BC_1 populations was used to produce testcrosses on $81A_1$ and $81A_4$. Similarly, testcrosses were produced from crosses of the individual plants of the (5054B × IPC 511) F_2 and BC_1 populations onto male-sterile lines $5054A_1$ and $5054A_4$. The BC_2 progenies [R × (B × R)] for both crosses were also produced in the rainy season 2002. During the summer 2003 season, testcrosses from individual plants of the BC_2 [R × (B × R)] populations in 81B and 5054B backgrounds were produced on $81A_1$ and $81A_4$, and $5054A_1$ and $5054A_4$, respectively.

All the testcrosses made with individual plants of the two (B × R) F_2 and their corresponding BC_1 populations were evaluated in summer 2003 in single-row plots of 4 m length with parents and F_1 progenies as control entries, and these entries were re-evaluated in rainy season 2003 (Table 5). The BC_2 testcrosses were evaluated during the rainy season 2003 only in single-row plots of 4 m length. Plants were thinned to a spacing of 5-8 cm to accommodate approximately 40 plants per row.

3.4 Molecular mapping of fertility restorer gene(s)

For mapping the fertility restorer gene(s) of A_1 and A_4 CMS systems, part of the material produced for the linkage studies (section 3.3.3) was used i.e. the (81B × IPC 804) F_2 population was used as a mapping population (Figure 3). The presence of contrasting and easily distinguishable morphological traits between the two parents (tall/dwarf, d_2/non-d_2, leaf hairiness/non-hairiness, panicle bristling/no-bristling) facilitates the mapping of these traits as well as fertility restorer gene(s) for the A_1 and A_4 CMS systems from a single mapping population.

Table 5. Number of testcross progenies evaluated in linkage and test of allelism experiments

Material	Summer season 2003			Rainy season 2003		
	A_1	A_4	Total	A_1	A_4	Total
Linkage experiment						
81 (B × R) F_2*	412	412	824	405	405	810
81 (B × R) BC_1	149	149	298	146	146	292
81 (B × R) BC_2	-	-	-	36	36	72
5054 (B × R) F_2	404	404	808	396	396	792
5054 (B × R) BC_1	140	140	280	139	139	278
5054 (B × R) BC_2	-	-	-	42	42	84
Test of allelism						
Parental R-lines	4	4	8	4	4	8
(R × R) F_1	6	6	12	6	6	12

*mapping population also for mapping fertility restorer gene(s) in A_1 and A_4 CMS systems

3.4.1 Female parent

The female parent of this mapping population cross, 81B (=ICMB 1) is a gamma radiation-induced downy mildew resistant selection from Tift $23D_2B_1$ (Anand Kumar *et al.*, 1984) and is the dwarf (d_2) maintainer of the A_1 CMS system cytoplasm; has hairy leaf blades, hairy leaf sheaths and hairy leaf margin; and has non-bristled panicles. The nuclear genome of 81B has been backcrossed into cytoplasms of different CMS systems

(Rai, 1995; Rai *et al.*, 1996; Rai *et al.*, 2001) to develop near-isonuclear A-lines. 81B is the seed parent of many commercially released hybrids in India including ICMH 451, HHB 50, HHB 60 and RHB 58. 81B is also the female parent of two of the initial planned mapping populations (involving different single-plant selections of male parent ICMP 451) developed in pearl millet (Hash and Witcombe, 1994) to compare recombination rates in male and female gametes of pearl millet (Busso *et al.*, 1995) and to serve as the World Reference mapping population for this crop (Devos *et al.*, 2000; Qi *et al.*, 2004). Recently, Qi *et al.* (2004) have reported the development of new SSR markers in pearl millet using 81B as a source of DNA to generate an SSR-enriched library. It is likely that mutation was not the sole source of the improved downy mildew resistance of 81B as this line differs sufficiently from Tift 23DB to suggest that an outcross was involved in its parentage (Rai and Hanna, 1990b; Liu *et al.*, 1992)

3.4.2 Male (pollen) parent

IPC 804 is a selection from a breeding line developed at ICRISAT- Patancheru by crossing S 10LB (a long-bristled inbred line developed at Punjab Agricultural University, Ludhiana, India from a Serere Composite) and LCSN 1225-6-3-1 (a progeny identified at Kamboinse, Burkina Faso from ICRISAT's Late Composite). The male parents (ICMP 451 selections) of several mapping populations described by Hash and Witcombe (1994) are also derived from a near-inbred line from this ICRISAT's Late Composite, LCSN 21-1-2-1-1. IPC 804 is a triple-restorer of the A_1, A_4 and A_{egp} CMS systems, with profuse pollen producing capacity. It is tall (non-d_2), with non-hairy leaves and stem, and presence of long panicle bristles. IPC 804 has been used in earlier pearl millet CMS studies (Rai *et al.*, 1996; Rai *et al.*, 2001).

3.4.3 Development of mapping population

During the post-rainy season of October 2001-April 2002, a F_1 hybrid was produced by making plant × plant crosses between 81B and IPC 804 restorer line (Figure 3). The F_1 seed was sown in March 2002 in the greenhouse to produce F_2 mapping populations by selfing of individual F_1 plants. A single F_1 that produced the largest number of selfed seeds was selected for the mapping work. The (B × R) F_2 population seed (harvested in June 2002 from the greenhouse) and their parents were sown in the field in rainy season 2002 along with $81A_1$ and $81A_4$. The F_2 plants were numbered from 1-450 and leaf samples taken from them at the late seedling stage (25-30 days old) for marker analysis.

Pollen from each of the numbered F_2 plants was used to testcross onto both $81A_1$ and $81A_4$ to produce testcross seed for phenotyping studies. The testcross entries were evaluated in the summer and rainy seasons 2003 in single-row plot of 4 m length for F/S reaction (PS data only).

3.5 Observations

3.5.1 Inheritance and test of allelism

Fertility/sterility (F/S) reaction was recorded on an individual plant basis in all the (A × R)- and (R × R)-derived populations and testcrosses. Two criterion were followed:

3.5.1.1 Pollen shedding (PS)

When 50-75% portion of the panicle exhibited anthesis, plants were scored for pollen shedding between 0800 and 1100 h by tapping the uncovered heads and depending on the pollen cloud, were tagged with fertile (F), sterile (S) or shy labels accordingly and written on the selfing bag also with a permanent marker pen in cases where selfed seed set data was also being taken.

3.5.1.2 Selfed seed-set (SSS) data

The main tiller panicle/head was bagged in each plant, prior to stigma emergence, for recording the seed set data under selfing. This observation was recorded only in a few selected crosses in 81B- and 5054B-nuclear backgrounds (given in section 3.3.1). The bagged heads were harvested after seed setting and seed set data of each selfed head was scored following a rating scale (0-100%) of Thakur and Williams (1980) initially developed for scoring ergot (*Claviceps fusiformis* Loveless) in pearl millet. A selfed seed set score of 0% means no selfed seed set and 100% represents complete selfed seed set. The selfed seed-set classes were 0%, <1%, 1-5%, 6-10%, 11-20%, 21-30%, 31-40%, 41-50%, 51-60%, 61-70%, 71-80%, 81-90% and 91-100%.

3.5.2 Phenotyping of testcrosses for linkage and molecular analysis

F/S reaction was recorded on a plot basis when 75% of the plants in a plot had come to anthesis. The testcrosses from the F_2 plants exhibited three types of pattern: plants in a testcross entry were either all fertile (F), or all sterile (S), or segregating for fertility-sterility (F/S or S/F). Testcrosses from the BC_1 plants were either all sterile (S), or segregating for F/S reaction and for BC_2, were all fertile (F), or segregating for F/S reaction. The number of fertile and sterile plants was counted in the segregating testcrosses and recorded. For marker analysis, the data was converted to % fertile class.

3.5.3 Morphological observations on mapping population

The mapping population parents (81B and IPC 804) exhibited contrasting phenotypes for plant height (both quantitative and qualitative), leaf pubescence (hairy or non-hairy) and bristling (presence or absence). These morphological traits were recorded on the individual plants in the F_2 mapping population and its BC_1 [B × (B × R)]. Data was recorded for each F_2 plant for the three morphological traits. Plant height was recorded on the main tiller from the ground level to the tip of the panicle of individual F_2 plants. Individual F_2 plants and testcrosses from the F_2 mapping population progenies were scored for d_2/non-d_2 segregation behavior to distinguish the tall heterozygote F_2 plants from the tall homozygous F_2 individuals, as these could not be classified on the basis of F_2 data alone. Plant height was thus scored as a co-dominant marker. Leaf hairiness and panicle bristling were scored as dominant markers on the basis of presence/absence.

3.6 Molecular marker analysis

3.6.1 DNA extraction

Leaf tissues were harvested (5 g) from young green field-grown seedlings (25-30 days old) to isolate genomic DNA. Several procedures for genomic DNA isolation have been reported, but results obtained by following the S-buffer maxi-preparation DNA protocol given by Sharp *et al.* (1988) with modifications (Mace *et al.*, 2003) was found satisfactory (Appendix 2). The DNA purity and concentration of each sample was quantified by spectrophotometer readings of UV absorption at 260 nm and 280 nm. The ratio of OD_{260} to OD_{280} was calculated to check the purity and concentration of each sample. The DNA samples were analyzed in a 0.8% TBE-agarose gel to test DNA integrity and concentration for making dilutions. The final DNA concentration was adjusted to 1 µg/µL for RFLP analysis and 5 ng/µL for SSR genotyping.

3.6.2 SSR analysis

For the present study, the optimization of PCR reaction conditions was achieved by using a grid with varying amounts and concentrations of critical reagents at different annealing temperatures following the initial work of Allouis *et al.* (2001) and Qi *et al.* (2001, 2004). The list of polymorphic SSR markers used for parental screening and genotyping is given in Table 43 (between pages 77-78).

3.6.2.1 Parental polymorphism

The quality and quantity of DNA isolated from each tissue sample were checked using agarose gel electrophoresis for SSR analysis. The final DNA concentration was adjusted to 5 ng/µl by diluting each sample with an appropriate volume of $T_{10}E_1$ buffer.

A set of 70 pearl millet SSR primer pairs received from John Innes Centre (Norwich, UK) were used for PCR amplification using DNA from the two parents, 81B and IPC 804, and the F_1 as template in order to identify polymorphic SSR markers that could be used for genotyping the mapping population progenies.

3.6.2.2 Amplification of SSR markers

The PCR reactions were performed in volumes of 20 µL containing 15 ng genomic DNA, 30 ng/µL each of forward and reverse primers, 2 mM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 50 mM $MgCl_2$, and 5 unit/µL of *Taq* DNA polymerase. The annealing temperature for PCR amplification was maintained based on the specificity of the primer pair obtained after the optimization.

PCR reactions were conducted in 96-well plates in a Peltier thermocycler DNA Engine PTC 200 model and DYAD model from MJ Research with a standard PCR profile of 94°C for 3 min followed by 34 cycles of 1 min for denaturation at 94°C, 1 min for annealing (with ramping @ –0.5°C/second) at 48°C to 61°C (specific to the primer pairs used), and 30 seconds for extension at 72°C followed by final extension for 4 min at 72°C.

3.6.2.3 Poly Acrylamide Gel Electrophoresis (PAGE)

For separation and visualization of PCR products, 6% polyacrylamide gels were used. The gels were prepared using

52.5 mL of doubled distilled water
7.5 mL of 10X TBE buffer
15 mL of Acryl amide : Bis-acryl amide (29:1) solution
450 µL of Ammonium Per Sulphate (APS) and
90-100 µL of TEMED.
(Total volume 75 mL)

5 µL of loading dye (orange red + EDTA + NaCl + glycerol) was added to 20 µL of PCR product. From this mixture, 2 µL of sample was loaded into the 6% non-denaturing PAGE gel. Along with the PCR amplified products of parental, F_1 and progenies, a 100 bp ladder (50 ng/µL) was also loaded in the first and last lane of the gel

to ensure proper sizing of amplified PCR fragments. The gel was run at 550V of constant power in 0.5X TBE buffer for 3 hours using a BioRad gel sequencing apparatus.

For a few SSR markers, post-PCR multiplexing was done either through differences in the size of amplified products between two primers or by loading the amplified products of a single primer with a time gap of 20-25 minutes. This enabled screening of 192 to 284 progeny samples in a single gel run, as compared to the normal 96 samples in a single PAGE run, thus providing an opportunity to save on time and reduce cost per data point generated.

3.6.2.4 Silver staining

After running the PAGE gels for the required time, the gels were developed by silver staining method (Panaud *et al.*, 1996). The gel was passed through a series of sequential steps in the order given below:

a) water for 3-5 minutes

b) 0.1% CTAB solution for 20 minutes (2 g in 2 L of water)

c) 0.3% ammonia solution for 15 minutes (26 mL of 25% ammonia solution in 2 L of water)

d) 0.1% silver nitrate solution for 15 minutes (2 g of silver nitrate + 8 mL of 1M NaOH in 2 lit of water and added 6-8 mL ammonia solution until the solution became colorless)

e) water for 1 minute

f) developer (30 g of sodium carbonate + 400 µL of formaldehyde in 2 L of water) until the bands became visible

g) rinsed in water for 1 minute and placed in fixer (30 mL glycerol in 2 L of water) for a few seconds

All the steps were performed on a shaker, as continuous shaking is required throughout the silver staining procedure.

After silver staining of the PAGE gels, the size (base pair) of the intensely amplified specific bands or alleles for each SSR marker was estimated based on its migration relative to the 100 bp DNA ladder (fragments ranging from 100 bp to 1000 bp) and scored either as parental or F_1 (heterozygote) bands.

3.6.3 RFLP analysis

3.6.3.1 Probes for RFLP work

Probes were selected from a *Pst*1 genomic library (PgPSM1 to PgPSM1000) generated by Liu *et al.* (1994) from total DNA of pearl millet genotype 7042(S). These RFLP probes were received as stab culture stocks from John Innes Centre, U.K. For use, plasmid DNA extraction and insert purification were done following the protocol given in Appendix 3 and their size and concentration checked on a 0.8% agarose gel.

3.6.3.2 Polymorphism between parents

Initial screening of parental lines was done before the actual genotyping of the mapping population progenies to identify polymorphic combinations of probes and restriction enzymes. The DNA from the two parents, 81B and IPC 804, and the F_1 was restricted with four endonuclease restriction enzymes and probed against ~60 selected pearl millet PgPSM probes. From the parental screening, polymorphic combinations identified were used for screening the mapping population.

3.6.3.3 Restriction enzyme digestion

For each sample, 20 µg of DNA in sterile distilled water was digested with *Dra*I, *Eco*RI, *Eco*RV and *Hind*III restriction endonucleases following the endonucleases supplier's instructions (Amersham Pharmacia Biotech, Ltd.). The digestion was performed in a total volume of 30 µL and the reaction was terminated by addition of 5 µL of loading buffer (25% sucrose, 0.1% bromophenol-blue and 20 mM EDTA) to each 30 µL sample.

3.6.3.4 Electrophoresis

Fragments of digested DNA obtained after enzyme digestion were separated by electrophoresis in 0.8% TAE-agarose on a horizontal slab gel unit (Owl Separation Systems Model No. A-1) for 16 h at 38 V/cm in TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.8) buffer. Gels were prepared in the same buffer that was used for electrophoresis. *Hind*III digested Lambda DNA (λ DNA) was used as the standard molecular size markers. Ethidum bromide was added at the time of gel preparation at a concentration of 0.5 µg/mL. After the run completion, the gels were viewed on a UV-transilluminator and photographed to assess the quality of digestion.

3.6.3.5 Southern blot hybridization

3.6.3.5.1 Preparation of southern blots

DNA fragments, separated electrophoretically after digestion, were transferred from agarose gels onto a Nylon transfer membrane (Hybond-N^+, Amersham Pharmacia Biotech, Ltd.) following the procedure of Reed and Mann (1985) (Appendix 4). Transferred membranes were soaked in 2X SSC for 2–5 min to neutralize the alkali, washed in dH_2O twice, air dried and wrapped with a cling film and stored at –20°C for future use.

3.6.3.5.2 Labeling of probes

The random-primed method of Feinberg and Vogelstein (1983) was used for labeling DNA with α-^{32}P. Purified insert DNA was denatured by heating at 95°C for 5 min, put on ice for 3 min before the labeling reaction mixture was added and incubated at 37°C for 3 hours. The reaction was terminated by adding 5 µL of 0.2 M EDTA pH 8.0 and 145 µL distilled H_2O, mixed properly, denatured at 90°C for 5 min followed by 3 min on ice and then added to hybridization bottles. The labeling reaction mixture was prepared using NE Blot® kit from New England Biolab Inc.

3.6.3.5.3 Prehybridization

Southern blots were pre-hybridized at 65°C with 15–20 mL of prehybridization solution and 1.0 mL of denatured salmon sperm DNA for six hours in case of new blots and 2–3 hours for stripped blots. Prehybridization was performed in a Techne Hybridizer bottles.

3.6.3.5.4 Hybridization

Labeled probe was added to the prehybridization mixture and incubated at 65°C in a hybridization oven for at least 16 hours. Care was taken to remove air bubbles present between the blots and the hybridization bottle.

3.6.3.6 Washing of blots

Following hybridization, the blots were washed following four changes of 60 mL each of ^{32}P wash solutions. Each wash was done for 15 min at 65°C in hybridization bottles using hybridization oven. The first two washes were done using wash 1 solution (100 mL 20X SSC, 25 mL 20% SDS and distilled water to 1 L). The second two washes were done using wash 2 solution (10 mL 20X SSC, 25 mL 20% SDS and distilled water to 1 L). Membranes were air dried and enclosed in cling film.

3.6.3.7 Autoradiography

Autoradiography was conducted at –80°C by exposing the membrane to photographic film (Kodak, X-OMAT™, XK-5) using Kodak intensifying screens in a cassette for varying exposure times depending on counts. The X-ray films were developed in a dark room with infra-red light with a Kodak developer for 2 minutes followed by a stop bath treatment with water for 1 minute and then fixed with Kodak fixer for 2 minutes, washed in running tap water and air-dried.

3.6.3.8 Filter Stripping and reuse

After the development of autorads, the filters were stripped to remove the incorporated ^{32}P-α-dATP for use with the next probe. The filters were put in a plastic box and boiling stripping solution (0.1X SSC, 0.5 SDS) was poured in until it covered the top filter. The box was covered and kept on a shaker for 5 min. The solution was poured off and the process repeated three more times. After stripping, filters were dried in between blotting paper sheets, saran-wrapped and kept at –20°C, or hybridized again immediately. Filters were reused 4–5 times.

3.6.4 Scoring of RFLP/SSR amplified bands

The banding patterns obtained for the F_2 mapping progenies were scored as:

A = homozygote for allele 'a' from parent P_1 (81B)

B = homozygote for allele 'b' from parent P_2 (IPC 804)

H = heterozygote carrying alleles from both P_1 and P_2 *i.e.* F_1

C = not a homozygote for allele 'a' (*i.e.* either B or H)

D = not a homozygote for allele 'b' (*i.e.* either A or H)

– = missing data

3.7 Statistical analysis

3.7.1 Testing goodness of fit of genetic ratios

Chi square (χ^2) method with Yates' correction factor (Steel and Torrie, 1980) was applied on the observed data to test the goodness of fit of different genetic ratios. The calculated χ^2 values were compared with tabulated χ^2 values with (n-1) degrees of freedom at 5% and 1% probability level. The null hypothesis was rejected if the calculated χ^2 value exceeded the corresponding tabulated χ^2 value. Exact probability value at (n-1) degrees of freedom for the best-fit hypothetical ratio was calculated in the Excel spreadsheet using the statistical function 'CHIDIST'.

$$\chi^2_{cal} = \Sigma (|O-E| - 0.5)^2 / E$$

where,

O = observed number of plants

E = expected number of plants

Σ = summation over all classes

n = number of independent classes in the hypothetical distribution

To test all possible monogenic, digenic, and trigenic Mendelian ratios for F_2 and BC_1 populations simultaneously, a program was made in an Excel spreadsheet for analysis of all the crosses.

3.7.2 Test of Homogeneity of genetic ratios

The χ^2 test for homogeneity of genetic ratios for a cross across the summer and rainy seasons was done. The step-by-step procedure is:

a) The χ^2 test for goodness of fit (section 3.7.1) was applied to individual seasons' data separately for the F_2 and BC_1 generations

b) Compute the χ^2_{sum} values from the sum of all the χ^2 values computed in step (a)

c) Compute the totals of observed values and of expected values for a given F_2 and BC_1 population over both the seasons and calculate χ^2_p for these totals.

d) The χ^2 value for heterogeneity (χ^2_h) was computed as the difference between the χ^2_{sum} (calculated in step b) and the χ^2_p value (computed in step c)

e) Compare the computed χ^2_h value with the tabulated χ^2 value with (s-1) degrees of freedom (where s = number of seasons) at 5% and 1% probability level. If the computed χ^2_h value is greater than the corresponding tabulated χ^2 value, it indicates that the data from both the seasons are heterogeneous with respect to the genetic ratio being tested and hence, cannot be pooled. In such cases, individual χ^2 values were examined.

3.7.3 Linkage estimation

The data obtained from the two (B × R) F_2 and BC_1 populations in 81B and 5054B nuclear backgrounds was arranged according to the joint segregation pattern of the A_1 and A_4 testcross entries originating from the same F_2 or BC_1 plant. In case of F_2, plants in a testcross entry were all fertile (F), all sterile (S), or segregating for fertility-sterility (F+S) whereas testcrosses from the BC_1 plants were all sterile (S), or segregating for F/S reaction and for BC_2, were all fertile (F), or segregating for F/S reaction. Joint segregation analysis using χ^2 method was done to detect linkage between fertility restorer gene(s) of A_1 and A_4 CMS systems.

3.7.4 Linkage mapping

Linkage analysis was done using the programs MAPMAKER/Exp version 3.0b supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA (Lincoln *et al.*, 1992a) and JoinMap® 3.0 (Stam, 1993; van Ooijen and Voorrips, 2001) with 36 polymorphic markers. The goodness of fit of markers to the expected Mendelian ratio of 1:2:1 for co-dominant markers and 3F:1S or 1F:3S for the dominant markers was done using χ^2 analysis (described in section 3.7.1). LOD threshold value of 3.0 and 2.0 were taken in MAPMAKER and JoinMap, respectively, to detect linkage among markers. The Haldane mapping function was used to calculate linkage map distances in centiMorgan (cM). The 'build' command was used in MAPMAKER to place new markers from the genotypic data set at the most appropriate position within the identified linkage group. The consensus map given by Qi *et al.* (2004) was used for reference and comparison.

3.7.5 QTL mapping

QTL analysis was done using the linkage map developed using 36 polymorphic markers and phenotyping data produced from the testcrosses made on $81A_1$ and $81A_4$ from each of the 397 individual F_2 plant. QTL analysis was performed using both MAPMAKER/QTL version 1.1b (Lander and Botstein, 1989; Lincoln *et al.*, 1992b) and PLABQTL version 1.2 (Utz and Melchinger, 2000), based on map produced with or without the distorted markers. For identifying QTL for fertility restoration, the additive model was enforced (as required for the testcross phenotyping data sets) whereas for plant height a free model (as allowed for the F_2 population phenotyping data sets) was used for analysis in MAPMAKER as well as PLABQTL. MAPMAKER uses a maximum likelihood approach for interval mapping (Lander and Botstein, 1989) whereas PLABQTL uses a multiple regression approach with flanking markers (Haley and Knott, 1992) to perform both interval mapping and composite interval mapping (CIM) (Zeng, 1994).

3.7.6 Marker-trait association analysis

With few polymorphic markers available, a recently developed map-free approach reported by Chandra *et al.* (2004) was also used to identify markers potentially linked to QTL. Basically, three approaches were used:

a) Single-marker approach (SMA) uses simple linear regression and is based on the following model

$$y_k = \alpha + \beta x_k + \varepsilon_k \; k = 1,\ldots., n \qquad (1)$$

b) Two multiple-marker approaches viz., step-wise regression (SWR) and Bayesian information criterion (BIC), based on the following model

$$y_k = \alpha + \Sigma_l \beta_l x_{kl} + \varepsilon_k \qquad k = 1,\ldots,n \qquad l = 1,\ldots,m \qquad (2)$$

where x_{kl} is the marker score (x= 0, 1 or 2 for marker genotype A, H or B, respectively) of k-th F_2 plant at l-th marker, β_l is the partial regression coefficient (additive genetic effect in the case of F_2) of a putative QTL linked to l-th marker, m is the number of markers and l is the number of F_2 or testcross individuals. The above model assumes that QTL act additively. Model 2 (point b) helps in identifying the smallest number of markers simultaneously significantly linked to the trait ($\beta_l \neq 0$) as compared to the conventional model selection approach that minimizes prediction error by selecting a subset of markers (Broman and Speed, 2002). This was achieved through step-wise regression (SWR) using $F_{in}=F_{out}=4$ as threshold for partial F-statistic to include linked and exclude unlinked markers. The threshold was increased up to 10 to retain only the most important markers.

The minimum Bayesian information criterion, applied on all possible 2^m regression models, was used for comparison and to identify QTL-associated markers that are consistently selected by the different approaches. BIC was used to identify the model with maximum posterior probability (assuming uniform prior) based on the following equation:

$$BIC = n \log (1-R^2) + k \log (n)$$

Where, R^2 is the coefficient of determination

For the three approaches, 26 distortion-free markers were included for the analysis. The genotypic data for the analysis was converted to numeric codes with A=0, H=1, B=2 and C, D or '–' to '*'.

From the single-marker analysis, the top 16 markers were selected based on F-probability value for further analysis with SWR and BIC criterion. The adjusted R^2 values for markers selected by SWR and BIC criterion were calculated using multiple linear regression in GenStat (6^{th} edition) package (Payne, 2002).

4. RESULTS

4. RESULTS

The present study was carried out with the objective of resolving the genetics of five well-documented CMS systems in pearl millet and identifying molecular markers for fertility restoration of A_1 and A_4 CMS systems. The results have been presented for each of the four objectives of the study as under:

4.1 Inheritance of cytoplasmic-nuclear male-sterility

The inheritance of cytoplasmic-nuclear male-sterility (CMS) was investigated in (A × R) crosses produced using a set of isonuclear A-lines developed by more than seven generations of backcrossing of the nuclear genomes of 81B, 5054B and ICMB 88004 into the cytoplasms of five different CMS systems viz., A_1, A_4, A_v, A_{egp} and A_5 and seven single-, dual- or triple-restorer lines of the five CMS systems. The F_1 hybrids produced on A-lines from IPC 492 were segregating for male-sterility and male-fertility in all the CMS systems tested indicating the unstable segregation behaviour. Therefore, the results from the studies involving this restorer are not presented. The F_1 hybrids and BC_2 populations produced from all other A × R crosses for all the five CMS systems had fully fertile plants across the two test environments of summer and rainy seasons at ICRISAT- Patancheru, indicating that male-fertility was dominant over male-sterility. Thus, this result will not be repeated in the discussion of individual CMS systems. The details of the plant material and the development of different populations have been described in the chapter 3 (Materials and Methods).

4.1.1 Inheritance of A_1 CMS system

The inheritance of A_1 CMS system was investigated based on segregation pattern of F_2 and BC_1 in a total of 15 (A × R) crosses. Information on inheritance of A_1 CMS system was also derived from the segregation pattern obtained from the F_2 and BC_1 testcross data as well as the segregation behavior observed within the segregating F_2 and BC_1 testcrosses of the two (B × R) crosses developed mainly for linkage and molecular mapping experiment. The (A × R) crosses were produced by crossing six restorer lines restoring fertility of A_1 CMS system with A-lines in three diverse nuclear backgrounds of 81B, 5054B and ICMB 88004. All the crosses were evaluated in two environments (summer and rainy seasons 2003) at ICRISAT- Patancheru for fertility/sterility (F/S) reaction on the basis of pollen shed data. A few selected crosses in 81B and 5054B background involving two restorer lines IPC 804 and IPC 511 were also evaluated on

the basis of selfed seed set (SSS) data. In crosses where SSS data was recorded, genetic ratios were worked out for two cases viz., one based on plants with 0-5% SSS classified as sterile irrespective of whether plants were tagged as fertile or shy on the basis of pollen shed data and plants with 6-100% SSS as fertile irrespective of whether plants were tagged as sterile or shy on the basis of pollen shed data. Similarly, another classification of plants was based upon 0-10% SSS classified as sterile and 11-100% SSS as fertile irrespective of whether plants were tagged as fertile or sterile or shy, respectively, on the basis of pollen shed data. The classification of plants as sterile following these two selfed seed-set data scenario were followed considering a low frequency of plants in these A-lines do set up to 10% seed when selfed. The results of inheritance of A_1 CMS system with respect to each of the fertility restorer parents have been presented in the account to follow.

4.1.1.1 Male-fertility restorer parent IPC 1518

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) plants in the three crosses produced with this restorer are provided in Table 6.

4.1.1.1.1 81B nuclear background

In the summer season, 304 F_2 plants segregated into 212 fertile (F) and 92 sterile (S) plants and gave a good fit (χ^2= 0.02; P = 0.89) to the expected trigenic ratio of 45F:19S that results from interaction between one basic gene and two duplicate-complimentary genes (Table 6). However, 156 BC_1 plants segregated into 99 fertile and 57 sterile plants giving a poor fit (χ^2= 10.78; $P < 0.01$) to the 1F:1S ratio expected for the above-mentioned trigenic ratio. In the rainy season, 365 F_2 plants (289 fertile and 76 sterile) showed departure from the expected trigenic ratio of 45F:19S (χ^2= 13.32; $P < 0.01$) but the observed 152 BC_1 plants (89 fertile and 63 sterile) gave a good fit to a 1F:1S ratio with χ^2 value of 4.11 (P = 0.04). When the data were pooled for both the seasons, the F_2 data agreed to the expected trigenic ratio of 45F:19S (χ^2= 6.49; P = 0.01) whereas the BC_1 pooled data deviated significantly from the expected numbers as evident from a significant χ^2 value of 14.57 ($P < 0.01$). The heterogeneity χ^2 value for F_2 data (χ^2= 6.85; P = 0.01) exhibited good agreement whereas the BC_1 agreed strongly with the expected numbers as evidenced by χ^2 value of 0.32 (P = 0.57).

Table 6. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1-system A-lines with the restorer parent IPC 1518, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_1 × IPC 1518-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	212	92	289	76	99	57	89	63
Expected	214	90	257	108	78	78	76	76
χ^2	0.02 (0.89)*		13.32 (< 0.01)		10.78 (< 0.01)		4.11 (0.04)	
χ^2_p	6.49 (0.01)				14.57 (< 0.01)			
χ^2_h	6.85 (0.01)				0.32 (0.57)			
$5054A_1$-P_2 × IPC 1518-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	228	67	190	37	120	48	115	22
Expected	249	46	192	35	126	42	103	34
χ^2	10.71 (< 0.01)		0.04 (0.84)		0.96 (0.33)		5.37 (0.02)	
χ^2_p	6.99 (0.01)				0.58 (0.45)			
χ^2_h	3.76 (0.05)				5.75 (0.02)			
$88004A_1$-P_3 × IPC 1518-P_2 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	197	129	245	74	93	77	64	56
Expected	229	97	224	95	85	85	60	60
χ^2	14.78 (< 0.01)		6.13 (0.01)		1.32 (0.25)		0.41 (0.52)	
χ^2_p	0.90 (0.34)				1.82 (0.18)			
χ^2_h	20.01 (< 0.01)				-0.09 (0.76)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

4.1.1.1.2 5054B nuclear background

The number of F_2 plants scored for F/S varied from 295 in the summer season to 227 plants in the rainy season (Table 6). In the BC_1 generation, 168 and 137 plants were scored in summer for F/S and rainy seasons, respectively. The observed number of plants in summer and rainy seasons as well as in the pooled data gave a good fit to the F_2 ratio of 54F:10S (except F_2 during summer) and BC_1 ratio of 3F:1S expected for the trigenic inheritance mechanism governed by any two of the three duplicate-complimentary genes restore fertility. The segregation in F_2 during summer season agreed poorly with the hypothesized ratio of 54F:10S as evident from the significant χ^2 value of 10.71 ($P < 0.01$). However, the corresponding BC_1 segregation gave a good fit to the expected 3F:1S ratio with a χ^2 value of 0.96 ($P = 0.33$). In rainy season, the F_2 segregated into 190 fertile and 37 sterile plants to give a good fit to the expected trigenic ratio of 54F:10S as evident from a χ^2 value of 0.04 ($P = 0.84$) and the BC_1 also showed a good agreement with the expected ratio of 3F:1S as indicated by a χ^2 value of 5.37 ($P = 0.02$). The pooled data showed the segregation in F_2 giving a good fit to the expected 54F:10S ratio ($\chi^2= 6.99$; $P = 0.01$) and in the BC_1 a good fit to the expected ratio of 3F:1S with a χ^2 value of 0.58 ($P = 0.45$). The heterogeneity χ^2 value was non-significant for F_2 and BC_1 indicating similar segregation pattern across the seasons.

4.1.1.1.3 ICMB 88004 nuclear background

During the summer season, 326 F_2 plants from the cross ICMA 88004 × IPC 1518 segregated into 197 fertile and 129 sterile plants exhibiting a poor fit to the expected trigenic ratio of 45F:19S ($\chi^2= 14.78$; $P < 0.01$) but in the BC_1, the segregation pattern of 170 plants (93 fertile and 77 sterile) were in good agreement with the expected ratio of 1F:1S as indicated by a χ^2 value of 1.32 ($P = 0.25$) (Table 6). In the rainy season, 319 F_2 plants segregated into 245 fertile and 74 sterile plants to give a good fit to the expected trigenic ratio of 45F:19S ($\chi^2= 6.13$; $P = 0.01$) and in the BC_1, 120 plants segregated into 64 fertile and 56 sterile plants to give a good fit to the expected ratio of 1F:1S as evident from a χ^2 value of 0.41 ($P = 0.52$). The pooled data also gave a good fit to the expected trigenic F_2 ratio of 45F:19S ($\chi^2= 0.90$; $P = 0.34$) and BC_1 ratio of 1F:1S ($\chi^2= 1.82$; $P = 0.18$). The heterogeneity χ^2 value indicated large variation in the segregation of F_2 as evident from a highly significant χ^2 value of 20.01 ($P < 0.01$) but indicated uniform segregation in BC1 data ($\chi^2= -0.09$; $P = 0.76$) across the seasons.

4.1.1.2 Male-fertility restorer parent IPC 804

In 81B and 5054B background, the F_2 and BC_1 were also evaluated on the basis of selfed seed set (SSS) data besides pollen-shed data. The 81B × IPC 804 cross, mainly produced for linkage analysis and molecular mapping, also provided information about inheritance from the testcross data (segregation pattern between testcrosses and within the segregating testcrosses). The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) plants in the three crosses produced with this restorer are provided in Table 7.

4.1.1.2.1 81B nuclear background

In the summer season, 274 F_2 plants segregated into 179 fertile and 95 sterile plants and agreed to the expected trigenic ratio of 45F:19S with a χ^2 value of 3.03 (P = 0.08) (Table 7). The 158 plants in BC_1 segregated into 74 fertile and 84 sterile plants and gave a good fit to the hypothesized 1F:1S ratio as evident from the χ^2 value of 0.51 (P = 0.48). In the rainy season, 312 F_2 (231 fertile and 81 sterile) and 188 BC_1 (105 fertile and 83 sterile) plants segregated according to the expected ratio of 45F:19S (χ^2= 1.90; P = 0.17) and 1F:1S (χ^2= 2.35; P = 0.13), respectively. The pooled data for both the seasons confirmed even more strongly to the expected F_2 (χ^2= 0.02; P = 0.89) and BC_1 (χ^2= 0.35; P = 0.55) ratios as compared to individual seasons. The non-significant heterogeneity χ^2 for F_2 and BC_1 indicated uniform segregation pattern across the two seasons.

On the basis of SSS, when plants with 0-5% SSS were classified as sterile and those with 6-100% SSS classified as fertile, the observed data for F_2 and BC_1 were in accordance with the expected ratio of 45F:19S and 1F:1S, respectively, during both the seasons (Table 8). In summer season, 274 F_2 plants segregated into 176 fertile and 98 sterile plants giving a good fit to the expected trigenic ratio of 45F:19S with a χ^2 value of 4.56 (P = 0.03). In the BC_1, 158 plants segregated into 73 fertile and 85 sterile plants giving a good fit to the hypothesized 1F:1S ratio as evident from the χ^2 value of 0.92 (P = 0.34). Similarly, in the rainy season, 312 F_2 (221 fertile and 91 sterile) and 188 BC_1 (97 fertile and 91 sterile) plants segregated according to the expected trigenic ratio of 45F:19S (χ^2= 0.02; P = 0.89) and 1F:1S (χ^2= 0.13; P = 0.72), respectively. The pooled data for both the seasons also confirmed to the same expected F_2 (χ^2= 1.73; P = 0.19) and BC_1 (χ^2= 0.10; P = 0.75) ratio. The non-significant heterogeneity χ^2 for F_2 and BC_1 indicated uniform segregation pattern across the two seasons.

Table 7. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1-system A-lines with the restorer parent IPC 804, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_1-P_3 × IPC 804-P_4 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	179	95	231	81	74	84	105	83
Expected	193	81	219	93	79	79	94	94
χ^2	3.03 (0.08)*		1.90 (0.17)		0.51 (0.48)		2.35 (0.13)	
χ^2_p	0.02 (0.89)				0.35 (0.55)			
χ^2_h	4.91 (0.03)				2.51 (0.11)			
5054A_1-P_3 × IPC 804-P_3 (Hypothetical ratio in S 03 F_2: 45F:19S; BC_1: 1F:1S)								
(Hypothetical ratio in K 03 and pooled data F_2: 54F:10S; BC_1: 3F:1S)								
Observed	197	95	273	67	113	54	164	38
Expected	205	87	287	53	83.5	83.5	152	50
χ^2	1.00 (0.32)		3.99 (0.05)		20.14 (< 0.01)		3.80 (0.05)	
χ^2_p	47.26 (< 0.01)				0.00 (1.00)			
χ^2_h	-				-			
88004A_1 –P_5 × IPC 804-P_1 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	217	79	293	116	88	72	129	93
Expected	208	88	288	121	80	80	111	111
χ^2	1.14 (0.29)		0.28 (0.60)		1.41 (0.24)		5.52 (0.02)	
χ^2_p	1.29 (0.26)				6.81 (0.01)			
χ^2_h	0.13 (0.72)				0.12 (0.73)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 8. Segregation for male-fertile (F) (6-100% SSS) and male-sterile (S) (0-5% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_1-system A-lines with the restorer parent IPC 804, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_3 × IPC 804-P_4 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	176	98	221	91	73	85	97	91
Expected	193	81	219	93	79	79	94	94
χ^2	4.56 (0.03)*		0.02 (0.89)		0.92 (0.34)		0.13 (0.72)	
χ^2_p	1.73 (0.19)				0.10 (0.75)			
χ^2_h	2.85 (0.09)				0.95 (0.33)			
$5054A_1$-P_3 × IPC 804-P_3 (No hypothetical ratio fits)								
Observed	153	139	252	88	106	61	153	49
Expected	-	-	-	-	-	-	-	-
χ^2	-		-		-		-	
χ^2_p	-				-			
χ^2_h	-				-			

*values in parenthesis are exact probability (*P*) values
χ^2_p is the Chi square value of the pooled data for both the seasons
χ^2_h is the heterogeneity Chi square value
- ratio not fitted

Table 9. Segregation for male-fertile (F) (11-100% SSS) and male-sterile (S) (0-10% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_1-system A-lines with the restorer parent IPC 804, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_3 × IPC 804-P_4 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	162	112	212	100	69	89	92	96
Expected	193	81	219	93	79	79	94	94
χ^2	-		0.73 (0.39)*		-		0.05 (0.82)	
χ^2_p	11.52 (< 0.01)				1.40 (0.24)			
χ^2_h	-				-			
$5054A_1$-P_3 × IPC 804-P_3 (No hypothetical ratio fits)								
Observed	139	153	243	97	92	75	149	53
Expected	-	-	-	-	-	-	-	-
χ^2								
χ^2_p								
χ^2_h								

*values in parenthesis are exact probability (*P*) values
χ^2_p is the Chi square value of the pooled data for both the seasons
χ^2_h is the heterogeneity Chi square value
- ratio not fitted

When plants with 0-10% of SSS were classified as sterile and 11-100% as fertile, the summer season data was not in agreement with the expected trigenic ratio of 45F:19S in the F_2 and the corresponding ratio of 1F:1S in BC_1, but the segregation pattern in rainy season was in good agreement with the expected numbers as indicated by a non-significant χ^2 value for both F_2 (0.73; $P = 0.39$) and BC_1 (0.05; $P = 0.82$) (Table 9). The F_2 pooled data showed a significant χ^2 value ($P < 0.01$) for the expected trigenic ratio of 45F:19S whereas the BC_1 pooled numbers agreed well ($\chi^2 = 1.40$; $P = 0.24$) with the expected 1F:1S ratio.

Segregation pattern of the testcrosses produced on $81A_1$ from individual plants of the (81B × IPC 804)-derived F_2 and BC_1 populations also provided information on the inheritance of A_1 CMS system (Table 10). Testcrosses were scored as fully fertile (F), segregating for fertile and sterile plants (F+S) and fully sterile (S). The testcrosses that segregated for F and S plants were scored for the exact number of F and S plants. In summer season, of the 397 testcrosses made with F_2 plants, 82 were fully fertile, 234 segregated for (F+S) plants and 81 were sterile, giving a poor fit to the expected ratio of 1F:2 (F+S):1S ($\chi^2 = 11.82$; $P<0.01$); and of the 146 BC_1 testcrosses, 62 segregated for F+S plants and 84 were sterile, giving a good fit to the expected 1 (F+S):1S ratio ($\chi^2 = 3.02$; $P = 0.08$). In rainy season, F_2 testcrosses segregated into 91 fully fertile, 226 segregating for (F+S) and 80 sterile giving a good fit to the expected ratio of 1F:2 (F+S):1S ($\chi^2 = 7.55$; $P = 0.02$) and BC_1 testcrosses again segregated into 62 segregating for F+S and 84 sterile, and agreed with the expected 1 (F+S):1S ratio ($\chi^2 = 3.02$; $P = 0.08$). The pooled data didn't match the expected F_2 ratio of 1F:2 (F+S):1S ($P < 0.01$) but agreed to the expected BC_1 ratio of 1F:1S ($\chi^2 = 6.33$; $P = 0.01$). The heterogeneity χ^2 value was non-significant for F_2 as well as BC_1 across the two seasons.

The segregation pattern within the segregating testcrosses was also looked at critically to further confirm the segregation ratio observed between the testcrosses. The number of plants in these testcrosses generally varied from 30 to 50 plants. Majority of the segregating testcrosses are expected to segregate in a 1F:1S ratio. In the observed the number of segregating F_2 testcrosses exhibiting a 1F:1S ratio was 180 out of 234 in the summer season (76.9% of the segregating testcrosses) and 188 out of 226 in the rainy season (83.2% of the segregating testcrosses) (Table 11). Of the total 62 segregating BC_1 testcrosses in summer and rainy seasons, 43 (69.4%) and 50 (80.6%) testcrosses, respectively, segregated for the expected 1F:1S ratio. Further, individual χ^2 values for the expected 1F:1S ratio for each of the segregating testcrosses was summed up and a

Table 10. Segregation of testcrosses produced on A_1-system A-lines in two nuclear backgrounds from individual plants of F_2 and BC_1 populations derived from (B × R) crosses with two restorer lines IPC 804 and IPC 511 and test of goodness of fit for hypothetical Mendelian ratios in summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2-TC						No. of BC_1-TC			
	Summer 2003			Rainy 2003			Summer 2003		Rainy 2003	
	Fertile	F+S	Sterile	Fertile	F+S	Sterile	F+S	Sterile	F+S	Sterile
$81A_1$ × (81B-P_8 × IPC 804-P_4)										
(Hypothetical ratio in F_2-TC: 1:2:1; BC_1-TC: 1:1)										
Observed	82	234	81	91	226	80	62	84	62	84
Expected	99	199	99	99	199	99	73	73	73	73
χ^2	11.82 (< 0.01)*			7.55 (0.02)			3.02 (0.08)		3.02 (0.08)	
χ^2_p	19.26 (< 0.01)						6.33 (0.01)			
χ^2_h	0.11 (0.95)						-0.29 (0.59)			
5054A_1 × (5054B-P_4 × IPC 511-P_3)										
(Hypothetical ratio in F_2-TC: 7:8:1; BC_1-TC: 3:1)										
Observed	87	241	65	78	243	72	94	44	83	55
Expected	172	197	25	172	197	25	103	35	103	35
χ^2	116.26 (< 0.01)			151.24 (< 0.01)			3.05 (0.08)		15.29 (<0.01)	
χ^2_p	268.77 (< 0.01)						16.81 (< 0.01)			
χ^2_h	-1.27 (0.26)						1.53 (0.22)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 11. Segregation pattern within the segregating F_2 and BC_1 testcrosses of A_1 CMS system

Parameter	Total segregating testcrosses	No of testcrosses		% of Total	
		1F:1S	3F:1S	1F:1S	3F:1S
$81A_1$ × (81B × IPC 804) F_2-testcross population Expected ratio within the segregating testcrosses: 1F:1S					
Summer	234	180	-	76.9	-
Rainy	226	188	-	83.2	-
$81A_1$ × [81B × (81B × IPC 804)] BC_1-testcross population					
Summer	62	43	-	69.4	-
Rainy	62	50	-	80.6	-
5054 A_1 × (5054B × IPC 511) F_2-testcross population Expected ratio within the segregating testcrosses: 1F:1S					
Summer	241	136	86	56.4	35.7
Rainy	243	182	109	74.9	44.9
5054 A_1 × [5054B × (5054B × IPC 511)] BC_1-testcross population					
Summer	94	49	11	52.1	11.7
Rainy	83	63	15	75.9	18.1

Table 12. Pooled and heterogeneity Chi square analysis of the segregation pattern within the segregating F_2 and BC_1 testcrosses of A_1 CMS system

Parameter	Total segregating testcrosses	Number of plants			χ^2_p	χ^2_h
		Fertile	Sterile	Total		
Hypothesized ratio: 1F:1S $81A_1$ × (81B × IPC 804) F_2-testcross population						
Summer	234	4178	4361	8539	**3.9**	1265.7
Rainy	226	4463	5303	9766	72.1	843.4
$81A_1$ × [81B × (81B × IPC 804)] BC_1-testcross population						
Summer	62	1020	1260	2280	25.1	377.7
Rainy	62	944	1469	2413	113.8	170.9
Hypothesized ratio: 1F:1S 5054 A_1 × (5054B × IPC 511) F_2-testcross population						
Summer	241	4313	4761	9074	22.0	1916.8
Rainy	243	6595	5704	12299	64.4	1355.7
5054 A_1 × [5054B × (5054B × IPC 511)] BC_1-testcross population						
Summer	94	1199	2571	3770	498.6	475.2
Rainy	83	1908	2405	4313	57.0	401.2

χ^2_p is the pooled Chi square of the fertile and sterile plants across the segregating testcrosses
χ^2_h is the heterogeneity Chi square value across the segregating testcross entries
Figures in bold represent non-significant Chi square values

pooled χ^2 value was also calculated by adding the fertile and sterile plants of the segregating testcrosses (Table 12). The pooled F_2 testcross data gave a good fit to the expected 1F:1S ratio in the summer season but poor fit in rainy season. In the BC_1 testcrosses, the pooled data gave a poor fit to the expected 1F:1S ratio in both the seasons. The heterogeneity χ^2 for the F_2 and BC_1 testcross data was significant.

4.1.1.2.2 5054B nuclear background

The restorer line IPC 804 in this genetic background gave different segregation ratios in the two seasons (Table 7). The F_2 segregation pattern of 197 fertile and 95 sterile plants in summer season gave a good fit to the hypothesized trigenic ratio of 45F:19S (χ^2= 1.00; P = 0.32). However, the corresponding expected BC_1 ratio of 1F:1S didn't fit the observed number of plants (113 fertile and 54 sterile) as indicated by a significant χ^2 value of 20.14 ($P < 0.01$). In the rainy season, an expected trigenic F_2 ratio of 54F:10S (χ^2= 3.99; P = 0.05) and BC_1 ratio of 3F:1S (χ^2= 3.80; P = 0.05) ratio was found to fit with the observed segregation pattern of 273 fertile and 67 sterile plants in the F_2 and 164 fertile and 38 sterile plants in the BC_1, respectively. The pooled data didn't fit the expected 54F:10S F_2 ratio ($P < 0.01$) but in the BC_1, gave an exact fit to the expected 3F:1S ratio (χ^2= 0.00; P = 1.00).

Based on the SSS data, when plants were classified according to 0-5% SSS as sterile and 6-100% SSS as fertile (Table 8) and 0-10% SSS as sterile and 11-100% SSS as fertile (Table 9), the observed numbers didn't fit the expected F_2 ratio of 45F:19S or 54F:10S and BC_1 ratio of 1F:1S or 3F:1S in any of the two seasons individually as well as when the data was pooled.

4.1.1.2.3 ICMB 88004 nuclear background

During the summer season, 296 F_2 plants of the cross ICMA 88004 × IPC 804 segregated into 217 fertile and 79 sterile plants, giving a good fit to the expected trigenic ratio of 45F:19S (χ^2= 1.14; P = 0.29) and in the BC_1 generation, 160 observed plants segregated into 88 fertile and 72 sterile plants exhibiting good agreement with the expected ratio of 1F:1S as indicated by a χ^2 value of 1.41 (P = 0.24) (Table 7). Similarly, in the rainy season, the observed segregation pattern in the F_2 (293 fertile and 116 sterile) and BC_1 (129 fertile and 93 sterile) gave a good fit to the expected trigenic ratio of 45F:19S (χ^2= 0.28; P = 0.60) and 1F:1S (χ^2= 5.52; P = 0.02), respectively. The pooled data exhibited a good fit to the expected F_2 trigenic ratio of 45F:19S (χ^2= 1.29; P = 0.26) but a good agreement with the BC_1 ratio of 1F:1S (χ^2= 6.81; P = 0.01). The non-

significant heterogeneity χ^2 indicated that the segregation pattern in both F_2 and BC_1 is uniform across the two seasons.

4.1.1.3 Male-fertility restorer parent IPC 511

In 81B and 5054B background, the F_2 and BC_1 were also scored for selfed seed set (SSS) data besides pollen-shed data. The 5054B × IPC 511 cross (primarily produced for linkage analysis) also provided information about inheritance of A_1 CMS system from the testcross data (segregation pattern between testcrosses and within the segregating testcrosses). The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S), in the three crosses produced with this restorer are presented in Table 13.

4.1.1.3.1 81B nuclear background

In the summer season, 241 F_2 plants segregated into 184 fertile and 57 sterile plants giving a good fit to the expected trigenic ratio of 45F:19S with a χ^2 value of 3.92 (P = 0.05) and 173 BC_1 plants segregated into 97 fertile and 76 sterile plants confirming to the expected 1F:1S ratio with χ^2 value of 2.31 (P = 0.13) (Table 13). In the rainy season, 335 F_2 plants (258 fertile and 77 sterile) exhibited a good agreement with the expected trigenic 45F:19S segregation ratio (χ^2= 6.89; P = 0.01). Similarly, 187 BC_1 plants (115 fertile and 72 sterile) fit poorly to the expected 1F:1S ratio with χ^2 value of 9.43 (P < 0.01). When the data was pooled across the seasons, the F_2 as well as BC_1 observed data deviated significantly from the expected ratios as evident from χ^2 values of 11.08 and 11.03, respectively (P < 0.01). The heterogeneity χ^2 was non-significant for both F_2 and BC_1 indicating uniformity in the segregation pattern across the two seasons.

On the basis of SSS data, plants with 0-5% SSS classified as sterile and 6-100% as fertile, the observed number of plants in both the seasons as well as in the pooled data across the seasons showed good agreement with the expected trigenic F_2 ratio of 45F:19S and the corresponding BC_1 ratio of 1F:1S (Table 14). In the summer season, 241 F_2 plants segregated into 164 fertile and 77 sterile plants and confirmed to the expected trigenic 45F:19S ratio with a χ^2 value of 0.49 (P = 0.48). Similarly, 173 BC_1 plants segregated into 96 fertile and 77 sterile plants and gave a good fit to the expected 1F:1S ratio with χ^2 value of 1.87 (P = 0.17). In the rainy season, 335 F_2 plants (246 fertile and 89 sterile) exhibited a good fit to the expected trigenic 45F:19S segregation ratio (χ^2= 1.42; P = 0.23). Similarly, the observed 187 BC_1 plants (105 fertile and 82 sterile) agreed with the expected 1F:1S ratio with a χ^2 value of 2.59 (P = 0.11). When the data was pooled for both the seasons, the F_2 as well as BC_1 exhibited non-significant

Table 13. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1-system A-lines with the restorer parent IPC 511, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_1-P_5 × IPC 511-P_1 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	184	57	258	77	97	76	115	72
Expected	169	72	236	99	86.5	86.5	93.5	93.5
χ^2	3.92 (0.05)*		6.89 (0.01)		2.31 (0.13)		9.43 (< 0.01)	
χ^2_p	11.08 (< 0.01)				11.03 (< 0.01)			
χ^2_h	-0.27 (0.60)				0.71 (0.40)			
5054A_1-P_4 × IPC 511-P_3 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	224	44	367	77	104	57	104	51
Expected	226	42	375	69	121	40	116	39
χ^2	0.07 (0.79)		0.87 (0.35)		8.75 (< 0.01)		4.75 (0.03)	
χ^2_p	0.91 (0.34)				13.71 (< 0.01)			
χ^2_h	0.03 (0.86)				-0.21 (0.65)			
S 04	493	84	0.04 (0.84)		124	46	0.28 (0.60)	
(5054A_1-P_4 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3) (F_1 × F_1) (Hypothetical ratio in F_2: 45F:19S)								
Observed	211	84	296	113	-	-		
Expected	207	88	288	121	-	-		
χ^2	0.15 (0.70)		0.74 (0.39)		-			
χ^2_p	0.90 (0.34)							
χ^2_h	-0.01 (0.92)				-			
88004A_1-P_8 × IPC 511-P_2 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	220	73	250	79	68	77	55	68
Expected	206	87	231	98	72.5	72.5	61.5	61.5
χ^2	2.12 (0.15)		4.81 (0.03)		0.44 (0.51)		1.63 (0.20)	
χ^2_p	6.62 (0.01)				1.99 (0.16)			
χ^2_h	0.31 (0.58)				0.08 (0.78)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 14. Segregation for male-fertile (F) (6-100% SSS) and male-sterile (S) (0-5% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_1-system A-lines with the restorer parent IPC 511, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_5 × IPC 511-P_1 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	164	77	246	89	96	77	105	82
Expected	169	72	236	99	86.5	86.5	93.5	93.5
χ^2	0.49 (0.48)*		1.42 (0.23)		1.87 (0.17)		2.59 (0.11)	
χ^2_p	0.17 (0.68)				4.67 (0.03)			
χ^2_h	1.74 (0.19)				-0.21 (0.65)			
$5054A_1$-P_4 × IPC 511-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	196	72	335	109	81	80	106	49
Expected	188	80	312	132	80.5	80.5	77.5	77.5
χ^2	0.89 (0.35)		5.37 (0.02)		0.00 (1.00)		12.82 (< 0.01)	
χ^2_p	6.01 (0.01)				6.78 (0.01)			
χ^2_h	0.25 (0.62)				6.04 (0.01)			
($5054A_1$-P_4 × IPC 511-P_3) × ($5054B$-P_4 × IPC 511-P_3) (F_1 × F_1) (Hypothetical ratio in S 03 & Pooled F_2: 9F:7S & K 03 45F:19S)								
Observed	154	141	264	145	-	-	-	-
Expected	166	129	288	121	-	-	-	-
χ^2	1.80 (0.18)		6.24 (0.01)		-		-	
χ^2_p	2.67 (0.10)				-			
χ^2_h	-				-			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

χ^2 values for the expected ratios as evident from a χ^2 value of 0.17 (P = 0.68) and 4.67 (P = 0.03), respectively. The heterogeneity χ^2 was non-significant for F_2 and BC_1 across the seasons indicating uniform segregation pattern.

When plants were classified based on SSS as sterile (0-10% SSS) and fertile (11-100% SSS), the observed number of fertile and sterile plants in both the seasons as well as in the pooled data showed good agreement with the expected number of plants in F_2 and BC_1 with ratios of 45F:19S and 1F:1S, respectively (Table 15). In the summer season, the F_2 segregated into 156 fertile and 85 sterile plants and gave a good fit to the expected 45F:19S ratio with a χ^2 value of 3.34 (P = 0.07). Similarly, the BC_1 segregated into 97 fertile and 76 sterile plants and gave a good agreement with the expected 1F:1S ratio with a χ^2 value of 2.31 (P = 0.13). In the rainy season, 335 F_2 plants (234 fertile and 101 sterile) exhibited a good fit to the expected 45F:19S segregation ratio (χ^2= 0.02; P = 0.89). Similarly, 187 BC_1 plants (96 fertile and 91 sterile) gave a good fit to the expected 1F:1S ratio with a χ^2 value of 0.09 (P = 0.76). When the data was pooled for both the seasons, the F_2 as well as BC_1 data exhibited non-significant χ^2 values for the expected ratios as evident from a χ^2 value of 1.75 (P = 0.19) and 1.74 (P = 0.19), respectively. The heterogeneity χ^2 square was non-significant for both F_2 and BC_1 indicating uniform segregation pattern of data across the seasons.

4.1.1.3.2 5054B nuclear background

In 5054B nuclear background, IPC 511 gave a good fit to 54F:10S F_2 and 3F:1S BC_1 ratio in both summer and rainy seasons as well as when the data were pooled except in the BC_1 of summer season and the pooled data where the hypothesized ratio didn't agree well with the observed number of plants (Table 13). In summer season, the observed F_2 segregation pattern of 224 fertile and 44 sterile plants was in good agreement with the expected trigenic ratio of 54F:10S as revealed by χ^2 value of 0.07 (P = 0.79). However, in BC_1, the observed number of plants (104 fertile and 57 sterile) was in poor agreement with the expected segregation ratio of 3F:1S as indicated by a significant χ^2 value of 8.75 (P < 0.01). In rainy season, the observed F_2 segregation pattern of 367 fertile and 77 sterile plants was in good agreement with the expected 54F:10S ratio as revealed by χ^2 value of 0.87 (P = 0.35) and similarly in BC_1, the observed number of plants (104 fertile and 51 sterile) was in agreement with the expected segregation ratio of 3F:1S as indicated by a χ^2 value of 4.75 (P = 0.03). The pooled data gave a good fit to the expected ratio with F_2 data (χ^2= 0.91; P = 0.34) but not so with BC_1 data (χ^2= 13.71; P <

Table 15. Segregation for male-fertile (F) (11-100% SSS) and male-sterile (S) (0-10% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_1-system A-lines with the restorer parent IPC 511, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_5 × IPC 511-P_1 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	156	85	234	101	97	76	96	91
Expected	169	72	236	99	86.5	86.5	93.5	93.5
χ^2	3.34 (0.07)*		0.02 (0.89)		2.31 (0.13)		0.09 (0.76)	
χ^2_p	1.75 (0.19)				1.74 (0.19)			
χ^2_h	1.61 (0.20)				0.66 (0.42)			
$5054A_1$-P_4 × IPC 511-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	176	92	307	137	65	96	88	67
Expected	188	80	312	132	80.5	80.5	77.5	77.5
χ^2	2.55 (0.11)		0.24 (0.62)		5.59 (0.02)		2.58 (0.11)	
χ^2_p	1.97 (0.16)				0.26 (0.61)			
χ^2_h	0.82 (0.37)				7.91 (< 0.01)			
($5054A_1$-P_4 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3) ($F_1 \times F_1$) (Hypothetical ratio in F_2: 9F:7S)								
Observed	136	159	240	169	-			
Expected	-	-	230	179	-			
χ^2	-		0.88 (0.35)		-			
χ^2_p	2.19 (0.14)							
χ^2_h	-							

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

0.01). The non-significant heterogeneity χ^2 value for F_2 and BC_1 indicated uniformity in the segregation pattern across the seasons.

On the basis of SSS data, when plants with 0-5% SSS were classified as sterile and 6-100% as fertile, during summer season, the segregation in F_2 into 196 fertile and 72 sterile plants was in good agreement with the expected trigenic ratio of 45F:19S as revealed by χ^2 value of 0.89 ($P = 0.35$) and in BC_1, the observed number of plants (81 fertile and 80 sterile) gave a perfect match (χ^2 value of 0.00; $P = 1.00$) to the expected segregation ratio of 1F:1S (Table 14). In rainy season, the observed F_2 segregation pattern of 335 fertile and 109 sterile plants was in good agreement with the expected ratio as revealed by χ^2 value of 5.37 ($P = 0.02$) whereas in BC_1, the observed number of plants (106 fertile and 49 sterile) were in poor agreement with the expected segregation as indicated by a significant χ^2 value of 12.82 ($P < 0.01$). The pooled data gave a good fit to the expected ratio in F_2 ($\chi^2= 6.01$; $P = 0.01$) and BC_1 ($\chi^2= 6.78$; $P = 0.01$). The heterogeneity χ^2 value was non-significant for F_2 ($\chi^2= 0.25$; $P = 0.62$) indicating uniform segregation pattern whereas in BC_1, a comparatively higher χ^2 value of 6.04 ($P = 0.01$) point out to different segregation pattern across the two seasons.

When plants were classified based on SSS as sterile (0-10% SSS) and fertile (11-100% SSS), the observed number of plants in both the seasons as well as across the seasons showed good agreement with the expected trigenic F_2 and the corresponding BC_1 ratios of 45F:19S and 1F:1S, respectively (Table 15). In the summer season, the F_2 segregation pattern of 176 fertile and 92 sterile plants was in agreement with the expected ratio of 45F:19S as revealed by χ^2 value of 2.55 ($P = 0.11$). In BC_1, the observed number of plants (65 fertile and 96 sterile) was deviating from the expected segregation ratio of 1F:1S as indicated by a comparatively higher χ^2 value of 5.59 ($P = 0.02$). In rainy season, the observed F_2 segregation pattern of 307 fertile and 137 sterile plants was in good agreement with the expected ratio as revealed by χ^2 value of 0.24 ($P = 0.62$). In BC_1 also, the observed number of plants (106 fertile and 49 sterile) gave a good fit to the expected segregation ratio as indicated by a χ^2 value of 2.58 ($P = 0.11$). The pooled observed data gave a good fit to the expected ratio in F_2 ($\chi^2= 1.97$; $P = 0.16$) as well as BC_1 ($\chi^2= 0.26$; $P = 0.61$). The heterogeneity χ^2 value was non-significant for F_2 (0.25; $P = 0.62$) but significant in case of BC_1 ($\chi^2= 7.91$; $P < 0.01$) indicating contrasting segregation pattern in BC_1.

The F_2 produced from $F_1 \times F_1$ cross [(5054A$_1$-P$_4$ × IPC 511-P$_3$) × (5054B-P$_4$ × IPC 511-P$_3$)] segregated into 211 fertile and 84 sterile plants in summer season and into

296 fertile and 113 sterile plants in the rainy season to give a good fit to the expected trigenic F_2 45F:19S ratio with χ^2 value of 0.15 (P = 0.70) and 0.74 (P = 0.39), respectively (Table 13). The pooled data also gave a good fit to the hypothesized ratio as indicated by χ^2 value of 0.90 (P = 0.34).

When plants were classified based on SSS as sterile (0-5% SSS) and fertile (6-100% SSS), the observed number of plants in the F_2 produced from F_1 × F_1 cross [(5054A_1-P_4 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3)] segregated according to the digenic ratio of 9F:7S (two complimentary genes required for fertility restoration) in summer (χ^2= 1.80; P = 0.18) and to the trigenic ratio of 45F:19S in rainy (χ^2= 6.24; P = 0.01) seasons (Table 14). The pooled data agreed with the expected digenic segregation ratio of 9F:7S with a χ^2 value of 2.67 (P = 0.10).

On the basis of SSS data, plants with 0-10% SSS classified as sterile and 11-100% as fertile, the observed number of plants in the F_2 produced from F_1 × F_1 cross [(5054A_1-P_4 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3)] didn't fit any of the hypothesized ratios in summer season (Table 15). However, the observed number of plants was in agreement with the expected digenic ratio of 9F:7S in the rainy season (χ^2= 0.88; P = 0.35) as well as in the pooled data (χ^2= 2.19; P = 0.14).

Segregation pattern of the testcrosses produced from individual plants of the (5054B × IPC 511)-derived F_2 and BC_1 populations on 5054A_1 CMS line provided additional information on the inheritance of A_1 CMS system (Table 10). In summer season, of the 393 F_2 testcrosses scored for F/S segregation pattern, 87 were fully fertile, 241 were segregating for F+S and 65 were sterile and gave a poor fit to the expected segregation ratio of 7F:8 (F+S):1S (P < 0.01) but, of the 138 BC_1 testcrosses, 94 segregated for F+S plants and 44 were sterile, giving a good χ^2 fit to the expected 3 (F+S):1S ratio (P < 0.01). In the rainy season, the F_2 and BC_1 observed testcross segregation pattern didn't fit the expected pattern. The F_2 testcrosses segregated into 78 completely fertile, 243 segregating for F+S plants and 72 sterile plants and of the 138 BC_1 testcrosses, 83 were segregating for (F+S) and 55 were sterile. As expected, the pooled data also gave a poor fit to the expected segregation pattern but the non-significant heterogeneity χ^2 values for F_2 and BC_1 across the seasons indicated uniformity in the segregation pattern.

Majority of the segregating testcrosses were expected to give a segregating ratio of 1F:1S and 3F:1S in equal proportion within the segregating testcrosses. The number of plants in these testcrosses generally varied from 35 to 60 plants. The number of

segregating F_2 testcrosses exhibiting a 1F:1S ratio was 136 out of 241 (56.4% of the segregating testcrosses) in the summer season and 182 out of 243 (74.9%) in the rainy season; and the testcrosses exhibiting a 3F:1S ratio was 86 out of 241 (35.7%) in the summer season and 109 out of 243 (44.9%) in the rainy season (Table 11). Of the 94 segregating BC_1 testcrosses in summer season, 49 (52%) exhibited a 1F:1S ratio and 11 (11.7%) had a 3F:1S ratio and in the rainy season, of the 83 segregating testcrosses, 63 (76%) segregated for 1F:1S ratio whereas 15 (18.1%) segregated for the 3F:1S ratio. Further, individual χ^2 values for the expected 1F:1S ratio for each of the segregating testcrosses was summed up and a pooled χ^2 value was also calculated by adding the fertile and sterile plants of the segregating testcrosses (Table 12). The pooled F_2 and BC_1 data of the segregating testcrosses didn't fit the expected 1F:1S ratio in both the summer and in rainy season and the heterogeneity χ^2 was also significant across the segregating testcrosses.

4.1.1.3.3 ICMB 88004 nuclear background

During the summer season, 293 F_2 plants of the cross ICMA 88004 × IPC 511 segregated into 220 fertile and 73 sterile plants and gave a good fit to the hypothesized trigenic ratio of 45F:19S (χ^2= 2.12; P = 0.15) (Table 13). In BC_1, the observed number of 145 plants segregated into 68 fertile and 77 sterile plants giving a good fit to the expected 1F:1S ratio as evident from χ^2 value of 0.44 (P = 0.51). The observed segregation pattern gave a good fit to the trigenic expected ratio of 45F:19S in F_2 (χ^2= 4.81; P = 0.03) and corresponding 1F:1S ratio in BC_1 (χ^2= 1.63; P = 0.20) in the rainy season. The number of plants scored for F/S reaction in F_2 and BC_1 was 329 (250 fertile and 79 sterile) and 123 (55 fertile and 68 sterile), respectively, during the rainy season. The pooled data across the seasons gave a good fit to the expected F_2 ratio of 45F:19S (χ^2= 6.62; P = 0.01) and even more strongly to the BC_1 ratio of 1F:1S (χ^2= 1.99; P = 0.16). The non-significant heterogeneity χ^2 indicated uniformity in the segregation pattern of F_2 as well as BC_1 data across the two seasons.

4.1.1.4 Male-fertility restorer parent IPC 382

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) plants in the three crosses produced with this restorer are provided in Table 16.

Table 16. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1-system A-lines with the restorer parent IPC 382, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_7 × IPC 382-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	251	43	337	72	88	46	158	63
Expected	248	46	345	64	101	34	166	55
χ^2	0.15 (0.70)*		1.07 (0.30)		5.73 (0.02)		1.27 (0.26)	
χ^2_p	0.23 (0.63)				5.86 (0.02)			
χ^2_h	0.99 (0.32)				1.14 (0.29)			
$5054A_1$-P_7 × IPC 382-P_3 (Hypothetical ratio in F_2: 63F:1S; BC_1: 7:1)								
Observed	299	1	458	7	179	4	224	7
Expected	295	5	458	7	160	23	202	29
χ^2	2.20 (0.14)		0.01 (0.92)		16.87 (< 0.01)		18.08 (< 0.01)	
χ^2_p	1.01 (0.31)				35.78 (< 0.01)			
χ^2_h	1.20 (0.27)				-0.83 (0.36)			
$88004A_1$-P_{12} × IPC 382-P_2 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	225	62	352	56	94	53	64	43
Expected	242	45	344	64	110	37	76	27
χ^2	7.33 (0.01)		0.98 (0.32)		9.00 (< 0.01)		12.36 (< 0.01)	
χ^2_p	0.87 (0.35)				21.50 (< 0.01)			
χ^2_h	7.44 (0.01)				-0.14 (0.71)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

4.1.1.4.1 81B nuclear background

In the summer season, of the 294 F_2 plants scored for F/S reaction, 251 were fertile and 43 sterile, giving a good fit to the expected trigenic 54F:10S ratio with a χ^2 value of 0.15 ($P = 0.70$) (Table 16). In BC_1, 134 plants segregated into 88 fertile and 46 sterile giving a good fit to the expected 3F:1S ratio with χ^2 value of 5.73 ($P = 0.02$). In the rainy season, 409 F_2 plants (337 fertile and 72 sterile) and 221 BC_1 plants (158 fertile and 63 sterile) plants gave a good fit to the expected trigenic ratio of 54F:10S (χ^2 value of 1.07; $P = 0.30$) and the BC_1 ratio of 3F:1S (χ^2 value of 1.27; $P = 0.26$), respectively. When the data was pooled for both the seasons, the F_2 (χ^2= 0.23; $P = 0.63$) and the BC_1 (χ^2= 5.86; $P = 0.02$) observed segregation pattern agreed with the expected ratios of 54F:10S and 3F:1S, respectively. The heterogeneity χ^2 was non-significant for both F_2 and BC_1 with χ^2 values of 0.99 ($P = 0.32$) and 1.14 ($P = 0.29$), respectively, indicating uniformity in the segregation pattern across the seasons.

4.1.1.4.2 5054B nuclear background

In the summer season, a trigenic expected ratio of 63F:1S that results from three duplicate genes governing fertility restoration, gave a good fit (χ^2 value of 2.20; $P = 0.14$) to the observed F_2 segregation of 299 fertile and one sterile plant (Table 16). However, in the BC_1, the observed segregation pattern of 179 fertile and four sterile plants agreed poorly to the corresponding expected ratio of 7F:1S ($P < 0.01$). The same trend was repeated in BC_1 in the rainy season (224 fertile and seven sterile plants) as well as in the pooled data with the observed number of plants giving a poor fit ($P < 0.01$) to the expected 7F:1S ratio. In rainy season, the F_2 segregated into 458 fertile and seven sterile plants from a total of 465 plants scored and gave a good fit to the hypothesized 63F:1S ratio with χ^2 value of 0.01 ($P = 0.92$). The F_2 pooled data also gave a good fit to the expected ratio as evident from a χ^2 value of 1.01 ($P = 0.31$). The heterogeneity χ^2 value was non-significant for the F_2 and BC_1 across the seasons indicating uniformity in the trend of segregation pattern across the seasons

4.1.1.4.3 ICMB 88004 nuclear background

In the summer season, among the 287 plants segregating for F/S reaction in the F_2, 225 were fertile and 62 were sterile, giving a poor fit to the hypothesized 54F:10S trigenic ratio as indicated by a χ^2 value of 7.33 ($P = 0.01$) (Table 16). The BC_1 segregated into 94 fertile and 53 sterile plants and agreed poorly to the expected 3F:1S ratio with χ^2 value of 9.00 ($P < 0.01$). In the rainy season, segregation pattern of 408 F_2 plants (352

fertile and 56 sterile) agreed well with the expected ratio of 54F:10S χ^2= 0.98; P = 0.32) but the segregation pattern of 107 BC_1 plants (64 fertile and 43 sterile) gave a poor fit to the expected 3F:1S ratio as seen from the χ^2 value of 12.36 ($P < 0.01$). When the data was pooled over seasons, similar as that of rainy season were obtained *i.e.* the F_2 (χ^2= 0.87; P = 0.35) agreeing well, whereas the BC_1 (χ^2= 21.50; $P < 0.01$) giving a poor fit to the expected ratio. The heterogeneity χ^2 value for the F_2 was found to be significant (χ^2= 7.44; P = 0.01) indicating variation in the segregation pattern across the seasons whereas in BC_1, it was found to be non-significant indicating the uniformity in the trend of segregation pattern across the seasons.

4.1.1.5 Male-fertility restorer parent L 67B

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) plants in the three crosses produced with this restorer are provided in Table 17.

4.1.1.5.1 81B nuclear background

In the summer season, of the 323 plants scored in the F_2, 272 were fertile and 51 were sterile, giving an exact fit to the hypothesized trigenic ratio of 54F:10S with a χ^2 value of 0.00 (P = 1.00) (Table 17). The BC_1 segregated into 114 fertile and 42 sterile plants and gave a good fit to the corresponding expected 3F:1S ratio with χ^2 value of 0.21 (P = 0.65). In the rainy season, 388 F_2 (339 fertile and 49 sterile) and 205 BC_1 (141 fertile and 64 sterile) observed number of plants segregated according to the expected 54F:10S ratio (χ^2= 2.42; P = 0.12) and 3F:1S ratio (χ^2= 3.90; P = 0.05), respectively. The pooled data across the seasons again gave a good fit to the expected ratios in the F_2 (χ^2= 1.20; P = 0.27) and in the BC_1 (χ^2= 3.44; P = 0.06) gave good fit to the expected ratios. The heterogeneity χ^2 was also non-significant for both F_2 and BC_1 with χ^2 values of 1.22 (P = 0.27) and 0.67 (P = 0.41), respectively, indicating the uniformity of segregation pattern across the seasons.

4.1.1.5.2 5054B nuclear background

In the summer season, 309 F_2 plants (282 fertile and 27 sterile) gave a good fit to the expected digenic ratio of 15F:1S with a χ^2 value of 2.85 (P = 0.09) (Table 17). However, the corresponding expected ratio of 3F:1S in BC_1 agreed poorly (χ^2= 7.85; P = 0.01) with the observed number of 147 fertile and 27 sterile plants. Similar pattern was observed in the rainy season and when the data pooled over seasons. The segregation pattern of F_2 plants in rainy season (384 fertile and 22 sterile plants) gave a good fit to the 15F:1S ratio (χ^2= 0.35; P = 0.55) whereas the BC_1 segregation into 187 fertile and

Table 17. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_1-system A-lines with the restorer parent L 67B, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_1$-P_8 × L 67B-P_3 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	272	51	339	49	114	42	141	64
Expected	273	50	327	61	117	39	154	51
χ^2	0.00 (1.00)*		2.42 (0.12)		0.21 (0.65)		3.90 (0.05)	
χ^2_p	1.20 (0.27)				3.44 (0.06)			
χ^2_h	1.22 (0.27)				0.67 (0.41)			
$5054A_1$-P_8 × L 67B-P_2 (Hypothetical ratio in F_2: 15:1; BC_1: 3F:1S)								
Observed	282	27	384	22	147	27	187	35
Expected	290	19	381	25	131	44	167	56
χ^2	2.85 (0.09)		0.35 (0.55)		7.85 (0.01)		9.61 (< 0.01)	
χ^2_p	0.35 (0.55)				17.94 (< 0.01)			
χ^2_h	2.85 (0.09)				-0.48 (0.49)			
$88004A_1$-P_{14} × L 67B-P_1 (Hypothetical ratio in F_2: 15:1; BC_1: 3F:1S)								
Observed	294	20	339	22	130	31	177	41
Expected	294	20	338	23	121	40	164	55
χ^2	0.00 (1.00)		0.00 (1.00)		2.54 (0.11)		4.13 (0.04)	
χ^2_p	0.00 (1.00)				6.97 (0.01)			
χ^2_h	0.00 (1.00)				-0.30 (0.58)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

35 sterile plants gave a poor fit to the expected 3F:1S ratio as evident from a significant χ^2 value ($P < 0.01$). The pooled data again gave a good fit to the hypothesized ratio in the F_2 ($\chi^2 = 0.35$; $P = 0.55$) but poor fit in the BC_1 ($P < 0.01$). The heterogeneity χ^2 value was found to be non-significant for both the F_2 and BC_1 across the two seasons indicating uniformity in the segregation pattern.

4.1.1.5.3 ICMB 88004 nuclear background

In the summer season, the F_2 segregated into 294 fertile and 20 sterile plants out of a total of 314 plants scored, giving an exact fit to the hypothesized 15F:1S ratio with a χ^2 value of 0.00 ($P = 1.00$) (Table 17). The BC_1 segregated into 130 fertile and 31 sterile plants according to the expected 3F:1S ratio as shown by a χ^2 value of 2.54 ($P = 0.11$). Similarly, in the rainy season, the F_2 observed segregation pattern of 339 fertile and 22 sterile plants was exactly matched the expected numbers according to the expected 15F:1S ratio ($P = 1.00$). The segregation pattern of observed 218 BC_1 plants (177 fertile and 41 sterile) gave a good fit to the expected 3F:1S ratio with χ^2 value of 4.13 ($P = 0.04$). When the data was pooled for both the seasons, the F_2 again gave an exact fit to the hypothesized ratio ($P = 1.00$) whereas the BC_1 gave a good fit (χ^2 value of 6.97; $P = 0.01$) to the expected 3F:1S ratio. Heterogeneity χ^2 values were also non-significant for both F_2 and BC_1 across the seasons.

4.1.2 Inheritance of A_4 CMS system

The inheritance of A_4 CMS system was investigated based on segregation pattern of F_2 and BC_1 in a total of 9 (A × R) crosses. Information on inheritance of A_4 CMS system was also derived from the segregation pattern obtained from the F_2 and BC_1 testcross data as well as the segregation behavior observed within the segregating F_2 and BC_1 testcrosses of the two (B × R) crosses developed mainly for linkage and molecular mapping experiment. The (A × R) crosses were produced by crossing four A_4 restorer lines with A_4 CMS lines in three diverse nuclear backgrounds of 81B, 5054B and ICMB 88004. All the crosses were evaluated in two environments (summer and rainy seasons, 2003) at ICRISAT- Patancheru for fertility/sterility (F/S) reaction on the basis of pollen shed data and a few selected crosses in 81B and 5054B background involving two restorer lines IPC 804 and IPC 511 were also evaluated on the basis of selfed seed set (SSS) data. In crosses where SSS data was also recorded, genetic ratios were worked out for two cases viz., based on plants showing 0-5% SSS classified as sterile and 6-100% as fertile irrespective of whether plants were tagged as sterile or shy on the basis of pollen shed data. Similarly, another classification was based plants showing 0-10% SSS

as sterile and 11-100% as fertile class irrespective of whether plants were tagged as shy or sterile on the basis of pollen shed data.

4.1.2.1 Male-fertility restorer parent IPC 1518

The F/S observed and expected data and the goodness of fit for the hypothesized Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) plants are presented in Table 18.

4.1.2.1.1 81B nuclear background

During the summer season, 265 plants (222 fertile and 43 sterile) in the F_2 of the cross $81A_4$ × IPC 1518 segregated according to the hypothesized trigenic ratio of 54F:10S (χ^2= 0.03; P = 0.86) but in the corresponding BC_1, 148 plants segregated into 90 fertile and 58 sterile to give a poor fit to the hypothesized ratio of 3F:1S (χ^2= 15.14; $P < 0.01$) (Table 18). In the rainy season, the F_2 observed data (367 fertile and 67 sterile) gave an exact fit to the hypothesized ratio of 54F:10S as evident from a χ^2 value of 0.00 (P = 1.00) but the BC_1 observed segregation data (122 fertile and 71 sterile) deviated from the expected segregation as revealed by a poor fit to the expected ratio of 3F:1S (χ^2= 13.68; $P < 0.01$). The F_2 pooled data across the two seasons gave an exact fit to the expected ratio of 54F:10S (χ^2= 0.00; P = 1.00) but the BC_1 pooled data deviated significantly from the expected 3F:1S ratio ($P < 0.01$). The heterogeneity χ^2 value for the F_2 (χ^2= 0.03; P = 0.86) and BC_1 (χ^2= -0.44; P = 0.51) was non-significant indicating uniformity in the segregation pattern across the two seasons.

4.1.2.1.2 5054B nuclear background

In summer season, segregation of 358 F_2 plants into 310 fertile and 48 sterile gave a good fit to the hypothesized trigenic ratio of 54F:10S (χ^2= 1.17; P = 0.28). In the BC_1, 148 plants segregated into 100 fertile and 48 sterile plants and gave a good fit to the corresponding expected ratio of 3F:1S (χ^2= 3.97; P = 0.05) (Table 18). In the rainy season, the observed F_2 segregation into 283 fertile and 53 sterile plants was exactly according to the hypothesized 54F:10S ratio (χ^2= 0.00; P = 1.00). However, the observed BC_1 segregation (73 fertile and 45 sterile) didn't fit the expected 1F:1S ratio (χ^2= 10.17; $P < 0.01$). The aggregate data gave a good fit to the expected 54F:10S F_2 ratio (χ^2= 0.53; P = 0.47) but didn't fit to the expected 3F:1S BC_1 ratio ($P < 0.01$). The heterogeneity χ^2 value was found to be non-significant for the F_2 (P = 0.42) and BC_1 (χ^2= 0.59; P = 0.44) indicating uniformity in the segregation pattern across the two seasons.

Table 18. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_4-system A-lines with the restorer parent IPC 1518, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_4$-P_1 × IPC 1518-P_3 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	222	43	367	67	90	58	122	71
Expected	224	41	366	68	111	37	145	48
χ^2	0.03 (0.86)*		0.00 (1.00)		15.14 (< 0.01)		13.68 (< 0.01)	
χ^2_p	0.00 (1.00)				29.26 (< 0.01)			
χ^2_h	0.03 (0.86)				-0.28 (0.60)			
$5054A_4$-P_1 × IPC 1518-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	310	48	283	53	100	48	73	45
Expected	302	56	283	53	111	37	88.5	29.5
χ^2	1.17 (0.28)		0.00 (1.00)		3.97 (0.05)		10.17 (< 0.01)	
χ^2_p	0.53 (0.47)				13.55 (< 0.01)			
χ^2_h	-0.64 (0.42)				0.59 (0.44)			
$88004A_4$-P_2 × IPC 1518-P_2 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	278	65	277	47	102	62	99	67
Expected	289	54	273	51	123	41	124.5	41.5
χ^2	2.63 (0.10)		0.23 (0.63)		13.67 (< 0.01)		20.08 (< 0.01)	
χ^2_p	0.60 (0.44)				34.20 (< 0.01)			
χ^2_h	2.26 (0.13)				-0.45 (0.50)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

4.1.2.1.3 ICMB 88004 nuclear background

In the summer season, of the 343 F_2 plants evaluated for F/S reaction, 278 were fertile and 65 were sterile, giving a good fit to the expected trigenic ratio of 54F:10S with a χ^2 value of 2.63 (P = 0.10) (Table 18). However, the BC_1 observed segregation pattern (102 fertile and 62 sterile) significantly deviated from the expected 3F:1S ratio as evident from a χ^2 value of 13.67 ($P < 0.01$). In the rainy season, of the 324 F_2 plants evaluated, 277 were fertile and 47 sterile, exhibiting good fit to the expected 54F:10S ratio (χ^2= 0.23; P = 0.63). The observed number of 166 BC_1 plants (99 fertile and 67 sterile) didn't fit to the expected 3F:1S ratio ($P < 0.01$). The segregation pattern of the pooled data across the seasons was a repeat of individual seasons with the F_2 giving a good fit to the expected 54F:10S ratio (χ^2= 0.60, P = 0.44) whereas the BC_1 not fitting the expected 3F:1S ratio ($P < 0.01$). The heterogeneity χ^2 was non-significant across the two seasons in case of F_2 and also for BC_1.

4.1.2.2 Male-fertility restorer parent IPC 804

The restorer IPC 804 produced fertile hybrids with the A-lines of A_4 CMS system in the three nuclear backgrounds of 81B, 5054B and ICMB 88004. Similarly, the three BC_2 populations produced with this restorer were also completely fertile. In 81B and 5054B background, the F_2 and BC_1 were also evaluated on the basis of selfed seed set (SSS) data besides pollen-shed data. The 81B × IPC 804 cross (mainly produced for linkage analysis and molecular mapping) also provided information about inheritance from the testcross data (segregation pattern between testcrosses and within the segregating testcrosses). The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) plants in the three crosses produced with this restorer are provided in Table 19.

4.1.2.2.1 81B nuclear background

The cross $81A_4$ × IPC 804 gave a good fit to the hypothesized F_2 trigenic ratio of 45F:19S and the corresponding BC_1 ratio of 1F:1S in both the seasons as well as in case of pooled data (Table 19). In summer season, the segregation pattern of F_2 into 197 fertile and 108 sterile plants was in agreement with the expected 45F:19S ratio as revealed by χ^2 value of 4.51 (P = 0.03). In the BC_1, the observed numbers (63 fertile and 61 sterile) were in good agreement with the expected 1F:1S ratio as indicated by a non-significant χ^2 value of 0.01 (P = 0.92). Similarly, in rainy season, the observed F_2 segregation pattern of 249 fertile and 102 sterile plants was in good agreement with the expected 45F:19S ratio as evident from a χ^2 value of 0.04 (P = 0.84). In BC_1, the

Table 19. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_4-system A-lines with the restorer parent IPC 804, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_4$-P_2 × IPC 804-P_4 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	197	108	249	102	63	61	113	128
Expected	214	91	247	104	62	62	120.5	120.5
χ^2	4.51 (0.03)*		0.04 (0.84)		0.01 (0.92)		0.81 (0.37)	
χ^2_p	1.59 (0.21)				0.39 (0.53)			
χ^2_h	2.96 (0.09)				0.43 (0.51)			
$5054A_4$-P_2 × IPC 804-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	190	87	150	64	89	91	90	100
Expected	195	82	150	64	90	90	95	95
χ^2	0.31 (0.58)		0.00 (1.00)		0.01 (0.92)		0.43 (0.51)	
χ^2_p	0.22 (0.64)				0.33 (0.57)			
χ^2_h	0.09 (0.76)				0.11 (0.74)			
$88004A_4$-P_3 × IPC 804-P_1 (Hypothetical ratio in F_2: 9F:7S; BC_1: 1F:3S)								
Observed	168	124	280	149	22	142	39	127
Expected	164	128	241	188	41	123	41.5	124.5
χ^2	0.15 (0.70)		13.81 (< 0.01)		11.13 (< 0.01)		0.13 (0.72)	
χ^2_p	9.91 (< 0.01)				7.13 (0.01)			
χ^2_h	4.05 (0.04)				4.13 (0.04)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

observed number of plants (113 fertile and 128 sterile) was also in good agreement with the 1F:1S expected ratio as indicated by a non-significant χ^2 value of 0.81 (P = 0.37). The pooled data gave a good χ^2 fit to the expected ratio in F_2 (χ^2= 1.59; P = 0.21) and in BC_1 (χ^2= 0.39; P = 0.53). The heterogeneity χ^2 across the seasons was found to be non-significant for F_2 and BC_1 indicating uniformity in the segregation pattern.

When plants with 0-5% and 0-10% SSS were classified as sterile and 6-100% and 11-100% SSS as the fertile, the observed data remained unchanged for F_2 and BC_1 in both the classifications except shift of two fertile plants to the sterile class in the rainy season in the F_2 (0-10% sterile category, Table 21) and BC_1 (0-5% and 0-10% sterile category, Table 20, 21). The observed data still gave a good fit to the expected ratios of 45F:19S and 1F:1S, respectively, in both the individual seasons as well as in the pooled data.

Segregation pattern of the testcrosses produced on $81A_4$ from individual plants of the (81B × IPC 804)-derived F_2 and BC_1 populations also provided information on the inheritance of A_4 CMS system (Table 22). In summer season, of the 397 F_2 testcrosses scored for F/S segregation pattern, five were uniformly fertile, 230 were segregating for F+S plants and 162 were sterile, giving a poor χ^2 fit to the expected ratio of 1F:2 (F+S):1S (P < 0.01). Of the 146 BC_1 testcrosses, eight segregated for (F+S) and 138 were sterile, giving a poor fit to the expected 1F:3S ratio (P < 0.01). The segregation pattern in the rainy season was similar to the summer season with both the F_2 and BC_1 giving a poor χ^2 fit. The pooled F_2 and BC_1 testcross segregation data also gave a poor fit to the expected segregation as indicated by significant χ^2 value (P < 0.01). However, the heterogeneity χ^2 value was non-significant for F_2 as well as BC_1 across the seasons.

The segregating F_2 testcrosses are expected to give a within segregation ratio of 1F:1S. The number of plants in these testcrosses varied from 30 to 50 plants. The observed segregation pattern doesn't depict the expected pattern as majority of testcrosses exhibit 1F:3S ratio. Of the 230 segregating F_2 testcrosses in summer season, only two (1%) gave a 1F:1S ratio whereas 189 (82.2%) segregated according to 1F:3S ratio (Table 23). In the rainy season, of the 228 segregating testcrosses, only two (0.9%) exhibited a 1F:1S ratio whereas 165 (72.4%) segregated according to 1F:3S ratio. Of the eight segregating BC_1 testcrosses in both summer and rainy season each, four (50%) and five (62.5%) exhibited a 1F:3S ratio, respectively. Further, individual χ^2 values for the expected 1F:3S ratio for each of the segregating testcrosses was summed up and a pooled χ^2 value was also calculated by adding the fertile and sterile plants of the

Table 20. Segregation for male-fertile (F) (6-100% SSS) and male-sterile (S) (0-5% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_4-system A-lines with the restorer parent IPC 804, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_4-P_2 × IPC 804-P_4 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	197	108	249	102	63	61	111	130
Expected	214	91	247	104	62	62	120.5	120.5
χ^2	4.51 (0.03)*		0.04 (0.84)		0.01 (0.92)		1.34 (0.25)	
χ^2_p	1.59 (0.21)				0.70 (0.40)			
χ^2_h	2.96 (0.09)				0.65 (0.42)			
5054A_4-P_2 × IPC 804-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	190	87	150	64	88	92	90	100
Expected	195	82	150	64	90	90	95	95
χ^2	0.31 (0.58)		0.00 (1.00)		0.05 (0.82)		0.43 (0.51)	
χ^2_p	0.22 (0.64)				0.46 (0.50)			
χ^2_h	0.09 (0.76)				0.02 (0.89)			

*values in parenthesis are exact probability (*P*) values
χ^2_p is the Chi square value of the pooled data for both the seasons
χ^2_h is the heterogeneity Chi square value

Table 21. Segregation for male-fertile (F) (11-100% SSS) and male-sterile (S) (0-10% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_4-system A-lines with the restorer parent IPC 804, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_4-P_2 × IPC 804-P_4 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	197	108	247	104	63	61	111	130
Expected	214	91	247	104	62	62	120.5	120.5
χ^2	4.51 (0.03)*		0.00 (1.00)		0.01 (0.92)		1.34 (0.25)	
χ^2_p	2.05 (0.15)				0.70 (0.40)			
χ^2_h	2.46 (0.12)				0.65 (0.42)			
5054A_4-P_2 × IPC 804-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	189	88	150	64	88	92	90	100
Expected	195	82	150	64	90	90	95	95
χ^2	0.48 (0.49)		0.00 (1.00)		0.05 (0.82)		0.43 (0.51)	
χ^2_p	0.32 (0.57)				0.46 (0.50)			
χ^2_h	0.16 (0.69)				0.02 (0.89)			

*values in parenthesis are exact probability (*P*) values
χ^2_p is the Chi square value of the pooled data for both the seasons
χ^2_h is the heterogeneity Chi square value

Table 22. Segregation of testcrosses produced on A-lines of A_4 CMS system in two nuclear backgrounds from individual plants of F_2 and BC_1 populations derived from (B × R) crosses with two restorer lines IPC 804 and IPC 511 and test of goodness of fit for hypothetical Mendelian ratios in summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2-TC						No. of BC_1-TC			
	Summer 2003			Rainy 2003			Summer 2003		Rainy 2003	
	Fertile	F+S	Sterile	Fertile	F+S	Sterile	F+S	Sterile	F+S	Sterile
$81A_4$ × (81B-P_8 × IPC 804-P_4)										
(Hypothetical ratio in F_2-TC: 1:2:1; BC_1-TC: 1:1)										
Observed	5	230	162	5	228	164	8	138	8	138
Expected	99	199	99	99	199	99	73	73	73	73
χ^2	132.44 (< 0.01)*			134.38 (< 0.01)			113.98 (< 0.01)		113.98 (< 0.01)	
χ^2_p	268.53 (< 0.01)						229.73 (< 0.01)			
χ^2_h	-1.71 (0.19)						-1.77 (0.18)			
$5054A_4$ × (5054B-P_4 × IPC 511-P_3)										
(Hypothetical ratio in F_2-TC: 1:2:1; BC_1-TC: 1:1)										
Observed	82	228	83	82	230	81	82	56	82	56
Expected	98	197	98	98	197	98	69	69	69	69
χ^2	9.32 (0.01)			10.59 (0.01)			4.53 (0.03)		4.53 (0.03)	
χ^2_p	20.35 (< 0.01)						9.42 (< 0.01)			
χ^2_h	-0.44 (0.80)						-0.36 (0.55)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 23. Segregation pattern within the segregating F_2 and BC_1 testcrosses of A_4 CMS system

Parameter	Total segregating testcrosses	No of testcrosses		% of Total	
		1F:1S	1F:3S	1F:1S	1F:3S
$81A_4$ × (81B × IPC 804) F_2-testcross population Expected ratio within the segregating testcrosses: 1F:1S & 1F:3S					
Summer	230	2	189	0.9	82.2
Rainy	228	2	165	0.9	72.4
$81A_4$ × [81B × (81B × IPC 804)] BC_1-testcross population Expected ratio within the segregating testcrosses: 1F:3S					
Summer	8	-	4	-	50.0
Rainy	8	-	5	-	62.5
5054 A_4 × (5054B × IPC 511) F_2-testcross population Expected ratio within the segregating testcrosses: 1F:1S					
Summer	228	193	-	84.6	-
Rainy	230	191	-	83.0	-
5054 A_4 × [5054B × (5054B × IPC 511)] BC_1-testcross population					
Summer	82	64	-	78.0	-
Rainy	82	65	-	79.3	-

Table 24. Pooled and heterogeneity Chi square analysis of the segregation pattern within the segregating F_2 and BC_1 testcrosses of A_4 CMS system

Parameter	Total segregating testcrosses	Number of plants			χ^2_p	χ^2_h
		Fertile	Sterile	Total		
Hypothesized ratio: 1F:3S $81A_4$ × (81B × IPC 804) F_2-testcross population						
Summer	230	1006	7438	8444	770.5	381.3
Rainy	228	1179	8598	9777	872.6	**243.3**
$81A_4$ × [81B × (81B × IPC 804)] BC_1-testcross population						
Summer	8	56	245	301	**6.2**	50.8
Rainy	8	47	292	339	21.8	22.7
Hypothesized ratio: 1F:1S 5054 A_4 × (5054B × IPC 511) F_2-testcross population						
Summer	228	4445	4004	8449	22.9	840.0
Rainy	230	6049	5253	11302	55.9	1196.1
5054 A_4 × [5054B × (5054B × IPC 511)] BC_1-testcross population						
Summer	82	1682	1593	3275	**2.4**	410.3
Rainy	82	1983	2063	4046	**1.5**	427.1

χ^2_p is the pooled Chi square of the fertile and sterile plants across the segregating
χ^2_h is the heterogeneity Chi square value across the segregating testcross entries
Figures in bold represent non-significant Chi square values

segregating testcrosses (Table 24). The pooled F_2 and BC_1 data of the segregating testcrosses didn't fit the expected 1F:3S ratio in both the summer and in rainy season and the heterogeneity χ^2 was significant in the summer season but non-significant in the rainy season across the segregating testcrosses.

4.1.2.2.2 5054B nuclear background

The cross $5054A_4$ × IPC 804 gave a good fit to the hypothesized F_2 trigenic ratio of 45F:19S and the corresponding BC_1 ratio of 1F:1S in both the seasons as well as in the pooled data (Table 19). During summer season, the F_2 segregated into 190 fertile and 87 sterile plants, giving a good χ^2 fit to the hypothesized ratio of 45F:19S (χ^2= 0.31; P = 0.58). In the BC_1, the observed number of plants (89 fertile and 91 sterile) were in good agreement with the expected segregation ratio of 1F:1S as indicated by a χ^2 value of 0.01 (P = 0.92). In rainy season, the observed F_2 segregation pattern of 150 fertile and 64 sterile plants was exactly according to the expected ratio of 45F:19S as revealed by χ^2 value of 0.00 (P = 1.00). Similarly, in BC_1, the observed numbers (90 fertile and 100 sterile) were in good agreement with the expected 1F:1S segregation ratio as indicated by a non-significant χ^2 value of 0.43 (P = 0.51). The aggregate data gave a good fit to the expected ratio in F_2 (χ^2= 0.22; P = 0.64) and BC_1 (χ^2= 0.33; P = 0.57). The heterogeneity χ^2 was non-significant for F_2 and BC_1 across the seasons indicating uniform segregation pattern.

When plants with 0-5% and 0-10% SSS were classified as sterile and 6-100% and 11-100% SSS as fertile, the observed data witnessed no change for F_2 and BC_1 in both the classifications except shift of one fertile plant to the sterile class in the each of the F_2 (0-10% sterile category, Table 21) and BC_1 (0-5% and 0-10% sterile category, Table 20, 21) in the summer season. The observed data still gave a good fit to the expected ratios of 45F:19S and 1F:1S, respectively, in both the individual seasons as well as in the pooled data.

4.1.2.2.3 ICMB 88004 nuclear background

In summer season, the F_2 data (168 fertile and 124 sterile) exhibited a good χ^2 fit to the hypothesized 9F:7S digenic ratio (χ^2= 0.15; P = 0.70) but the observed data in the BC_1 (22 fertile and 142 sterile) deviated significantly from the expected number of fertile and sterile plants expected according to ratio of 1F:3S as indicated from a significant χ^2 value of 11.13 ($P < 0.01$) (Table 19). In contrast, during the rainy season, the observed F_2 data (280 fertile and 149 sterile) deviated significantly from the expected numbers to give a poor fit to the 9F:7S ratio ($P < 0.01$) but in the BC_1, the observed number of 39

fertile and 127 sterile plants exhibited good fit to the expected segregation ratio of 1F:3S as evident from a non-significant χ^2 value of 0.13 ($P = 0.72$). The pooled data did not fit according to the expected ratios in F_2 as well as BC_1 both ($P < 0.01$). However, the heterogeneity χ^2 was non-significant for both F_2 and BC_1 generations.

4.1.2.3 Male-fertility restorer parent IPC 511

In 81B and 5054B background, the F_2 and BC_1 were also scored for selfed seed set (SSS) data besides pollen-shed data. The 5054B × IPC 511 cross (primarily produced for linkage analysis) also provided information about inheritance of A_4 CMS system from the testcross data (segregation pattern between testcrosses and within the segregating testcrosses). The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S), in the three crosses produced with this restorer are provided in Table 25.

4.1.2.3.1 81B nuclear background

In the summer season, 250 F_2 plants segregated into 213 fertile and 37 sterile plants to give a poor fit to the expected monogenic ratio of 3F:1S as indicated by a significant χ^2 value ($P < 0.01$) (Table 25). However, the BC_1 segregated into 81 fertile and 72 sterile plants to give a good fit to the hypothesized 1F:1S ratio with χ^2 value of 0.42 ($P = 0.52$). In the rainy season, 389 F_2 plants (310 fertile and 79 sterile) gave a good fit to the expected 3F:1S segregation ratio ($\chi^2 = 4.32$; $P = 0.04$) and segregation of 191 BC_1 plants into 107 fertile and 84 sterile plants gave a good fit to the expected 1F:1S ratio as suggested by a χ^2 value of 2.53 ($P = 0.11$). The pooled observed data in the F_2 gave a poor fit to the expected 3F:1S ratio ($P < 0.01$) but good fit to the expected 1F:1S ratio in the BC_1 ($\chi^2 = 2.79$; $P = 0.09$). The heterogeneity Chi square was non-significant for the F_2 and BC_1 across the two seasons.

When plants with 0-5% and 0-10% SSS were classified as sterile and 6-100% and 11-100% SSS as fertile, the observed data exhibited no change for F_2 and BC_1 in both the classifications except shift of one fertile plant to the sterile class in the F_2 of rainy season (0-10% sterile category, Table 27) and shift of two fertile plants to the sterile class in the BC_1 of rainy season (0-5% and 0-10% sterile category, Table 26, 27) in the summer season. The small shift in the number of plants lead to a better χ^2 fit as

Table 25. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_4-system A-lines with the restorer parent IPC 511, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_4-P_4 × IPC 511-P_1 (Hypothetical ratio in F_2: 3F:1S; BC_1: 1F:1S)								
Observed	213	37	310	79	81	72	107	84
Expected	187.5	62.5	292	97	76.5	76.5	95.5	95.5
χ^2	13.33 (< 0.01)*		4.32 (0.04)		0.42 (0.52)		2.53 (0.11)	
χ^2_p	15.61 (< 0.01)				2.79 (0.09)			
χ^2_h	2.04 (0.15)				0.16 (0.69)			
5054A_4-P_3 × IPC 511-P_3 (Hypothetical ratio in F_2: 3F:1S; BC_1: 1F:1S)								
Observed	245	69	393	87	104	71	124	99
Expected	235.5	78.5	360	120	87.5	87.5	111.5	111.5
χ^2	1.38 (0.24)		11.74 (< 0.01)		5.85 (0.02)		2.58 (0.11)	
χ^2_p	11.85 (< 0.01)				8.16 (< 0.01)			
χ^2_h	1.27 (0.26)				0.27 (0.60)			
(5054A_4-P_3 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3) (Hypothetical ratio in F_2: 54F:10S)								
Observed	269	39	321	72	-	-	-	-
Expected	260	48	332	61	-	-	-	-
χ^2	1.83 (0.18)		1.97 (0.16)		-		-	
χ^2_p	0.01 (0.92)				-			
χ^2_h	3.79 (0.05)				-			
88004A_4-P_4 × IPC 511-P_2 (Hypothetical ratio in S 03 F_2: 3F:1S; BC_1: 1F:1S) (Hypothetical ratio in K 03 & Pooled F_2: 54F:10S; BC_1: 3F:1S)								
Observed	230	53	418	55	101	73	166	74
Expected	212	71	399	74	87	87	180	60
χ^2	5.61 (0.02)		5.43 (0.02)		4.19 (0.04)		4.05 (0.04)	
χ^2_p	0.93 (0.33)				23.82 (< 0.01)			
χ^2_h	10.11 (< 0.01)				-15.58 (< 0.01)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 26. Segregation for male-fertile (F) (6-100% SSS) and male-sterile (S) (0-5% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_4-system A-lines with the restorer parent IPC 511, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_4$-P_4 × IPC 511-P_1 (Hypothetical ratio in F_2: 3F:1S; BC_1: 1F:1S)								
Observed	213	37	310	79	81	72	105	86
Expected	187.5	62.5	292	97	76.5	76.5	95.5	95.5
χ^2	13.33 (< 0.01)*		4.32 (0.04)		0.42 (0.52)		1.70 (0.19)	
χ^2_p	15.61 (< 0.01)				2.12 (0.15)			
χ^2_h	2.04 (0.15)				0.00 (1.00)			
$5054A_4$-P_3 × IPC 511-P_3 (Hypothetical ratio in F_2: 3F:1S; BC_1: 1F:1S)								
Observed	245	69	393	87	103	72	124	99
Expected	235.5	78.5	360	120	87.5	87.5	111.5	111.5
χ^2	1.38 (0.24)		11.74 (< 0.01)		5.14 (0.02)		2.58 (0.11)	
χ^2_p	11.85 (< 0.01)				7.60 (0.01)			
χ^2_h	1.27 (0.26)				0.12 (0.73)			
($5054A_4$-P_3 × IPC 511-P_3) × ($5054B$-P_4 × IPC 511-P_3) (Hypothetical ratio in F_2: 54F:10S)								
Observed	258	50	320	73	-	-	-	-
Expected	260	48	332	61	-	-	-	-
χ^2	0.05 (0.82)		2.38 (0.12)		-		-	
χ^2_p	1.82 (0.18)				-			
χ^2_h	0.61 (0.43)				-			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 27. Segregation for male-fertile (F) (11-100% SSS) and male-sterile (S) (0-10% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_4-system A-lines with the restorer parent IPC 511, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_4$-P_4 × IPC 511-P_1 (Hypothetical ratio in F_2: 3F:1S; BC_1: 1F:1S)								
Observed	213	37	309	80	81	72	105	86
Expected	187.5	62.5	292	97	76.5	76.5	95.5	95.5
χ^2	13.33 (< 0.01)*		3.85 (0.05)		0.42 (0.52)		1.70 (0.19)	
χ^2_p	14.90 (< 0.01)				2.12 (0.15)			
χ^2_h	2.28 (0.13)				0.00 (1.00)			
$5054A_4$-P_3 × IPC 511-P_3 (Hypothetical ratio in F_2: 3F:1S; BC_1: 1F:1S)								
Observed	243	71	393	87	103	72	124	99
Expected	235.5	78.5	360	120	87.5	87.5	111.5	111.5
χ^2	0.83 (0.36)		11.74 (< 0.01)		5.14 (0.02)		2.58 (0.11)	
χ^2_p	10.75 (< 0.01)				7.60 (0.01)			
χ^2_h	1.82 (0.18)				0.12 (0.73)			
($5054A_4$-P_3 × IPC 511-P_3) × ($5054B$-P_4 × IPC 511-P_3) (Hypothetical ratio in F_2: 54F:10S)								
Observed	258	50	320	73	-	-	-	-
Expected	260	48	332	61	-	-	-	-
χ^2	0.05 (0.82)		2.38 (0.12)		-		-	
χ^2_p	1.82 (0.18)				-			
χ^2_h	0.61 (0.43)				-			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

compared to the normal classification (see Table 25 for comparison) for the expected ratios.

4.1.2.3.2 5054B nuclear background

In summer season, the F_2 segregated into 245 fertile and 69 sterile plants to give a good fit to the expected monogenic ratio of 3F:1S as revealed by non-significant χ^2 value of 1.38 ($P = 0.24$) (Table 25). In the BC_1, the observed numbers (104 fertile and 71 sterile) were in agreement with the expected segregation as indicated by a χ^2 value of 5.85 ($P = 0.02$). In rainy season, the observed F_2 segregation pattern of 393 fertile and 87 sterile plants deviated from the number of plants expected of a 3F:1S ratio as revealed by significant χ^2 value of 11.74 ($P < 0.01$). In BC_1, the observed numbers (124 fertile and 99 sterile) were in good agreement with the expected 1F:1S segregation ratio as indicated by a χ^2 value of 2.58 ($P = 0.11$). The pooled data didn't fit the expected ratios in the F_2 and BC_1 both ($P< 0.01$). However, the heterogeneity χ^2 exhibited non-significant values in contrast to the significant χ^2 values for the pooled data indicating that segregation pattern is uniform across the seasons.

Based on plants with 0-5% and 0-10% SSS classified as sterile and 6-100% and 11-100% SSS as fertile, the observed data remained unchanged for F_2 and BC_1 in both the classifications except shift of two fertile plants to the sterile class in the F_2 of summer season (0-10% sterile category, Table 27) and shift of one fertile plant to the sterile class in the BC_1 of summer season (0-5% and 0-10% sterile category, Table 26, 27) in the summer season. The small shift in the number of plants lead to a better χ^2 fit as compared to the normal classification (see Table 25 for comparison) for the hypothesized ratios.

The F_2 produced from $F_1 \times F_1$ cross [(5054A_4-P_3 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3)] segregated according to the hypothesized trigenic ratio of 54F:10S with non-significant χ^2 square values in both summer (χ^2= 1.83; $P = 0.18$) and rainy (χ^2= 1.97; $P = 0.16$) seasons as well as in the pooled data (χ^2= 0.01; $P = 0.92$) (Table 25). In summer season, of the total 308 plants scored for F/S reaction, 269 were fertile and 39 were sterile and in the rainy season, 321 plants were fertile and 72 sterile out of the total 393 plants evaluated.

The F_2 produced from $F_1 \times F_1$ cross [(5054A_4-P_3 × IPC 511-P_3) × (5054B-P_4 × IPC 511-P_3)] exhibited a uniform shift of 11 fertile plants to sterile class in the summer season and one fertile plant to sterile class in the rainy season, in both the classification

categories viz., 0-5% SSS (Table 26) and 6-100% (Table 27) SSS as sterile class. This lead to a better χ^2 fit to the hypothesized trigenic 54F:10S F_2 ratio in the summer season as indicated by a lower value of χ^2 ($P = 0.82$) but in the rainy season with a higher value of χ^2 ($P = 0.12$) as compared to the classification based on pollen-shed data alone.

Segregation pattern of the testcrosses produced from individual plants of the (5054B × IPC 511)-derived F_2 and BC_1 populations on $5054A_4$ CMS line also provided information on the inheritance of A_4 CMS system (Table 22). In summer season, of the 393 F_2 testcrosses scored for F/S segregation pattern, 82 were fully fertile, 228 were segregating for F+S plants and 83 were sterile and gave a good χ^2 fit to the expected segregation ratio of 1F:2 (F+S):1S ($P = 0.01$). In the rainy season, the segregation pattern exhibited 82 completely fertile, 230 segregating and 81 completely sterile testcrosses, again giving a good χ^2 fit to the expected ratio with $P = 0.01$. Of the 138 BC_1 testcrosses, 82 segregated for F+S plants and 56 were sterile in the summer and rainy season each, giving a good fit to the expected 1 (F+S):1S ratio ($\chi^2 = 4.53$; $P = 0.03$). The pooled data gave a poor fit to the expected segregation pattern but the non-significant heterogeneity χ^2 values for F_2 and BC_1 across the seasons indicated uniformity in the segregation pattern.

Majority of the segregating testcrosses are expected to give a within testcross segregating ratio of 1F:1S. The number of plants in these testcrosses varied from 35 to 60 plants. The number of segregating F_2 testcrosses exhibiting a 1F:1S ratio was 193 out of 228 (84.6% of the segregating testcrosses) in the summer season and 191 out of 230 (83.0%) in the rainy season (Table 23). Of the 82 segregating BC_1 testcrosses in both summer and rainy seasons, 64 (78.0%) and 65 (79.3%) exhibited a 1F:1S ratio, respectively. Further, individual χ^2 values for the expected 1F:1S ratio for each of the segregating testcrosses was summed up and a pooled χ^2 value was also calculated by adding the fertile and sterile plants of the segregating testcrosses (Table 24). The pooled data of the segregating F_2 testcrosses didn't fit the expected 1F:1S ratio in both the summer and in rainy season whereas in the BC_1, gave a good fit in both summer ($\chi^2 = 2.40$; $P = 0.12$) and rainy season ($\chi^2 = 1.5$; $P = 0.22$). However, the heterogeneity χ^2 was significant across the segregating testcrosses.

4.1.2.3.3 ICMB 88004 nuclear background

Different ratios were found to fit the observed data in the two seasons (Table 25). During the summer season, the F_2 of the cross $ICMA_4$ 88004 × IPC 511 segregated into 230 fertile and 53 sterile plants out of a total of 283 plants evaluated and gave a good fit

to the hypothesized monogenic ratio of 3F:1S (χ^2= 5.61; P = 0.02). In the BC_1, observed segregation pattern of 101 fertile and 73 sterile plants was in agreement with the expected 1F:1S ratio as indicated by a χ^2 value of 4.19 (P = 0.04). In the rainy season, the expected trigenic ratio of 54F:10S in the F_2 (χ^2= 5.43; P = 0.02) and corresponding BC_1 ratio of 3F:1S (χ^2= 4.05; P = 0.04) gave a good fit to the observed segregation pattern. During the rainy season, the number of plants scored for F/S reaction in the F_2 and BC_1 was 473 (418 fertile and 55 sterile) and 240 (166 fertile and 74 sterile), respectively. The aggregate data showed a good agreement with the expected F_2 ratio of 54F:10S (χ^2= 0.93; P = 0.33) but a poor fit to the BC_1 1F:1S ratio (χ^2= 23.82; P< 0.01). The heterogeneity χ^2 value was significant for F_2 as well as BC_1 indicating differences in the segregation pattern across the seasons.

4.1.3 Inheritance of A_v CMS system

The inheritance of A_v CMS system was investigated based on segregation pattern of F_2 and BC_1 in a total in six (A × R) crosses produced by crossing three restorer lines viz., IPC 382 and L 67B restoring fertility of A_v CMS system with A-lines in three diverse nuclear backgrounds of 81B, 5054B and ICMB 88004. All the F_2 and BC_1 populations were evaluated in two environments (summer and rainy seasons, 2003) at ICRISAT, Patancheru. F/S reaction was observed on the basis of pollen shed data in all the crosses.

4.1.3.1 Male-fertility restorer parent IPC 382

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) in the three crosses produced with this restorer are provided in Table 28.

4.1.3.1.1 81B nuclear background

In the summer season, of the 264 plants evaluated in the F_2, 253 were fertile and 11 were sterile, giving a poor fit to the hypothesized trigenic ratio of 63F:1S as evident from the significant χ^2 value of 10.00 (P < 0.01) (Table 28). However, the corresponding BC_1 segregated into 142 fertile and 17 sterile plants out of a total of 159 plants scored for F/S reaction and gave a good fit to the expected 7F:1S ratio with χ^2 value of 0.32 (P = 0.57). In the rainy season, 418 F_2 plants segregated into 409 fertile and nine sterile plants according to the hypothesized 63F:1S ratio with χ^2 value of 0.60 (P = 0.44). The 220 BC_1 plants (200 fertile and 20 sterile) exhibited a good agreement with the expected 7F:1S ratio as shown by a χ^2 value of 2.04 (P = 0.15). The pooled data gave a poor fit in the F_2 (P < 0.01) but a good fit in the BC_1 (χ^2= 2.35; P = 0.13) to the

Table 28. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_v-system A-lines with the restorer parent IPC 382, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_v-P_5 × IPC 382-P_1 (Hypothetical ratio in F_2: 63F:1S; BC_1: 7:1)								
Observed	253	11	409	9	142	17	200	20
Expected	260	4	411	7	139	20	192.5	27.5
χ^2	10.01 (< 0.01)*		0.60 (0.44)		0.32 (0.57)		2.04 (0.15)	
χ^2_p	7.46 (0.01)				2.35 (0.13)			
χ^2_h	3.15 (0.08)				0.01 (0.92)			
5054A_v-P_3 × IPC 382-P_3 (Hypothetical ratio in F_2: 57F:7S; BC_1: 3F:1S)								
Observed	308	43	423	50	124	41	175	74
Expected	313	38	421	52	124	41	187	62
χ^2	0.49 (0.48)		0.03 (0.86)		0.00 (1.00)		2.71 (0.10)	
χ^2_p	0.07 (0.79)				1.56 (0.21)			
χ^2_h	0.45 (0.50)				1.15 (0.28)			
88004A_v-P_2 × IPC 382-P_2 (Hypothetical ratio in F_2: 57F:7S; BC_1: 3F:1S)								
Observed	256	35	424	45	112	47	179	49
Expected	259	32	418	51	119	40	171	57
χ^2	0.25 (0.62)		0.74 (0.39)		1.53 (0.22)		1.32 (0.25)	
χ^2_p	0.09 (0.76)				0.00 (1.00)			
χ^2_h	0.90 (0.34)				2.85 (0.09)			

* values in parenthesis are exact probability (P) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

expected ratios. The heterogeneity χ^2 value was non-significant for both F_2 and BC_1 across the seasons.

4.1.3.1.2 5054B nuclear background

In the summer season, a hypothesized trigenic 57F:7S F_2 ratio with a χ^2 value of 0.49 (P = 0.48) gave a good fit to the observed number of 308 fertile and 43 sterile plants (Table 28). The corresponding expected ratio of 3F:1S in BC_1 was also given a perfect fit (P = 1.00) by the observed number of 124 fertile and 41 sterile plants. In the rainy season, a total of 473 F_2 (423 fertile and 50 sterile) and 249 BC_1 (175 fertile and 74 sterile) plants gave a good fit to the expected trigenic ratio of 57F:7S (χ^2= 0.03; P = 0.86) and the corresponding 3F:1S ratio (χ^2= 2.71; P = 0.10), respectively. In the pooled data, the observed number of plants again gave a good fit to the expected ratios in the F_2 (χ^2= 0.07; 0.79) and BC_1 (χ^2= 1.56; P = 0.21). The heterogeneity χ^2 values were non-significant for the F_2 and BC_1 across the seasons exhibiting consistency in the segregation pattern.

4.1.3.1.3 ICMB 88004 nuclear background

In the summer season, of the 291 plants scored for F/S in the F_2, 256 were fertile and 35 were sterile, giving a good fit to the hypothesized trigenic ratio of 57F:7S with a χ^2 value of 0.25 (P = 0.62) (Table 28). Similarly, the BC_1 observed number of 112 fertile and 47 sterile plants segregated according to the expected 3F:1S ratio (χ^2= 1.53; P = 0.22). In the rainy season, 469 F_2 observed number of plants (424 fertile and 45 sterile) agreed well with a good fit to 57F:7S ratio (χ^2= 0.74; P = 0.39). Similarly, the observed number of 228 BC_1 plants (179 fertile and 49 sterile) gave a good fit to the hypothesized 3F:1S ratio with χ^2 value of 1.32 (P = 0.25). In the pooled data, the F_2 (χ^2 value of 0.09; P = 0.76) gave a good fit to whereas the BC_1 gave an exact fit (P = 1.00) to the expected ratios. The heterogeneity χ^2 values were non-significant for F_2 and BC_1 pointing to consistent segregation pattern across the two seasons.

4.1.3.2 Male-fertility restorer parent L 67B

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) in the three crosses produced with this restorer are provided in Table 29.

4.1.3.2.1 81B nuclear background

The observed data gave a good fit to the expected trigenic F_2 ratio of 54F:10S and the corresponding expected BC_1 ratio of 3F:1S in both the seasons (Table 29). In the

Table 29. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_v-system A-lines with the restorer parent L 67B, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_v-P_6 × L 67B-P_3 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	252	50	316	64	117	54	167	46
Expected	255	47	321	59	128	43	160	53
χ^2	0.13 (0.71)*		0.34 (0.56)		3.60 (0.06)		1.14 (0.29)	
χ^2_p	0.54 (0.46)				0.17 (0.68)			
χ^2_h	-0.07 (0.79)				4.57 (0.03)			
5054Av-P4 × L 67B-P2 (Hypothetical ratio in F2: 54F:10S; BC1: 3F:1S)								
Observed	254	42	337	74	118	65	114	85
Expected	250	46	347	64	137	46	149	50
χ^2	0.36 (0.55)		1.59 (0.21)		10.25 (< 0.01)		32.36 (< 0.01)	
χ^2_p	0.27 (0.60)				40.71 (< 0.01)			
χ^2_h	1.68 (0.19)				1.90 (0.17)			
88004A_v-P_5 × L 67B-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	229	50	367	63	102	48	110	74
Expected	235	44	363	67	112.5	37.5	138	46
χ^2	0.95 (0.33)		0.24 (0.62)		3.56 (0.06)		21.92 (< 0.01)	
χ^2_p	0.03 (0.86)				23.06 (< 0.01)			
χ^2_h	1.16 (0.28)				2.42 (0.12)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

summer season, of the 302 plants tagged for F/S reaction, the F_2 segregated into 252 fertile and 50 sterile plants, giving a good fit to the hypothesized trigenic ratio of 54F:10S as explained by χ^2 value of 0.13 ($P = 0.71$). The BC_1 segregated into 117 fertile and 54 sterile plants out of 171 plants evaluated and gave a good fit to the hypothesized 3F:1S ratio with χ^2 value of 3.60 ($P = 0.06$). In the rainy season, 380 F_2 plants (316 fertile and 64 sterile) gave a good fit to 54F:10S ratio with χ^2 value of 0.34 ($P = 0.56$). Similarly, 213 BC_1 plants segregated into 167 fertile and 46 sterile plants to give a good fit to the expected 3F:1S ratio as evident from a χ^2 value of 1.14 ($P = 0.29$). The pooled data exhibited a good fit to the F_2 expected ratio of 54F:10S ($\chi^2 = 0.54$; $P = 0.46$) and the corresponding BC_1 ratio of 3F:1S as indicated by χ^2 value of 0.17 ($P = 0.68$). The heterogeneity χ^2 value was non-significant for F_2 ($P = 0.79$) as well as BC_1 ($P = 0.03$) indicating a uniform segregation pattern across seasons.

4.1.3.2.2 5054B nuclear background

The observed data gave a good χ^2 fit to expected F_2 trigenic ratio in both the seasons but didn't fit the corresponding BC_1 ratio of 3F:1S in any of the seasons (Table 29). A 54F:10S F_2 ratio with a χ^2 value of 0.36 ($P = 0.55$) gave a good fit to the observed number of 254 fertile and 42 sterile plants in the summer season. However, the corresponding expected ratio of 3F:1S in BC_1 agreed poorly ($P < 0.01$) with the observed 118 fertile and 65 sterile plants. In the rainy season, a total of 411 F_2 plants segregated into 337 fertile and 74 sterile to give a good fit to the expected ratio of 54F:10S in the F_2 ($\chi^2 = 1.59$; $P = 0.21$) but the segregation pattern of 199 BC_1 plants into 114 fertile and 85 sterile plants gave a poor fit to the expected segregation ratio of 3F:1S ($P < 0.01$). In case of aggregate data, the F_2 gave a good fit to the expected F_2 ratio ($\chi^2 = 0.27$; $P = 0.60$) but not in the BC_1 ($P < 0.01$). The heterogeneity χ^2 values were non-significant for both the F_2 and BC_1 across the seasons.

4.1.3.2.3 ICMB 88004 nuclear background

The observed data gave a good fit to the expected trigenic F_2 ratio of 54F:10S in both the seasons whereas the corresponding expected BC_1 ratio of 3F:1S was found to give a good χ^2 fit in the summer season only (Table 29). In the summer season, the F_2 segregated into 229 fertile and 50 sterile plants and gave a good fit to the hypothesized trigenic 54F:10S ratio with a χ^2 value of 0.95 ($P = 0.33$). The corresponding BC_1 data (102 fertile and 48 sterile plants) segregated according to the expected 3F:1S ratio ($\chi^2 = 3.56$; $P = 0.06$). In the rainy season, 430 F_2 plants (367 fertile and 63 sterile) gave a good fit to the expected 54F:10S ratio with χ^2 value of 0.24 ($P = 0.62$). However, the

observed number of 184 BC_1 plants (110 fertile and 74 sterile) gave a poor fit to the hypothesized 3F:1S ratio as specified by a significant χ^2 value ($P < 0.01$). When the data were pooled for both the seasons, the F_2 gave a good fit (χ^2=0.03; $P = 0.86$) to the expected ratio whereas the BC_1 observed numbers didn't fit the expected ratio ($P < 0.01$). The heterogeneity χ^2 values were non-significant for both F_2 and BC_1 when analyzed across the seasons.

4.1.4 Inheritance of A_{egp} CMS system

The inheritance of A_{egp} CMS system was investigated based on segregation pattern of F_2 and BC_1 in three crosses produced by crossing the restorer line L 67B restoring fertility of A_{egp} CMS system with a-lines in three diverse nuclear backgrounds of 81B, 5054B and ICMB 88004. All the crosses were evaluated in two environments (summer and rainy seasons, 2003) at ICRISAT- Patancheru for F/S reaction on the basis of pollen shed data and also on the basis of selfed seed set (SSS) data in two crosses in the 81B and 5054B backgrounds. In crosses where SSS data was recorded, genetic ratios were worked out for two cases viz., when plants with 0-5% SSS were taken as sterile irrespective of whether plants were tagged as fertile or shy on the basis of pollen shed data and 6-100% as fertile irrespective of whether plants were tagged as sterile or shy on the basis of pollen shed data. Similarly, plants with 0-10% SSS data were classified as sterile and 11-100% SSS as fertile.

4.1.4.1 Male-fertility restorer parent L 67B

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) in the three crosses produced with this restorer are provided in Table 30.

4.1.4.1.1 81B nuclear background

In the summer season, out of 317 plants evaluated, the F_2 segregated exactly according to the hypothesized trigenic ratio of 54F:10S into 267 fertile and 50 sterile plants, as explained by a χ^2 value of 0.00 ($P = 1.00$) (Table 30). The corresponding BC_1 segregated into 103 fertile and 44 sterile plants and gave a good fit to the expected 3F:1S ratio with χ^2 value of 1.65 ($P = 0.20$). In the rainy season, 408 F_2 (305 fertile and 103 sterile) plants gave a good fit to the hypothesized 45F:19S ratio with χ^2 value of 3.65 ($P = 0.06$) and 109 BC_1 plants (64 fertile and 45 sterile) gave a good fit to the expected 1F:1S ratio with χ^2 value of 2.97 ($P = 0.08$), respectively. Neither of the expected F_2 and BC_1 ratios was found to fit the pooled data although 54F:10S F_2 and 3F:1S BC_1 ratios gave a comparatively lower significant χ^2 values.

Table 30. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_{egp}-system A-lines with the restorer parent L 67B, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_{egp}-P_4 × L 67B-P_3 (Hypothetical ratio in S 03 and pooled F_2: 54F:10S; BC_1: 3F:1S)								
(Hypothetical ratio in K 03 F_2: 45F:19S; BC_1: 1F:1S)								
Observed	267	50	305	103	103	44	64	45
Expected	267	50	287	121	110	37	54.5	54.5
χ^2	0.00 (1.00)*		3.65 (0.06)		1.65 (0.20)		2.97 (0.08)	
χ^2_p	16.09 (< 0.01)				12.51 (< 0.01)			
χ^2_h	-				-			
5054A_{egp}-P_3 × L 67B-P_2 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	264	69	429	102	88	57	21	14
Expected	281	52	448	83	109	36	26	9
χ^2	6.18 (0.01)		4.91 (0.03)		9.71 (< 0.01)		3.44 (0.06)	
χ^2_p	11.06 (< 0.01)				13.70 (< 0.01)			
χ^2_h	0.03 (0.86)				-0.55 (0.46)			
88004A_{egp}-P_3 × L 67B-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	263	34	425	64	100	33	155	44
Expected	251	46	413	76	100	33	149	50
χ^2	3.62 (0.06)		2.20 (0.14)		0.00 (1.00)		0.74 (0.39)	
χ^2_p	5.70 (0.02)				0.49 (0.48)			
χ^2_h	0.12 (0.73)				0.25 (0.62)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

When the SSS data was classified based on plants with 0-5% SSS as sterile and 6-100% as fertile, the change in numbers of the two classes was brought about by a shift of plants from fertile to sterile class in the F_2 and the BC_1 (Table 31). In the summer season, the F_2 observed data segregated into 251 fertile and 66 sterile plants giving a good χ^2 fit to the expected 54F:10S ratio as indicated by χ^2 value of 6.10 ($P = 0.01$). However, the BC_1 observed segregation of 94 fertile and 53 sterile plants gave a poor fit to the expected 3F:1S ratio as revealed by a significant χ^2 value ($P < 0.01$). In the rainy season, the F_2 segregated into 296 fertile and 112 sterile plants and gave a good fit to the expected 45F:19S ratio ($\chi^2= 0.87$; $P = 0.35$). The BC_1 segregated into 64 fertile and 45 sterile plants and gave a good fit to the expected 1F:1S ratio with χ^2 value of 2.97 ($P = 0.08$). The pooled data in F_2 as well as in BC_1 exhibited significant deviation from the expected numbers as revealed by a poor fit ($P < 0.01$) to the hypothesized ratio of 54F:10S and 3F:1S, respectively.

When the SSS data was classified into the 0-10% sterile and 11-100% fertile classes, the change in numbers of the two classes was brought about by a shift of plants from fertile to sterile class in the F_2 and the BC_1 (Table 32). In the summer season, the F_2 segregated into 243 fertile and 74 sterile plants and gave a good fit to the expected 45F:19S ratio as indicated by χ^2 value of 5.81 ($P = 0.02$). However, the BC_1 segregation into 94 fertile and 53 sterile plants gave a poor fit to the expected 1F:1S ratio as shown by a significant χ^2 value ($P < 0.01$). In the rainy season, the F_2 segregated perfectly according to the expected 45F:19S ratio into 288 fertile and 120 sterile plants ($P = 1.00$). The BC_1 segregated into 64 fertile and 45 sterile plants and gave a good fit to the expected 1F:1S ratio with χ^2 value of 2.97 ($P = 0.08$). The pooled data for F_2 gave a good fit to the expected ratio ($\chi^2= 2.84$; $P = 0.09$) but not in the BC_1 as revealed by significant χ^2 value ($P < 0.01$). The heterogeneity χ^2 values were non-significant for both F_2 ($\chi^2= 2.97$; $P = 0.08$) and BC_1 ($\chi^2= 0.25$; $P = 0.62$) indicating towards a consistent segregation behavior across the seasons.

4.1.4.1.2 5054B nuclear background

In the summer season, the observed number of 264 fertile and 69 sterile plants in the F_2 gave a good χ^2 fit to the hypothesized trigenic 54F:10S ratio with a χ^2 value of 6.18 ($P = 0.09$) (Table 30). However, the corresponding expected ratio of 3F:1S in BC_1 didn't fit well ($P < 0.01$) with the observed segregation into 88 fertile and 57 sterile plants. In the rainy season, a total of 531 F_2 (429 fertile and 102 sterile) and 35 BC_1 plants (21 fertile and 14 sterile) were evaluated for F/S reaction and found to give a good fit to the

Table 31. Segregation for male-fertile (F) (6-100% SSS) and male-sterile (S) (0-5% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_{egp}-system A-lines with the restorer parent L 67B, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_{egp}$-P_4 × L 67B-P_3 (Hypothetical ratio in S 03 F_2: 54F:10S; BC_1: 3F:1S)								
	(Hypothetical ratio in K 03 and pooled F_2: 45F:19S; BC_1: 1F:1S)							
Observed	251	66	296	112	94	53	64	45
Expected	267	50	287	121	110	37	54.5	54.5
χ^2	6.10 (0.01)*		0.87 (0.35)		9.00 (< 0.01)		2.97 (0.08)	
χ^2_p	8.92 (< 0.01)				13.60 (< 0.01)			
χ^2_h	-				-			
$5054A_{egp}$-P_3 × L 67B-P_2 (Hypothetical ratio in S 03 F_2: 45F:19S; BC_1: 1F:1S)								
	(Hypothetical ratio in K 03 & Pooled F_2: 3F:1S; BC_1: 1F:1S)							
Observed	245	88	417	114	83	62	20	15
Expected	234	99	398	133	72.5	72.5	17.5	17.5
χ^2	1.54 (0.21)		3.35 (0.07)		2.76 (0.10)		0.46 (0.50)	
χ^2_p	1.13 (0.29)				3.47 (0.06)			
χ^2_h	-				-			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

Table 32. Segregation for male-fertile (F) (11-100% SSS) and male-sterile (S) (0-10% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_{egp}-system A-lines with the restorer parent L 67B, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_{egp}$-P_4 × L 67B-P_3 (Hypothetical ratio in F_2: 45F:19S; BC_1: 1F:1S)								
Observed	243	74	288	120	94	53	64	45
Expected	223	94	287	121	73.5	73.5	54.5	54.5
χ^2	5.81 (0.02)*		0.00 (1.00)		10.88 (< 0.01)		2.97 (0.08)	
χ^2_p	2.84 (0.09)				13.60 (< 0.01)			
χ^2_h	2.97 (0.08)				0.25 (0.62)			
$5054A_{egp}$-P_3 × L 67B-P_2 (Hypothetical ratio in S 03 F_2: 45F:19S; BC_1: 1F:1S)								
(Hypothetical ratio in K 03 & Pooled F_2: 3F:1S; BC_1: 1F:1S)								
Observed	239	94	408	123	79	66	19	16
Expected	234	99	398	133	72.5	72.5	17.5	17.5
χ^2	0.27 (0.60)		0.86 (0.35)		0.99 (0.32)		0.11 (0.74)	
χ^2_p	0.00 (1.00)				1.25 (0.26)			
χ^2_h	-				-			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

expected ratio of 54F:10S (χ^2= 4.91; P = 0.03) and the corresponding 3F:1S ratio (χ^2= 3.44; P = 0.06), respectively. The pooled data didn't fit the expected F_2 and BC_1 ratios (P < 0.01). However, the heterogeneity χ^2 values were non-significant for both the F_2 and BC_1 across the seasons.

When plants with 0-5% SSS data were classified as sterile and 6-100% as fertile, the change in numbers of the two classes was brought about by a shift of plants from fertile to sterile class in the F_2 and the BC_1 (Table 31). In the summer season, the F_2 segregated into 245 fertile and 88 sterile plants giving a good agreement with the expected 45F:19S ratio as indicated by χ^2 value of 1.54 (P = 0.21). The BC_1 observed segregation (83 fertile and 62 sterile) also gave a good fit to the expected 1F:1S ratio with a χ^2 value of 2.76 (P = 0.10). In the rainy season, the F_2 segregated into 417 fertile and 114 sterile plants and gave a good fit to the expected monogenic 3F:1S ratio (χ^2= 3.35; P = 0.07). The BC_1 segregated into 20 fertile and 15 sterile plants and gave a good fit to the expected 1F:1S ratio with χ^2 value of 0.46 (P = 0.50). The pooled data in the F_2 (χ^2= 1.13; P = 0.29) as well as BC_1 (χ^2= 3.47; P = 0.06) exhibited good fit to the expected F_2 ratio of 3F:1S and BC_1 ratio of 1F:1S as revealed by non-significant χ^2 values in both cases.

When the SSS data was classified into 0-10% sterile and 11-100% fertile classes, the change in numbers of the two classes was brought about by a shift of plants from fertile to sterile class in the F_2 and the BC_1 (Table 32). In the summer season, the F_2 segregated into 239 fertile and 94 sterile plants giving a good fit to the expected 45F:19S ratio as indicated by χ^2 value of 0.27 (P = 0.60). The corresponding BC_1 segregation (79 fertile and 66 sterile) also gave a good fit to the expected 1F:1S ratio with a non-significant χ^2 value of 0.99 (P = 0.32). In the rainy season, the F_2 segregated into 408 fertile and 123 sterile plants and gave a good fit to the hypothesized 3F:1S ratio (χ^2= 0.86; P = 0.35). The BC_1 segregated into 19 fertile and 16 sterile plants and gave a good fit to the expected 1F:1S ratio with χ^2 value of 0.11 (P = 0.74). The pooled data for F_2 agreed exactly with the expected segregation of 3F:1S (χ^2= 0.00; P = 1.00) and in case of BC_1, exhibited good fit to the expected 1F:1S ratio as revealed by non-significant χ^2 of 1.25 (P = 0.26).

4.1.4.1.3 ICMB 88004 nuclear background

In the summer season, the F_2 segregated into 263 fertile and 34 sterile plants out of 297 plants evaluated for F/S reaction, giving a good fit to the hypothesized trigenic 54F:10S ratio with a χ^2 value of 3.62 (P = 0.06) (Table 30). The BC_1 segregated exactly

according to the expected 3F:1S ratio (χ^2= 0.00; P = 1.00) into 100 fertile and 33 sterile plants out of 133 plants scored for F/S reaction. In the rainy season, 489 F_2 plants (425 fertile and 64 sterile) gave a good fit to the expected 54F:10S ratio with χ^2 value of 2.20 (P = 0.14). The observed number of 199 BC_1 plants (155 fertile and 44 sterile) gave a good fit to the hypothesized 3F:1S ratio with χ^2 value of 0.74 (P = 0.39). In the pooled data, the F_2 agreed with 54F:10S ratio with a χ^2 value of 5.70 (P = 0.02) and the corresponding BC_1 gave a good fit to the expected 3F:1S ratio (χ^2= 0.49; P = 0.39). The heterogeneity χ^2 values were non-significant for both F_2 (χ^2= 0.12; P = 0.73) and BC_1 (χ^2= 0.25; P = 0.62) across the seasons pointing to a consistent segregation pattern.

4.1.5 Inheritance of A_5 CMS system

The inheritance of A_5 CMS system was investigated based on segregation pattern of F_2 and BC_1 in three crosses produced by crossing the restorer line LSGP A_5 R-line restoring fertility of A_5 CMS system with A-lines in three diverse nuclear backgrounds of 81B, 5054B and ICMB 88004. All the crosses were evaluated in two environments (summer and rainy seasons, 2003) at ICRISAT- Patancheru for F/S reaction on the basis of pollen shed data and also on the basis of selfed seed set (SSS) data in two crosses in the 81B and 5054B backgrounds. In crosses where SSS data was recorded, genetic ratios were worked out for two cases viz., when plants with 0-5% SSS data were classified as sterile and 6-100% as fertile. Similarly, another classification was done with 0-10% SSS data taken as sterile and 11-100% SSS as fertile.

4.1.5.1 Male-fertility restorer parent LSGP A_5 R-line

The goodness of fit for the hypothetical Mendelian ratios in the F_2 and BC_1 populations for the two classes viz., fertile (F) and sterile (S) in the three crosses produced with this restorer are provided in Table 33.

4.1.5.1.1 81B nuclear background

In the summer season, of the 293 plants evaluated, the F_2 segregated into 258 fertile and 35 sterile plants, giving a good fit to the hypothesized trigenic ratio of 54F:10S with a χ^2 value of 2.74 (P = 0.10) (Table 33). The corresponding BC_1 segregated into 107 fertile and 39 sterile plants and gave a good fit to the expected 3F:1S ratio with χ^2 value of 0.15 (P = 0.70). In the rainy season, the observed number of 417 F_2 plants (355 fertile and 62 sterile) exhibited good fit to the expected 54F:10S ratio as indicated by χ^2 value of 0.13 (P = 0.72). In the BC_1, 177 fertile and 58 sterile plants gave an exact fit to the expected 3F:1S segregation (P = 1.00). When the observed data for individual seasons was pooled, F_2 (χ^2= 1.93; P = 0.17) as well as BC_1 (χ^2= 0.02; P = 0.89) gave a good fit to the

Table 33. Segregation for male-fertile (F) and male-sterile (S) plants in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A_5-system A-lines with the restorer parent LSGP A_5 R-line, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 (A × F_1) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
81A_5-P_1 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	258	35	355	62	107	39	177	58
Expected	247	46	352	65	109.5	36.5	176	59
χ^2	2.74 (0.10)*		0.13 (0.72)		0.15 (0.70)		0.00 (1.00)	
χ^2_p	1.93 (0.17)				0.02 (0.89)			
χ^2_h	0.94 (0.33)				0.13 (0.72)			
5054A_5-P_1 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 63F:1S; BC_1: 7:1)								
Observed	97	2	452	15	143	8	158	10
Expected	97	2	460	7	132	19	147	21
χ^2	0.00 (1.00)		7.22 (0.01)		6.52 (0.01)		6.00 (0.01)	
χ^2_p	6.73 (0.01)				13.09 (< 0.01)			
χ^2_h	0.49 (0.48)				-0.57 (0.45)			
88004A_5-P_4 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 57F:7S; BC_1: 5F:3S)								
Observed	229	41	409	40	96	42	72	24
Expected	240	30	400	49	86	52	60	36
χ^2	4.57 (0.03)		1.69 (0.19)		2.65 (0.10)		3.88 (0.05)	
χ^2_p	0.05 (0.82)				8.23 (< 0.01)			
χ^2_h	6.21 (0.01)				-1.7 (0.19)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

expected ratios. The heterogeneity χ^2 value was non-significant for both F_2 and BC_1 populations across seasons indicating a consistent segregation pattern across the seasons.

When plants with 0-5% SSS data were classified as sterile and 6-100% as fertile, there was a minor shift of two and one plant from the fertile to the sterile class in the F_2 of summer and rainy season, respectively (Table 34). The BC_1 data didn't witness any change in either of the F/S classes. The observed F_2 data in both the seasons exhibited lower χ^2 values for the expected 54F:10S ratio when compared with χ^2 values obtained in the normal grouping (see Table 34). Similarly, the BC_1 segregation pattern gave same χ^2 values for the 3F:1S ratio as the normal grouping. The pooled data for F_2 (χ^2= 1.16; P = 0.28) as well as BC_1 (χ^2= 0.02; P = 0.89) exhibited good fit to the expected numbers. The heterogeneity χ^2 values for F_2 as well as BC_1 were non-significant indicating uniform segregation pattern of data across the seasons.

When plants with 0-10% SSS were classified as sterile and 11-100% as fertile, there was a minor shift of two and three plants from the fertile to the sterile class in the F_2 of summer and rainy season, respectively (Table 35). The rainy season BC_1 data exhibited shift of three fertile plants to the sterile class while there was no change in the summer season data. The F_2 and BC_1 gave a good fit to the expected 54F:10S and 3F:1S ratio, respectively, in the individual seasons as well as in the pooled data with comparatively lower χ^2 values in the F_2 but a slightly higher yet non-significant χ^2 value in the rainy season BC_1.

4.1.5.1.2 5054B nuclear background

The F_2 of this cross had a poor seedling stand (97 fertile and two sterile plants) in summer season but still gave a perfect fit to the expected trigenic ratio of 63F:1S (P = 1.00). The observed number of 143 fertile and eight sterile plants agreed with the corresponding expected BC_1 ratio of 7F:1S (χ^2= 6.52; P = 0.01) (Table 33). In the rainy season, a total of 467 F_2 plants (452 fertile and 15 sterile) gave a good fit to the expected 63F:1S ratio in the F_2 (χ^2= 7.22; P = 0.01) whereas 168 BC_1 (158 fertile and 10 sterile) plants agreed with the corresponding 3F:1S BC_1 ratio with a lower χ^2 value of 6.00 (P = 0.01). In the pooled data, the F_2 gave a good fit to the expected ratio (χ^2= 6.73; P = 0.01) but the BC_1 gave a poor fit (P< 0.01). The heterogeneity χ^2 values were non-significant for both the F_2 and BC_1 across the seasons.

Table 34. Segregation for male-fertile (F) (6-100% SSS) and male-sterile (S) (0-5% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_5-system A-lines with the restorer parent LSGP A_5 R-line, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_5$-P_1 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	256	37	354	63	107	39	177	58
Expected	247	46	352	65	109.5	36.5	176	59
χ^2	1.78 (0.18)*		0.05 (0.82)		0.15 (0.70)		0.00 (1.00)	
χ^2_p	1.16 (0.28)				0.02 (0.89)			
χ^2_h	0.67 (0.41)				0.13 (0.71)			
$5054A_5$-P_1 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 63F:1S; BC_1: 7:1)								
Observed	97	2	448	19	143	8	158	10
Expected	97	2	460	7	132	19	147	21
χ^2	0.00 (1.00)		17.47 (< 0.01)		6.52 (0.01)		6.00 (0.01)	
χ^2_p	15.61 (< 0.01)				13.09 (< 0.01)			
χ^2_h	1.86 (0.17)				0.57 (0.45)			

*values in parenthesis are exact probability (*P*) values
χ^2_p is the Chi square value of the pooled data for both the seasons
χ^2_h is the heterogeneity Chi square value

Table 35. Segregation for male-fertile (F) (11-100% SSS) and male-sterile (S) (0-10% SSS) plants on the basis of selfed seed set (SSS) in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_5-system A-lines with the restorer parent LSGP A_5 R-line, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter	No. of F_2 plants				No. of BC_1 ($A \times F_1$) plants			
	Summer 2003		Rainy 2003		Summer 2003		Rainy 2003	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
$81A_5$-P_1 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 54F:10S; BC_1: 3F:1S)								
Observed	256	37	352	65	107	39	174	61
Expected	247	46	352	65	109.5	36.5	176	59
χ^2	1.78 (0.18)*		0.00 (1.00)		0.15 (0.70)		0.07 (0.79)	
χ^2_p	0.76 (0.38)				0.25 (0.62)			
χ^2_h	1.02 (0.31)				-0.09 (0.76)			
$5054A_5$-P_1 × LSGP A_5-P_1 (Hypothetical ratio in F_2: 63F:1S; BC_1: 7:1)								
Observed	97	2	448	19	143	8	158	10
Expected	97	2	460	7	132	19	147	21
χ^2	0.00 (1.00)		17.47 (< 0.01)		6.52 (0.01)		6.00 (0.01)	
χ^2_p	15.61 (< 0.01)				13.09 (< 0.01)			
χ^2_h	1.86 (0.17)				0.57 (0.45)			

*values in parenthesis are exact probability (*P*) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value

When plants with the observed SSS data were classified into 0-5% and 0-10% sterile classes, the observed number of plants remain unchanged except for a shift of four fertile plants to the sterile class in the rainy season F_2 data in both the groupings as compared to the normal grouping (Table 34, 35). The observed data gave a good fit to the expected F_2 and BC_1 ratios of 63F:1S and 7F:1S, respectively, in both the groupings except the rainy season F_2 whose calculated χ^2 value increased from 7.22 ($P = 0.01$) to 17.47 ($P < 0.01$) for the 63F:1S ratio.

4.1.5.1.3 ICMB 88004 nuclear background

In the summer season, the F_2 segregated into 229 fertile and 41 sterile plants out of 270 plants evaluated, concurring with the hypothesized 57F:7S ratio with a χ^2 value of 4.57 ($P = 0.03$) (Table 33). The BC_1 segregated according to the expected 5F:3S ratio (χ^2= 2.65; $P = 0.10$) into 96 fertile and 42 sterile plants out of 138 plants evaluated. In the rainy season, 449 F_2 plants (409 fertile and 40 sterile) gave a good fit to the 57F:7S ratio with χ^2 value of 1.69 ($P = 0.19$). The observed number of 96 BC_1 plants (72 fertile and 24 sterile) gave a good fit to the hypothesized 5F:3S ratio with χ^2 value of 3.88 (P = 0.05). When the data was pooled, the F_2 agreed strongly with the expected 57F:7S ratio (χ^2= 0.05; $P = 0.82$) whereas the BC_1 gave a poor fit to the expected 5F:3S ratio (χ^2= 8.23; $P < 0.01$). The heterogeneity χ^2 values were non-significant for both F_2 and BC_1 across the seasons.

4.2 Test of allelism

The test of allelism was carried out to know whether two or more restorer lines possess same or different alleles of a restorer gene restoring fertility of the sterile cytoplasm. The absence of sterile or partially sterile plants among testcross progenies of a cross will indicate presence of same alleles of a restorer gene among the restorer lines involved the cross combinations, whereas the presence of sterile or partially sterile plants indicate presence of different alleles. The (R × R) F_1 and parental testcrosses involving three dual-restorer lines (IPC 1518, IPC 511 and IPC 804) were evaluated on an individual plant basis for pollen shedding (PS) and selfed-seed-set (SSS) score in summer 2003 and re-evaluated in rainy 2003 season.

4.2.1 Allelism among fertility restorer gene(s) of A_1 CMS system

Complete fertility restoration was observed in the three parental testcrosses produced on $81A_1$ in both summer and rainy seasons (Table 36). The number of plants evaluated in the testcrosses varied between 71 and 80 in summer season and between 78 and 120 in the rainy season. All of the (R × R) F_1 hybrids viz., IPC 804 × IPC 1518, IPC 511 × IPC 1518 and IPC 511 × IPC 804 produced fertile progenies in the testcrosses produced on $81A_1$ CMS line. The number of plants evaluated in these testcrosses in the summer season varied between 213 and 222 and in the rainy season between 330 and 498.

4.2.2 Allelism among fertility restorer gene(s) of A_4 CMS system

All three parental restorer lines viz., IPC 1518, IPC 804 and IPC 511 produced complete fertile progenies in testcross made on $81A_4$ A-line in both summer and rainy seasons (Table 37).

The (R × R) F_1 hybrids produced complete fertile progenies on $81A_4$ involving two cross combinations viz., IPC 511 × IPC 1518 (225 plants in summer season and 374 plants in rainy season) and IPC 511 × IPC 804 (222 plants in summer season and 367 plants in the rainy season) (Table 37). The testcross progenies produced from the cross IPC 804 and IPC 1518 segregated into 155 fertile and 66 sterile plants to give a good fit to the hypothesized ratio of 3F:1S (χ^2= 2.54; P = 0.11) in the summer season (Table 38). In the rainy season, this testcross segregated into 290 fertile and 78 sterile plants out of the total 368 evaluated, giving a good fit to the hypothesized 3F:1S ratio as indicated by a χ^2 value of 2.64 (P = 0.10). These results indicate that IPC 511 and IPC 1518 possess same alleles of the restorer genes for the A_4 system whereas IPC 804 and IPC 1518 were found to be non allelic.

4.3 Linkage between fertility restorer genes of A_1 and A_4 CMS systems

For detecting and estimating the linkage between fertility restorer genes of A_1 and A_4 CMS systems, two (B × R)-derived F_2 and BC_1 populations were produced involving 81B and IPC 804 and 5054B and IPC 511. The individual plants of these F_2 and BC_1 populations were testcrossed onto the A_1 and A_4 CMS lines in 81B and 5054B nuclear backgrounds. The testcrosses were scored on plot basis as either fully fertile or fully sterile or segregating for fertile and sterile plants. The testcrosses were classified as segregating even when a few plants of lesser class (fertile or sterile) were present. The joint segregation pattern of testcrosses for the two CMS systems produced nine classes viz., testcrosses that were fertile on A_1 and either fertile or sterile or segregating on A_4,

Table 36. Segregation pattern for fertility restoration in testcross F_1 progenies involving $81A_1$ × (Restorer × Restorer)

Testcross	Summer 2003			Rainy 2003		
	F	S	Total	F	S	Total
Parental testcrosses						
$81A_1$ × IPC 1518	71	0	71	94	0	94
$81A_1$ × IPC 804	76	0	76	120	0	120
$81A_1$ × IPC 511	79	0	79	78	0	78
(R x R) F_1 testcrosses						
$81A_1$ × (IPC 804 × IPC 1518)	222	0	222	330	0	330
$81A_1$ × (IPC 511 × IPC 1518)	216	0	216	389	0	389
$81A_1$ × (IPC 511 × IPC 804)	213	0	213	330	0	330

Table 37. Segregation pattern for fertility restoration in testcross F_1 progenies involving $81A_4$ × (Restorer × Restorer)

Testcross	Summer 2003			Rainy 2003		
	F	S	Total	F	S	Total
Parental testcrosses						
$81A_4$ × IPC 1518	82	0	82	106	0	106
$81A_4$ × IPC 804	78	0	78	74	0	74
$81A_4$ × IPC 511	82	0	82	112	0	112
(R x R) F_1 testcrosses						
$81A_4$ × (IPC 804 × IPC 1518)	155	66	221	290	78	368
$81A_4$ × (IPC 511 × IPC 1518)	225	0	225	374	0	374
$81A_4$ × (IPC 511 × IPC 804)	222	0	222	367	0	367

Table 38. Segregation pattern for fertility restoration in segregating testcross F_1 progenies involving $81A_4$ × (Restorer × Restorer)

Testcross	Season	A-line × (R × R)					
		F	S	Total	Ratio	χ^2 value	*P*-value
$81A_4$ × (IPC 804 × IPC 1518)	summer	155	66	221	3:1	2.54	0.11
	rainy	290	78	368	3:1	2.64	0.10

testcrosses that were sterile on A_1 and either fertile or sterile or segregating on A_4, and testcrosses segregating on A_1 and either fertile or sterile or segregating on A_4. The results for the two populations are presented separately as under:

4.3.1 81B × IPC 804 population

The (81B × IPC 804)-derived population had complete data from 397 F_2 and 146 BC_1 derived testcross progenies for both the seasons. First, the inheritance pattern of individual CMS systems was estimated and then a joint segregation analysis was done. The A_1 CMS system testcrosses segregated in a monogenic 1F:2 (F+S):1S ratio in the F_2-derived testcrosses and 1(F+S):1S in the BC_1-derived testcrosses (Table 10). However, for the A_4 CMS system, the expected segregation pattern of 1F:2 (F+S):1S ratio in the F_2 testcrosses and 1 (F+S):1S in the BC_1 testcrosses was found to give a poor χ^2 fit (Table 22). The joint segregation analysis of the individual ratios observed in the A_1 and A_4 CMS F_2 testcrosses gave significant Chi square values in both summer (χ^2= 163.1; $P < 0.01$) and rainy (χ^2= 153.4; $P < 0.01$) seasons indicating that linkage is present between the fertility restorer genes of these CMS systems (Table 39). The joint segregation analysis for the BC_1 testcross data also indicated that linkage was present as revealed by a significant χ^2 value in both the seasons ($P < 0.01$) (Table 40).

4.3.2 5054B × IPC 511 population

The (5054B × IPC 511)-derived population had complete data set for 393 F_2 and 138 BC_1 individuals for both the seasons. In the A_1 CMS system, the observed testcross segregation pattern gave a poor χ^2 fit to the expected pattern of 7F:8 (F+S):1S ratio in the F_2 in both the seasons and 3 (F+S):1S ratio in the corresponding BC_1 in rainy season only (Table 10). In the A_4 CMS testcrosses, a 1F:2 (F+S):1S ratio in the F_2 and 1 (F+S):1S ratio in the corresponding BC_1 was found to give a good χ^2 fit to the observed segregation pattern (Table 22). The joint segregation analysis in the F_2 testcrosses gave significant Chi square values in both summer (χ^2= 579.9) and rainy (χ^2= 729.0) seasons indicating that linkage is present between the fertility restorer genes of these systems (Table 41). Similarly, the joint segregation analysis in the BC_1 also indicated that linkage was present as revealed by poor χ^2 fit in both the seasons (Table 42).

4.4 Molecular mapping of fertility restorer genes of A_1 and A_4 CMS systems

The identification of molecular markers tightly linked to fertility restoration loci in pearl millet would permit the classification of lines as either maintainers (B-lines) or restorers (R-lines) without the need for field evaluation of test crosses. A molecular marker system would be helpful in identification of fertility restorer gene(s) and the subsequent

Table 39. Detection of linkage between fertility restorer genes of A_1 and A_4 CMS system in (81B × IPC 804) F_2 testcross population

Category	Joint segregation ratio	Expected No	No of testcrosses	
			Summer 2003	Rainy 2003
F on both A_1 and A_4	1	25	1	1
F on A_1 and S on A_4	1	25	28	30
F on A_1 and F/S on A_4	2	50	53	60
S on A_1 and F on A_4	1	25	1	1
S on both A_1 and A_4	1	25	27	34
S on A_1 and F/S on A_4	2	50	53	45
F/S on A_1 and F on A_4	2	50	3	3
F/S on A_1 and S on A_4	2	50	107	100
F/S on both A_1 and A_4	4	99	124	123
Total	16	397	397	397

Observed F_2 testcross ratio:

A_1 CMS system: 1F:2 (F+S):1S

A_4 CMS system: 1F:2 (F+S):1S

Joint segregation Chi square calculated value: 163.1** 153.4*

Table 40. Detection of linkage between fertility restorer genes of A_1 and A_4 CMS system in (81B × IPC 804) BC_1 testcross population

Category	Joint segregation ratio	Expected No	No of testcrosses	
			Summer 2003	Rainy 2003
S on both A_1 and A_4	1	37	78	79
S on A_1 and F/S on A_4	1	37	6	5
F/S on A_1 and S on A_4	1	37	60	59
F/S on both A_1 and A_4	1	37	2	3
Total	4	146	146	146

Observed BC_1 testcross ratio:

A_1 CMS system: 1 (F+S):1S

A_4 CMS system: 1 (F+S):1S

Joint segregation Chi square calculated value: 120.4** 121.3*

** Chi square value tested at $P = 0.01$

Table 41. Detection of linkage between fertility restorer genes of A_1 and A_4 CMS system in (5054B × IPC 511) F_2 testcross population

Category	Joint segregation ratio	Expected No	No of testcrosses	
			Summer 2003	Rainy 2003
F on both A_1 and A_4	7	43	59	74
F on A_1 and S on A_4	7	43	3	0
F on A_1 and F/S on A_4	14	86	25	4
S on A_1 and F on A_4	1	6	0	0
S on both A_1 and A_4	1	6	54	56
S on A_1 and F/S on A_4	2	12	11	16
F/S on A_1 and F on A_4	8	49	23	8
F/S on A_1 and S on A_4	8	49	26	25
F/S on both A_1 and A_4	16	98	192	210
Total	64	393	393	393

Observed F_2 testcross ratio:

A_1 CMS system: 7F:8 (F+S):1S

A_4 CMS system: 1F:2 (F+S):1S

Joint segregation Chi square calculated value: 579.9** 729.0**

Table 42. Detection of linkage between fertility restorer genes of A_1 and A_4 CMS system in (5054B × IPC 511) BC_1 testcross population

Category	Joint segregation ratio	Expected No	No of testcrosses	
			Summer 2003	Rainy 2003
S on both A_1 and A_4	1	17	39	48
S on A_1 and F/S on A_4	1	17	5	7
F/S on A_1 and S on A_4	3	52	17	8
F/S on both A_1 and A_4	3	52	77	75
Total	8	138	138	138

Observed BC_1 testcross ratio:

A_1 CMS system: 3 (F+S):1S

A_4 CMS system: 1 (F+S):1S

Joint segregation Chi square calculated value: 71.8** 108.3*

** Chi square value tested at $P = 0.01$

selection of lines having these gene(s) using marker-assisted selection. For mapping the fertility restorer gene(s) of A_1 and A_4 CMS systems, part of the material produced for the linkage studies (section 3.3.3), *i.e.* the (81B × IPC 804) F_2 population, was used as a mapping population. The results are presented for parental polymorphism, segregation distortion of markers, linkage map construction, QTL identification for A_1 and A_4 CMS systems and map-free methods for markers linked to the fertility restorer genesof the A_1 and A_4 CMS systems.

4.4.1 Parental polymorphism

The parental lines 81B and IPC 804 were screened for detecting polymorphic markers using SSR primer pairs, RFLP probe-enzyme combinations and three morphological markers. The parents were differentiating with respect to each other for plant height (dwarf, d_2 vs. non-dwarf, D_2) (Burton and Fortson, 1966), panicle bristling (long bristled, *Br* vs. non-bristled, *NBr*) (Ahluwalia and Shankar, 1964; Gill *et al.*, 1971) and leaf pubescence or hairiness (hairy leaf, *hl* vs. non-hairy leaf, *Hl*) (Gill *et al.*, 1971). A total of 70 SSR markers and 40 RFLP probes (160 probe-enzyme combinations from 40 probes × 4 restriction enzymes) were used to detect polymorphic markers. Of the 70 SSR markers, 32 detected polymorphism between the parents but when tested in combination with the F_1 and assessed for ease of scoring, 24 SSR markers were selected for genotyping the mapping population. Similarly, 11 RFLP probe-enzyme combinations were found to detect clear polymorphism with the parents and F_1. The level of polymorphism for SSR markers was 34% and for RFLP probes 28%. Most of these markers had been mapped previously by Liu *et al.* (1994) and Qi *et al.* (2004). A total of 38 polymorphic markers that included 24 SSR, 11 RFLP and 3 morphological markers (Table 43), were used to genotype the F_2 mapping population consisting of 397 individuals.

4.4.2 Goodness of fit of markers and segregation distortion

The goodness of fit of the markers used in the study was calculated by Chi square analysis as implemented in the program JoinMap 3.0 (van Ooijen and Voorrips, 2001). The observed segregation pattern of the marker loci was compared with the expected 1:2:1 (A:H:B) ratio for co-dominant markers and 1:3 (A:C or B:D) for dominant markers. The calculated χ^2 values for each of the 38 marker loci (24 SSR, 11 RFLP and 3 morphological markers) are given in Table 43. Segregation of ten out of the 38 markers showed significant deviation at $P = 0.01$ from the expected ratios of 1:2:1 (A:H:B) for the co-dominant markers or 1:3 (A:C or B:D) for the dominant markers.

Table 43. Goodness of fit of 38 pearl millet markers used for molecular mapping of fertility restorer genes in pearl millet

Marker		Band type									
Locus	Type[a]	A	H	B	C	D	Missing	χ^2	d.f.	Classes	Remarks
Linkage group 1 (length= 28.6 cM)											
Xpsm2273[b]	S	108	200	87	0	0	2	2.30	2	A:H:B	single-copy
Xpsm858	R	93	201	101	1	1	0	0.50	2	A:H:B	single-copy
Xpsm761	R	93	203	101	0	0	0	0.50	2	A:H:B	single-copy
Xpsm17	R	95	211	90	0	0	1	1.90	2	A:H:B	single-copy
Xpsmp2080	S	88	211	86	0	0	12	4.20	2	A:H:B	single-copy
Xpsm223	R	70	202	87	5	11	22	7.30	2	A:H:B	multi-copy
Br	M	66	0	0	331	0	0	14.41**	1	A:C	single-copy
Linkage group 2a (length= 88.3 cM)											
Xpsm708.1	R	117	181	86	0	2	11	6.30	2	A:H:B	multi-copy
Xpsmp2072	S	137	0	0	259	0	1	18.94**	1	A:C	single-copy
Xpsmp2077	S	137	246	12	0	1	1	102.90**	2	A:H:B	single-copy
Linkage group 2b (length= 77.4 cM)											
Xpsmp2059	S	0	0	151	0	242	4	37.05**	1	B:D	single-copy
Xpsmp2237	S	0	0	153	0	237	7	41.37**	1	B:D	single-copy
Linkage group 3 (length= 89.1 cM)											
Xpsmp2068	S	98	201	94	0	0	4	0.30	2	A:H:B	single-copy
Xpsmp2070	S	74	212	96	0	0	15	7.20	2	A:H:B	single-copy
Linkage group 4 (length= 140.3 cM)											
Xpsm409.1	R	48	203	139	2	3	2	43.10**	2	A:H:B	multi-copy
Xpsmp2225	S	60	216	111	1	8	1	18.70**	2	A:H:B	single-copy
Xpsm306	R	40	169	73	0	1	114	18.80**	2	A:H:B	single-copy
d_2	M	62	231	104	0	0	0	19.50**	2	A:H:B	single-copy
Xpsm837.2	R	75	210	108	1	0	3	7.40	2	A:H:B	multi-copy
Xpsmp2086	S	96	197	104	0	0	0	0.40	2	A:H:B	single-copy
Xpsmp2008[b]	S	0	0	95	0	295	12	0.00	1	B:D	single-copy
Linkage group 5 (length= 20.5 cM)											
Xpsmp2202	S	98	204	95	0	0	0	0.40	2	A:H:B	single-copy
Xpsmp2220	S	96	214	87	0	0	0	2.80	2	A:H:B	single-copy
Xpsmp2001	S	98	216	81	0	0	2	4.90	2	A:H:B	single-copy
Xpsmp2078	S	93	215	85	1	0	3	3.80	2	A:H:B	single-copy

(Contd...)

Table 43. Goodness of fit of 38 pearl millet marker loci used for molecular mapping of fertility restorer genes in pearl millet (contd...)

Marker		Band type									
Locus	Type[a]	A	H	B	C	D	Missing	χ^2	d.f.	Classes	Remarks
Linkage group 6 (length= 56.1 cM)											
*Xpsmp*2048	S	84	192	100	1	1	19	1.50	2	A:H:B	single-copy
*Xpsm*202	R	102	179	95	2	1	18	1.10	2	A:H:B	single-copy
*Xpsm*696	R	99	201	95	1	1	0	0.20	2	A:H:B	single-copy
*Xpsmp*2270	S	96	195	95	0	2	9	0.10	2	A:H:B	single-copy
hl	M	95	0	0	302	0	0	0.19	1	A:C	single-copy
*Xicmp*3022	S	69	127	53	1	7	140	2.20	2	A:H:B	single-copy
*Xpsmp*2018	S	86	215	93	1	1	1	3.50	2	A:H:B	single-copy
Linkage group 7 (length= 208.5 cM)											
*Xpsmp*2013	S	101	207	89	0	0	0	1.50	2	A:H:B	single-copy
*Xpsmp*2074	S	99	218	78	0	0	2	6.50	2	A:H:B	single-copy
*Xpsmp*2263	S	97	216	73	0	0	11	8.50	2	A:H:B	single-copy
*Xpsm*330.2	R	50	196	74	0	73	4	19.80**	2	A:H:B	multi-copy
*Xpsmp*2203	S	101	220	75	0	0	1	8.30	2	A:H:B	single-copy
*Xpsmp*2027	S	76	208	103	0	0	10	5.90	2	A:H:B	single-copy

[a] R= RFLP, S= SSR and M= morphological marker

[b] unmapped in the present study

d.f.: degree of freedom

**Markers exhibiting segregation distortion at a Chi square calculated value (P= 0.01)

A = homozygote for allele 'a' from parent P_1 (81B)

B = homozygote for allele 'b' from parent P_2 (IPC 804)

H = heterozygote carrying alleles from both P_1 and P_2 i.e. F_1

C = not a homozygote for allele 'a' (i.e. either B or H)

D = not a homozygote for allele 'b' (i.e. either A or H)

Five SSR (*Xpsmp*2072, *Xpsmp*2077, *Xpsmp*2059, *Xpsmp*2237, *Xpsmp*2225) three RFLP (*Xpsm*409.1, *Xpsm*306, *Xpsm*330.2) and two morphological markers (*Br* and d_2) showed segregation distortion. Most of the distorted markers were placed on linkage group 2 (LG 2), LG 4 and LG 7. All marker loci on LG 2 except *Xpsm*708.1 displayed significant segregation distortion at $P = 0.01$ whereas four out of seven loci on LG 4 exhibited significant distortion. On LG 7, *Xpsm*330.2 exhibited segregation distortion as revealed by a significant χ^2 at $P = 0.01$ level. Two marker loci had large number of missing data points viz., *Xpsm*306 had 114 missing values and *Xicmp*3022 had 140 missing values. Figures 4-9 illustrate SSR PAGE and RFLP autoradiograms of some selected marker loci and Figure 10 displays location of marker loci on linkage groups.

4.4.3 Genetic linkage map construction

Using a total of 36 marker loci (all except *Xpsmp*2273 and *Xpsmp*2008, which were not significantly linked to other marker loci in this study) a genetic linkage map of 708.8 cM length (Haldane) was constructed for the pearl millet F_2 mapping population based on cross 81B × IPC 804 (Figure 10). The pearl millet consensus map given by Qi *et al.* (2004) was used as a reference map for assigning linkage groups and confirming marker order. A Mapmaker/Exp version 3.0 (Lincoln *et al.*, 1992a) multipoint analysis was used to construct the linkage map using a log likelihood (LOD) threshold value of 2.0 and recombination fraction of 0.5. The markers were placed on linkage groups based on 'group', 'sequence' and 'map' commands of Mapmaker program. Unlinked markers were then placed in appropriate linkage groups using the 'build' command. Markers with satisfactory orders and fewer candidate errors and higher LOD values were then assigned and fixed to each linkage group using the 'anchor' and the 'framework' commands.

4.4.3.1 Linkage groups

The map length of individual linkage groups varied from a minimum of 20.5 cM (LG 5) to a maximum of 208.5 cM (LG 7), as shown in Figure 10. Linkage group 6 (LG 6) had a maximum of seven marker loci followed by six marker loci on LG 1, LG 4 and LG 7, five marker loci on LG 2 (three on LG 2a and two on LG 2b), four on LG 5 and a minimum of two marker loci on LG 3. All the markers were placed in their previously assigned positions on the linkage groups according to the pearl millet consensus map (Qi *et al.*, 2004) except *Xpsmp*2225, which was placed on LG 4 in this study between *Xpsm*409.1 and *Xpsm*306 instead of on LG 2. In the final map, two markers, *Xpsmp*2273 and *Xpsmp*2008 remain unmapped. Three markers that had remained unmapped till now

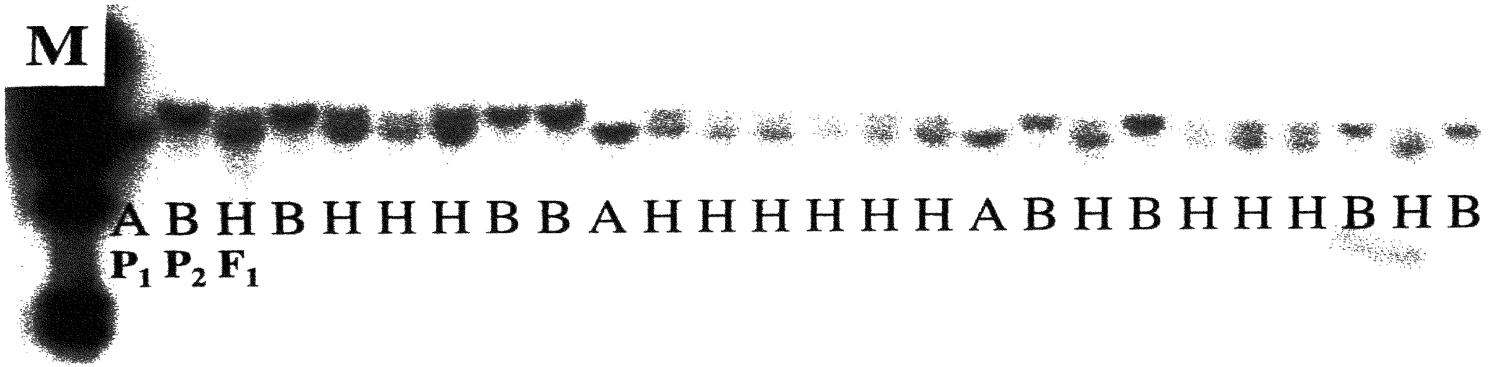

Figure 4. Autoradiogram showing banding pattern of parents, F_1 and F_2 segregating progenies based on cross 81B × IPC 804 with RFLP locus *Xpsm*17/*Dra*I

Figure 5. Autoradiogram showing banding pattern of F_2 segregating progenies based on cross 81B × IPC 804 with RFLP locus *Xpsm*17/*Dra*I

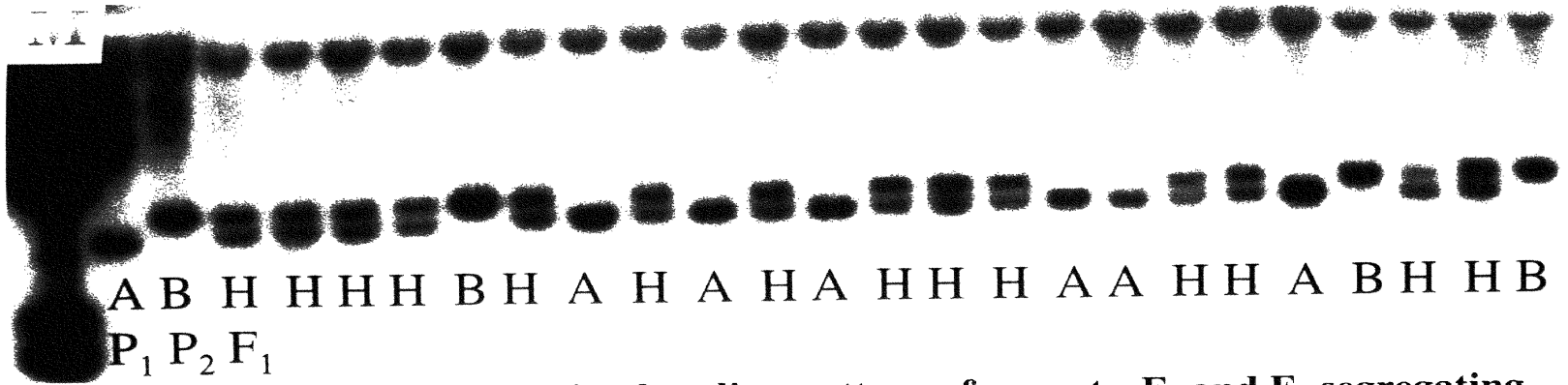

Figure 6. Autoradiogram showing banding pattern of parents, F_1 and F_2 segregating progenies based on cross 81B × IPC 804 with RFLP locus *Xpsm*708/*Eco*I

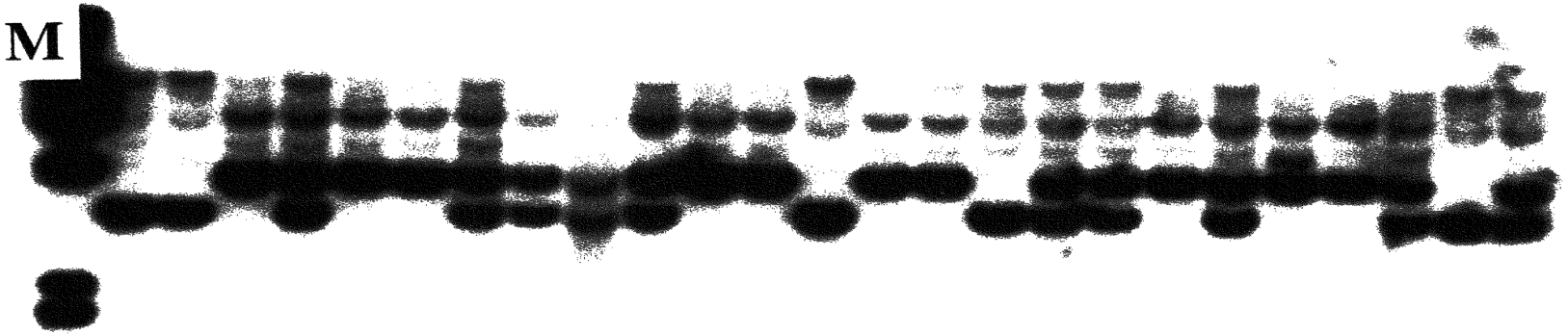

Figure 7. Autoradiogram showing banding pattern of F_2 segregating progenies based on cross 81B × IPC 804 with RFLP locus *Xpsm*409/*Hind*III

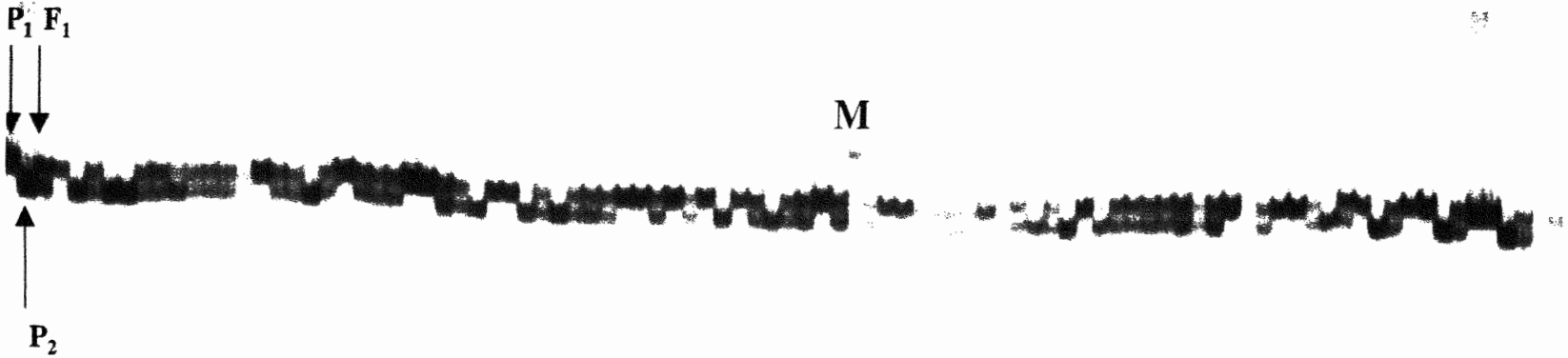

Figure 8. PAGE gel showing banding pattern of parents, F_1 and F_2 segregating progenies of the cross 81B × IPC 804 from SSR locus *Xpsmp*2080

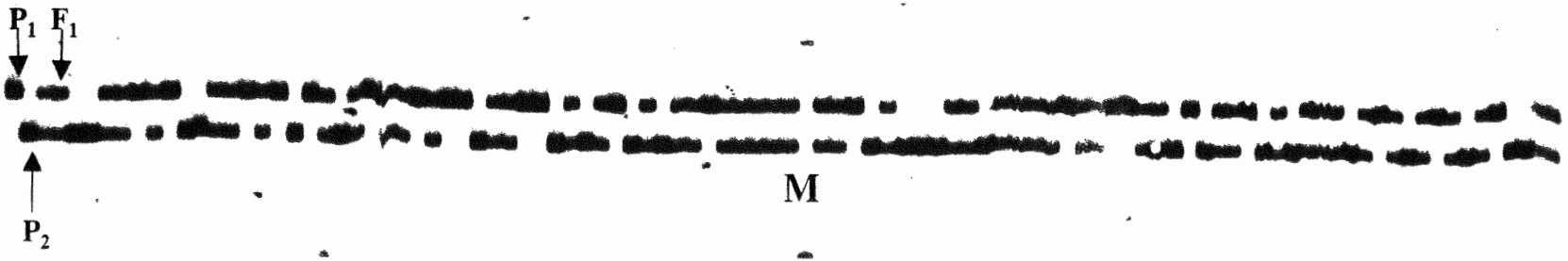

Figure 9. PAGE gel showing banding pattern of parents, F_1 and F_2 segregating progenies of the cross 81B × IPC 804 from SSR locus *Xpsmp*2202

Figure 10. Genetic linkage map and location of QTLs for fertility restorer genes of A_1 and A_4 CMS systems of pearl millet cross 81B × IPC 804

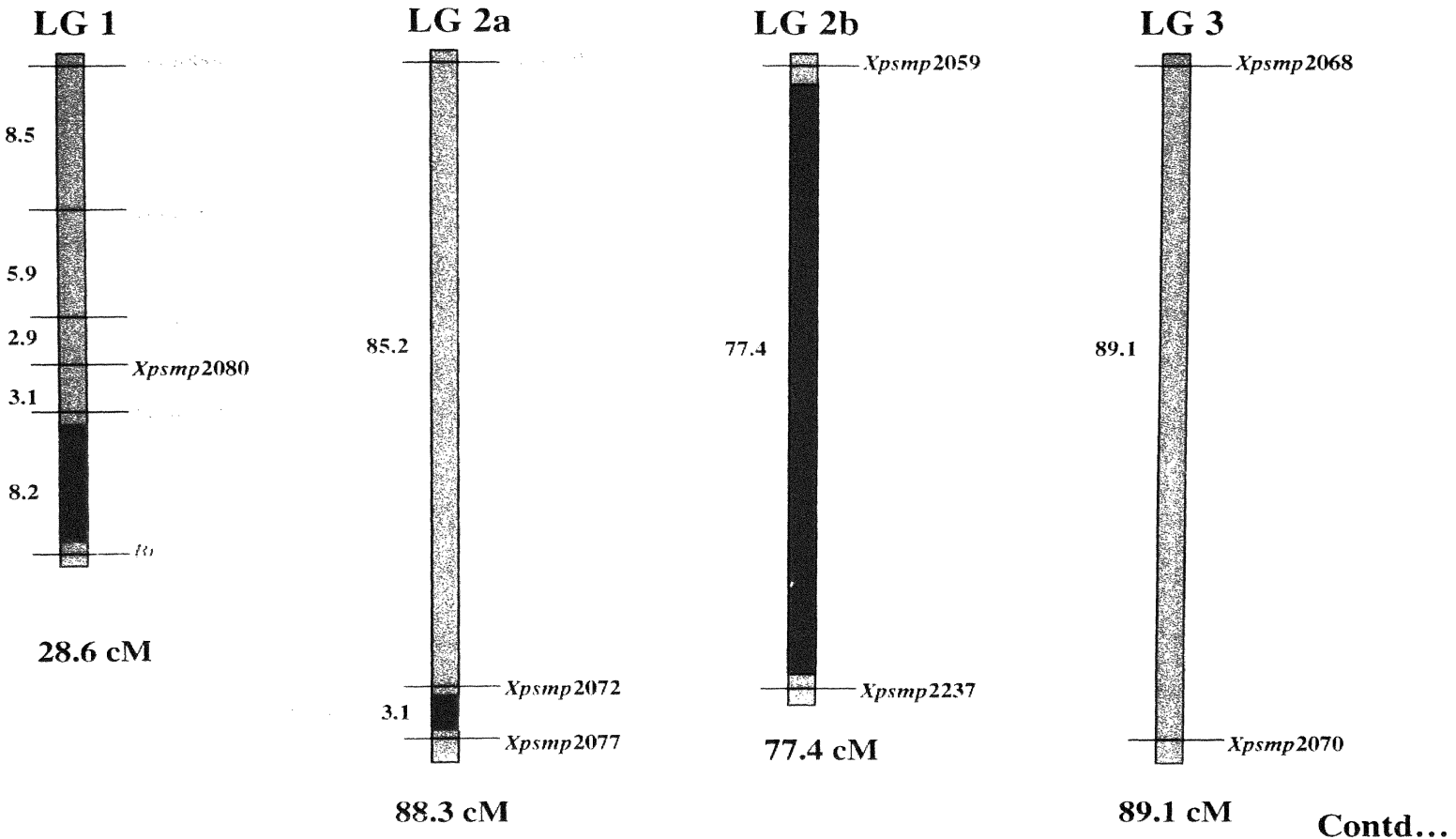

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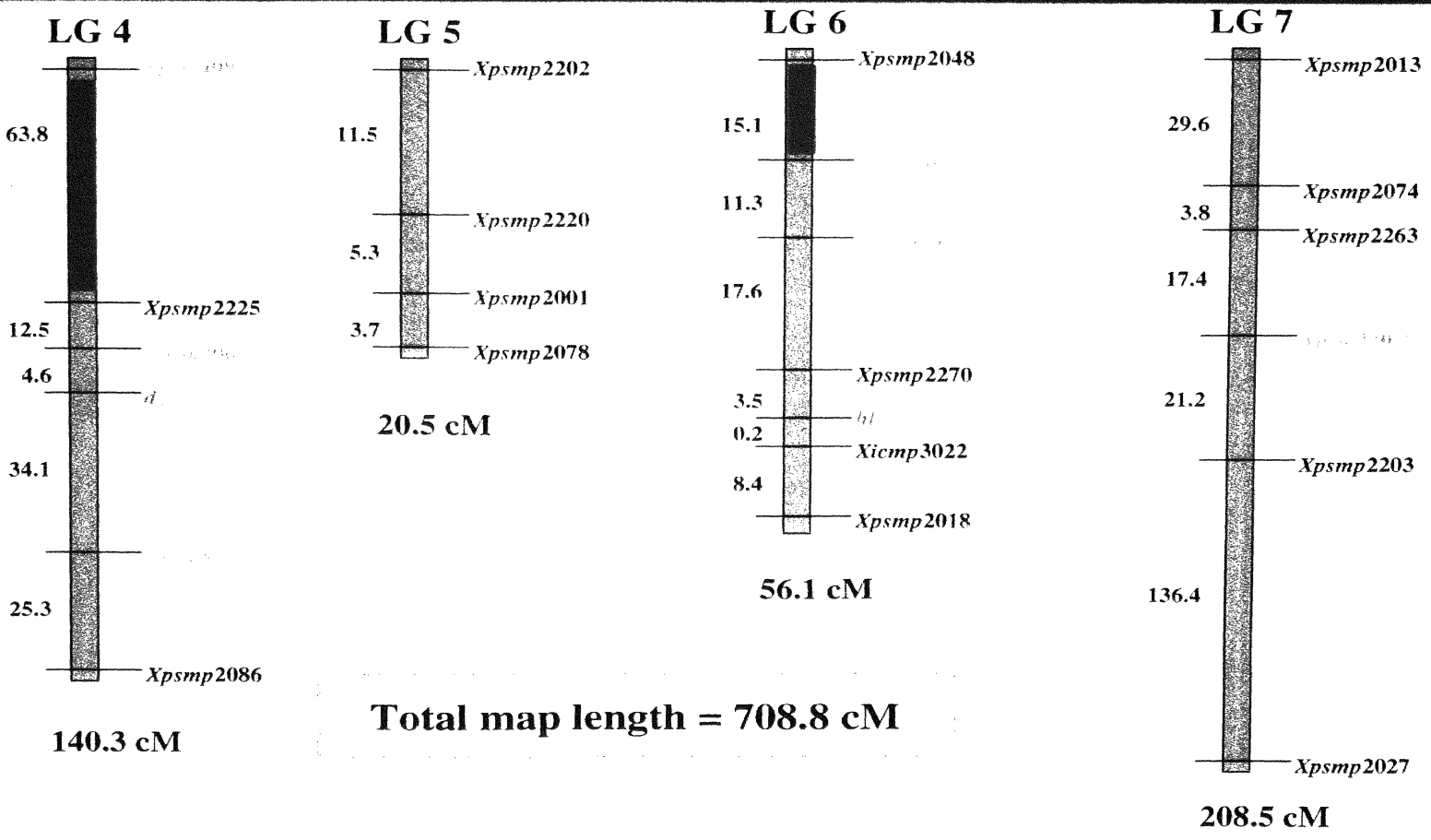
Figure 10. Genetic linkage map and location of QTLs for fertility restorer genes of A_1 and A_4 CMS systems of pearl millet cross 81B × IPC 804



viz., *Xpsmp*2080, *Xpsmp*2068 and *Xicmp*3022 were mapped to LG 1, LG 3 and LG 6, respectively, in this mapping population. The average distance between markers comes out to 19.7 cM. The LG 2 was divided into two sub-groups: LG 2a and LG 2b. LG 2a consisted of three marker loci and LG 2b was comprised of two markers. All markers on LG 2 except *Xpsm*708.1 exhibited segregation distortion. Linkage groups 5 and 6 were comprised of all distortion-free markers whereas LG 2 and LG 4 each had four distorted markers.

4.4.4 QTL mapping for fertility restoration

Complete data (*i.e.* fertility/sterility reaction of testcrosses and molecular data) was available for 397 F_2 individuals. Together with the linkage map based on the complete set of 397 F_2 plants, these data were analyzed using MAPMAKER/QTL version 1.1b (Lincoln *et al.*, 1992b) and PLABQTL version 1.1 (Utz and Melchinger, 2000). The phenotypic distribution of the mapping population has been depicted in Figures 11 and 12. Two types of interval mapping analysis were done: simple interval mapping (Lander and Botstein, 1989) as implemented in MAPMAKER/QTL and composite interval mapping (Zeng, 1993, 1994) as implemented in PLABQTL.

Simple interval mapping was performed in MAPMAKER/QTL with a LOD score of 2.0 as the threshold value for detecting significant QTL (Table 44). As the phenotyping was done in testcrosses produced from individual F_2 plants, an 'additive' model was used as required for a testcross phenotyping population. Based on estimated single QTL map positions, combined effects of two or more QTL were calculated for two-QTL and three-QTL models. The criteria chosen for accepting a multiple-QTL model was a LOD score of at least 2.0 units more than the highest LOD score of the best model having one less QTL:

$$LOD_n \geq LOD_{(n-1)} + 2.0$$

where,

LOD_n = minimum qualifying LOD score for acceptance in a multiple-QTL model with 'n' QTL

$LOD_{(n-1)}$ = maximum LOD score for any observed model with (n-1) QTL

Composite interval mapping as implemented in PLABQTL was used with a threshold likelihood ratio of 2.0 and the model meant for 'testcross case (RALPH= 2)' (Table 45). The LOD and % phenotypic variance values were taken from the 'final simultaneous fit' model in the PLABQTL. The mapping population in the present study was developed

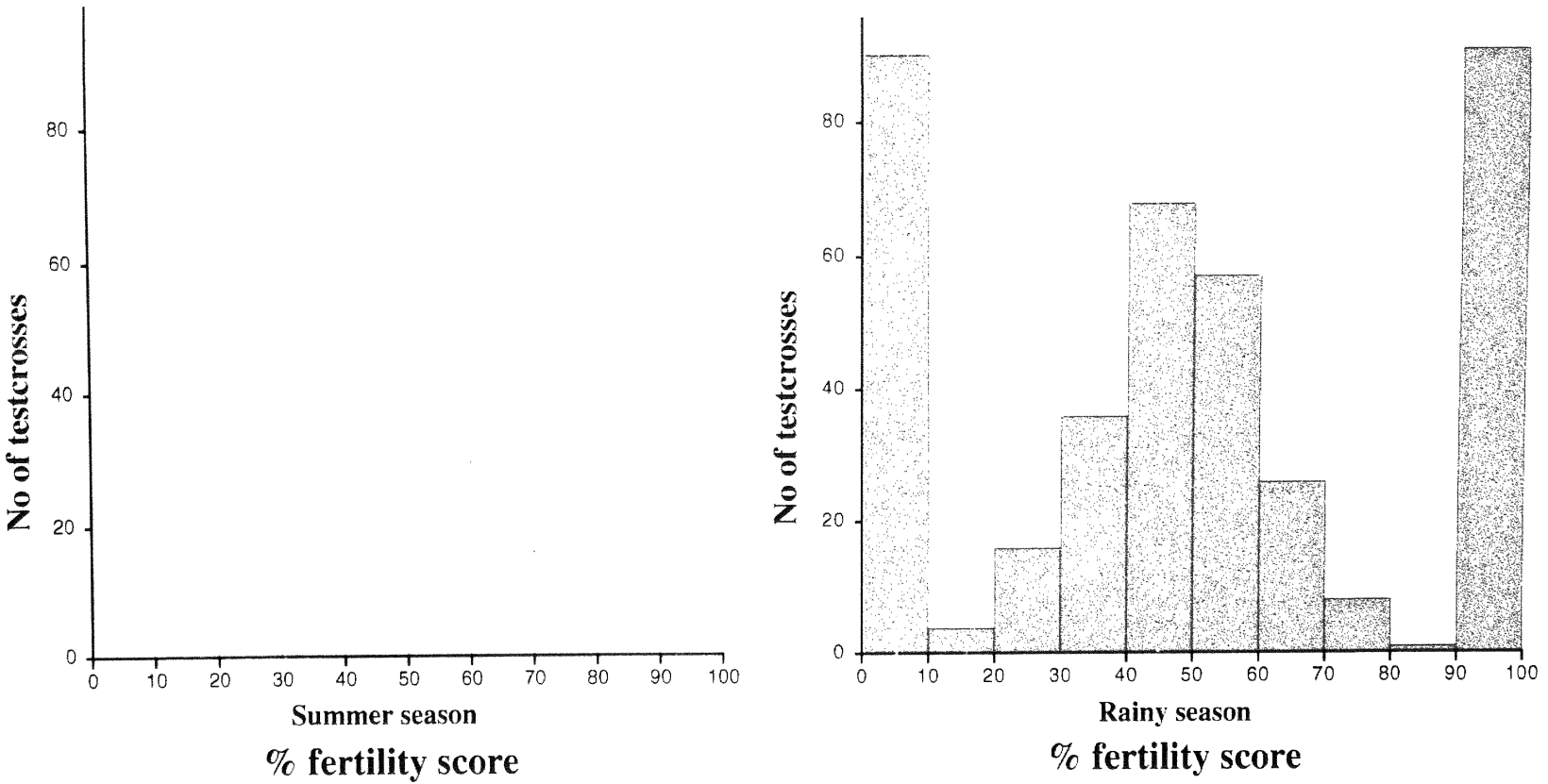


Figure 11. Fertility restoration segregation pattern of (81B × IPC 804)-derived F_2 testcrosses on CMS line $81A_1$ based on % fertility data in summer and rainy season 2003

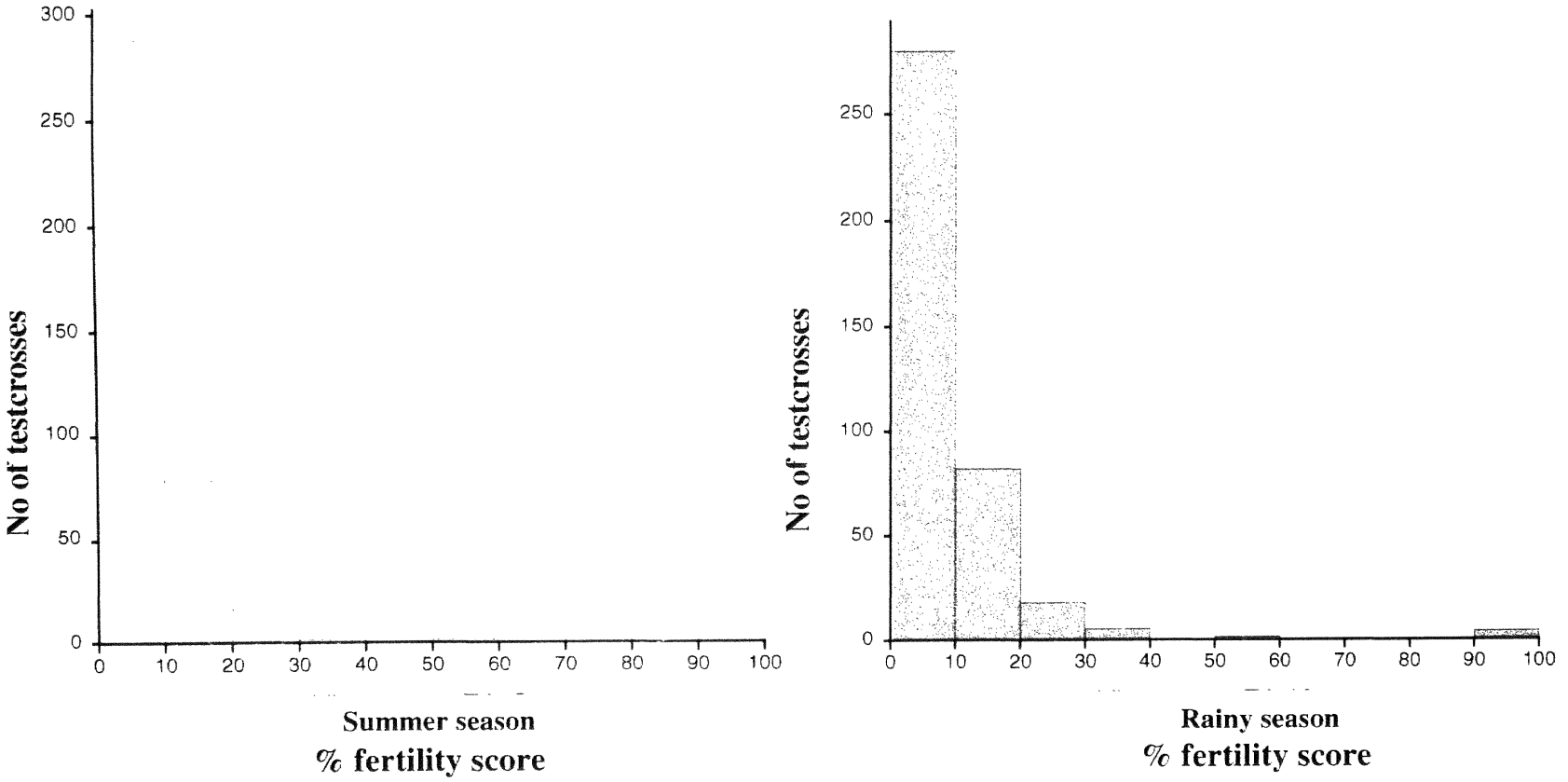


Figure 12. Fertility restoration segregation pattern of (81B × IPC 804)-derived F_2 testcrosses on CMS line $81A_4$ based on % fertility data in summer and rainy season 2003

with the twin objectives to map fertility restoration in A_1 and A_4 CMS systems. The results are, therefore, presented for the two CMS systems.

Table 44. QTL associated with fertility restoration of A_1 and A_4 CMS systems in pearl millet in summer and rainy seasons 2003 at ICRISAT- Patancheru, detected using MAPMAKER/QTL

CMS system	Season	Marker interval	LG	QTL position[a]	QTL length	LOD	Phenotypic variance explained (%)
A_1	Summer	*Xpsm*223-*Br*	1	4	8.2	23.88	29.9
		*Xpsm*409.1-*Xpsmp*2225	4	44	63.8	2.03	5.1
	Rainy	*Xpsmp*223-*Br*	1	4	8.2	26.34	33.1
A_4	Summer	*Xpsmp*2072-*Xpsmp*2077	2a	2	3.1	25.71	30
Two-QTL model		*Xpsmp*2072-*Xpsmp*2077	2a	0.9	3.1		
		*Xpsmp*2059-*Xpsmp*2237	2b	76	77.4	**36.1**	**56.1**
	Rainy	*Xpsmp*2072-*Xpsmp*2077	2a	2	3.1	28.74	30.2
		*Xpsmp*2059-*Xpsmp*2237	2b	64	77.4	3.35	11.4
Two-QTL model		*Xpsmp*2072-*Xpsmp*2077	2a	1.7	3.1		
		*Xpsmp*2059-*Xpsmp*2237	2b	76	77.4	**34.08**	**41.2**

[a] Distance (Haldane cM) from the marker on the left side of the interval
LG: Linkage group
LOD: log10 of the likelihood odds ratio

4.4.4.1 QTL for fertility restoration in A_1 CMS system

Two QTL for fertility restoration in the A_1 system were identified by the SIM approach in MAPMAKER/QTL in the summer season (Table 44, Figure 10). Of the two QTL, one was a major QTL located at a distance of 4 cM from the marker *Xpsm*223 at the bottom of LG 1 and flanked by the RFLP marker loci *Xpsm*223 and the morphological marker *Br* (Bristling). This major QTL was detected with a high log-likelihood or LOD value of 23.88 and accounting for 29.9% of the observed phenotypic variation. In the rainy season, only the major QTL, flanked by marker loci *Xpsm*223 and *Br*, could be detected but with a higher LOD score of 26.34 and observed phenotypic variation of 33.1% as compared to the summer season. The minor QTL in the summer season was located on the LG 4 between the marker loci *Xpsm*409.1 and *Xpsmp*2225 with a LOD value of 2.03 and explaining only 5.1% of the observed phenotypic variation. The putative major QTL on LG 1 is designated as *Rf*1a and the minor QTL on LG 4 as *Rf*1b (Figure 10).

Table 45. QTLs associated with fertility restoration of A_1 and A_4 CMS systems in pearl millet during summer and rainy seasons 2003 at ICRISAT- Patancheru detected using PLABQTL

CMS system	Season	Marker interval	LG	Position	LOD	R^2%	Additive effect
1. Simple Interval Mapping							
A_1	Summer	*Xpsmp*2080-*Xpsm*223	1	18	12.51	13.50	1.96**
		Final Simultaneous Fit			12.51	13.10	
	Rainy	*Xpsmp*2080-*Xpsm*223	1	20	10.55	11.50	1.81**
		Final Simultaneous Fit			10.55	11.10	
A_4	Summer	*Xpsmp*2072-*Xpsmp*2077	2a	88	83.93	62.30	5.94**
		*Xpsmp*2059-*Xpsmp*2237	2b	76	35.47	34.50	-3.67**
		Final Simultaneous Fit			87.94	63.60	
	Rainy	*Xpsmp*2072-*Xpsmp*2077	2a	88	78.79	60.00	5.85**
		*Xpsmp*2059-*Xpsmp*2237	2b	76	33.37	32.90	-3.60**
		*Xpsm*409.1-*Xpsmp*2225	4	62	5.21	5.90	1.54**
		*Xpsm*202-*Xpsm*696	6	16	2.71	3.10	-0.99
		Final Simultaneous Fit			84.64	61.80	
2. Composite Interval Mapping							
A_1	Summer	2 co-factors: *Br*, *Xpsm*708.1					
		*Xpsm*223-*Br*	1	22	11.47	12.50	1.99**
		Final Simultaneous Fit			11.78	12.30	
	Rainy	3 co-factors: *Br*, *Xpsm*708.1, *Xpsm*330.2					
		*Xpsm*223-*Br*	1	24	9.16	10.10	1.84**
		Final Simultaneous Fit			9.53	10.00	
A_4	Summer	2 cofactors: *Xpsmp*2077, *Xpsmp*2237					
		*Xpsmp*2072-*Xpsmp*2077	2a	88	36.55	51.30	4.87**
		*Xpsmp*2059-*Xpsmp*2237	2b	68	4.40	5.10	-1.28**
		Final Simultaneous Fit			87.99	63.60	
	Rainy	5 cofactors: *Xpsmp*2077, *Xpsmp*2237, *Xpsmp*2225, d_2, *Xpsmp*2048					
		*Xpsmp*2072-*Xpsmp*2077	2a	88	33.34	48.10	4.60**
		*Xpsmp*2059-*Xpsmp*2237	2b	76	3.17	3.70	-0.95**
		*Xpsmp*2225-*Xpsm*306	4	72	2.18	4.20	1.55
		*Xpsmp*2048-*Xpsm*202	6	0	2.12	4.30	-0.71*
		Final Simultaneous Fit			84.28	61.60	

LG: Linkage group

Position: Position of the QTL on the LG, in cM

LOD: log10 of the likelihood odds ratio

R^2%: Coefficient of determination; the percentage of the phenotypic variance explained by the putative QTL

The CIM approach in the program PLABQTL also detected one QTL on LG 1 and flanked by marker loci *Xpsm*223 and *Br* in both summer and rainy seasons. In the summer season, this QTL was located 2 cM from the marker locus *Xpsm*223 and accounted for 12.3% of observed phenotypic variation with a LOD value of 11.78 and a significant additive effect of 1.99 (Table 45). In the rainy season, the position of this QTL was 4 cM from *Xpsm*223 and exhibited a slightely lower contribution to phenotypic variation (10.0%) compared to summer season with a LOD score of 9.53 and a significant additive effect of 1.84.

4.4.4.2 QTL for fertility restoration in A_4 CMS system

The SIM analysis in MAPMAKER/QTL revealed a single genomic region of large effect on LG 2a, explaining 30% of the observed phenotypic variance for fertility restoration in the A_4 CMS system in the summer season (Table 44, Figure 10). This putative major QTL is designated as *Rf*4a. The LOD score for this QTL was 25.7, well above the threshold of 2.00. This QTL with a length of 3.1 cM is flanked by the marker loci *Xpsmp*2072 and *Xpsmp*2077. The best two-QTL model explaining 56.1% of the phenotypic variance with a high LOD score of 36.1 (>25.7+2.0) was accepted as better than the single-QTL model. The two-QTL model included the single QTL identified earlier and a second minor QTL on LG 2b flanked by marker loci *Xpsmp*2059 and *Xpsmp*2237. The putative minor QTL is designated as *Rf*4b.

In the rainy season, the same two QTL were detected as in summer season. The major QTL, *Rf*4a, accounted for 30.2% of phenotypic variance with a LOD score of 28.7. The minor QTL, *Rf*4b, with a LOD value of 3.3 accounts for 11.4% of the observed phenotypic variation. The best two-QTL model explaining 41.2% of the phenotypic variance with a high LOD score of 34.1 (>28.7+2.0) was accepted as better than the single-QTL model.

The CIM approach in the program PLABQTL (Table 45) detected the same QTL for A_4 CMS fertility restoration as detected by the SIM analysis in the summer and rainy seasons. In the summer season, the major QTL *Rf*4a, explains 51.3% of phenotypic variance with a LOD score of 36.55 and significant additive effect of 4.87 and is flanked by marker loci *Xpsmp*2072 and *Xpsmp*2077. In the rainy season, the major QTL *Rf*4a, explains 48.1% of phenotypic variance with a LOD score of 33.3 and significant additive effect of 4.60 and is flanked by marker loci *Xpsmp*2072 and *Xpsmp*2077. The minor QTL in the summer season is located on LG 2b between *Xpsmp*2059 and

*Xpsmp*2237, accounts for 5.1% of observed phenotypic variance and has a significant additive effect of -1.28. The LOD score for this QTL was 4.4. In the rainy season, this minor QTL accounts for 3.7% of observed phenotypic variance with an additive effect of -0.95. Two additional minor QTL were detected in the rainy season. One minor QTL was located on LG 4 between the marker loci *Xpsmp*2225 and *Xpsm*306. This QTL accounted for 4.2% of the observed phenotypic variance, and had a LOD score of 2.18 but a non-significant additive effect of 1.55. A fourth QTL detected in this season had a small but significant additive effect of –0.71, explained 4.13% of the phenotypic variance, and had a LOD score of 2.12. This QTL is designated as *Rf*4c. This QTL is flanked by the marker loci *Xpsmp*2048 and *Xpsm*202. The final simultaneous fit model for the two QTL in the summer season gave a combined LOD value of 87.8 and explained 63.6% of the total phenotypic variance. The final simultaneous fit model for the four QTL in rainy season gave a combined LOD value of 84.3 and explained 61.6% of the total phenotypic variance for the A_4 CMS fertility restoration.

4.4.5 Marker-trait association analysis

Three map-free approaches viz., single-marker approach (SMA) using simple linear regression, and two multiple marker approaches, namely step-wise regression (SWR) and Bayesian information criterion (BIC) were used to identify markers linked to putative QTL for fertility restoration in A_1 and A_4 CMS systems (Table 46). For these analyses, only the 26 distortion-free markers were included out of the total 38 markers for which genotypic data were generated. The genotypic data for these three approaches was converted into numeric codes from the alphabetical codes with 'A'= 0, 'H'= 1, 'B'= 2, 'C', 'D' or '—' to * (see sections 3.6.4 and 3.7.6 in the Chapter 3)

4.4.5.1 A_1 CMS system

Using summer season phenotypic data, SMA identified a total of seven markers linked to fertility restoration using simple linear regression with an F-probability threshold value of 10% (Table 46). Of the seven markers, five were located on LG 1 and exhibited significant linear regression coefficient values for fertility restoration of A_1 CMS system. The marker *Xpsm*223 explained the largest phenotypic contribution (R_a^2) with a value of 23.27 followed by *Xpsmp*2080 (19.39), *Xpsm*17 (17.36), *Xpsm*761 (12.86) and *Xpsm*858 (10.32). The remaining two markers viz., *Xpsmp*2068 and *Xpsmp*2070 showed non-significant linear regression coefficient values with fertility restoration and

Table 46. Markers linked to fertility restoration in A_1 and A_4 CMS system identified by different map free approaches

Season/Statistic	Marker	*Xpsm* 858	*Xpsm* 761	*Xpsm* 17	*Xpsmp* 2080	*Xpsm* 223	*Xpsmp* 2068	*Xpsmp* 2070	*Xpsmp* 2202	*Xpsmp* 2078	*Xpsmp* 2001	*Xpsmp* 2048	*Xpsmp* 2018	*Xpsmp* 3022	*Xpsm* 837.2	R_a^2 (%)	BIC
		+	+	+	+	+	++	++	+++	+++	+++	++++	++++	++++			
A_1 CMS system																	
Summer season																	
SMA	ß	16.46***	18.33***	21.85***	23.69***	26.08***	-4.81	-5.11									
	R_a^2 (%)	10.32	12.86	17.36	19.39	23.27	0.65	0.66									
SWR	ß					25.50*	-4.31									23.30	
BIC	ß					26.08*										22.46	326
Rainy season																	
SMA	ß	16.62***	18.01***	21.41***	23.88***	26.72***			-4.74						5.37		
	R_a^2 (%)	10.78	12.76	17.13	20.34	25.17			0.64						0.83		
SWR	ß					26.72*										25.20	
BIC	ß					26.72*										24.80	324
A_4 CMS system																	
Summer season																	
SMA	ß									-2.12	-1.90						
	R_a^2 (%)									0.70	0.51						
SWR	ß											-3.59	6.77	-6.67		2.30	
BIC	ß											-1.23				4.47	188
Rainy season																	
SMA	ß					-2.27*											
	R_a^2 (%)					0.83											
SWR	ß											-1.18				0.10	
BIC	ß											-1.18				3.56	188

Markers with same number of + signs are linked ($r \leq 0.35$) except 2068/2070 (0.417) and 2048/2018 (0.378); SMA= single marker analysis using simple linear regression; SWR= stepwise regression; BIC= Bayesian information criterion; R_a^2= adjusted coefficient of determination; ß= linear regression coefficient; *P<.05, **P<.01 ***P<.001 (with Bonferroni correction)

negligible R_a^2 values of 0.65 and 0.66, respectively. Using the rainy season phenotypic data set, SMA identified the same five significant markers, located on LG 1, as with the summer season data set. The largest contribution to phenotypic variance was provided by *Xpsm*223 (25.17) followed by *Xpsmp*2080 (20.34), *Xpsm*17 (17.13), *Xpsm*761 (12.76) and *Xpsm*858 (10.78). Two other non-significant markers, *Xpsmp* 2202 (LG 5) and *Xpsm*837.2 (LG 4), were also selected by SMA although with very small contributions to the phenotypic variance.

The step-wise regression analysis using $F_{in}=F_{out}= 4$ as threshold identified only one significant marker, *Xpsm*223 with both summer and rainy season phenotypic data sets with R_a^2 values of 23.30 and 25.20, respectively (Table 47). Using the summer season data set, a non-significant marker *Xpsmp*2068 was also selected by SWR but when the stringency level was increased further up to level 10, only *Xpsm*223 was identified in both the seasons.

The Bayesian information criterion also identified the marker locus *Xpsm*223 in both the summer and rainy seasons with phenotypic variance contributions of 22.46 and 24.80, respectively (Table 46).

4.4.5.2 A_4 CMS system

For the A_4 CMS system, the SMA failed to identify any significant markers contributing substantially to fertility restoration (Table 46). The SWR analysis at the threshold level of $F_{in}=F_{out}= 4$, selected *Xpsmp*2048 in both summer and rainy season but this marker was not selected by SMA. The BIC criterion also selected *Xpsmp*2048 but with a very small R_a^2 value (Table 48).

Table 47. Marker selection based on Forward and Backward stepwise regression for fertility restoration of A_1 CMS systems

Steps	A_1 CMS system			
	Summer season		Rainy season	
	Forward SWR	Backward SWR	Forward SWR	Backward SWR
$F_{in}=F_{out}= 2$	*Xpsm223*, *Xpsmp2273*, *Xpsmp2220*, *Xpsmp2080*, *Xpsmp2086*, *Xpsmp2070*, *Xpsmp2068*	*Xpsmp*2273, *Xpsm*223, *Xpsm*17, *Xpsmp*2068, *Xpsmp*2070, *Xpsmp*2220, *Xpsmp*2086	*Xpsm*223, *Xpsmp*2273, *Xpsmp*2220, *Xpsmp*2068, *Xpsm*837.2	*Xpsm*223, *Xpsmp*2273, *Xpsmp*2001, *Xpsmp*2068, *Xpsm*837.2
$F_{in}=F_{out}= 3$	*Xpsm223*, *Xpsmp2273*, *Xpsmp2068*, *Xpsmp2070*	*Xpsmp2273*, *Xpsm223*, *Xpsmp2068*, *Xpsmp2070*	*Xpsm223*, *Xpsmp2273*	*Xpsmp2273*, *Xpsm223*
$F_{in}=F_{out}= 4$	***Xpsm*223, *Xpsmp*2068**	***Xpsmp*2068, *Xpsm*223**	***Xpsm*223**	***Xpsm*223**
$F_{in}=F_{out}= 5$	*Xpsm*223, *Xpsmp*2068	*Xpsmp*2068, *Xpsm*223	*Xpsm*223	*Xpsm*223
$F_{in}=F_{out}= 6$	*Xpsm*223	*Xpsm*223	*Xpsm*223	*Xpsm*223
$F_{in}=F_{out}= 7$	*Xpsm*223	*Xpsm*223	*Xpsm*223	*Xpsm*223
$F_{in}=F_{out}= 8$	*Xpsm*223	*Xpsm*223	*Xpsm*223	*Xpsm*223
$F_{in}=F_{out}= 9$	*Xpsm*223	*Xpsm*223	*Xpsm*223	*Xpsm*223
$F_{in}=F_{out}= 10$	*Xpsm*223	*Xpsm*223	*Xpsm*223	*Xpsm*223

Table 48. Marker selection based on Forward and Backward stepwise regression for fertility restoration of A_4 CMS system

Steps	A_4 CMS system			
	Summer season		Rainy season	
	Forward SWR	Backward SWR	Forward SWR	Backward SWR
$F_{in}=F_{out}= 2$	*Xpsmp*2048, *Xpsmp*2078	*Xpsmp*2078, *Xpsmp*2048, *Xpsm*696, *Xpsmp*2018, *Xpsmp*3022	*Xpsmp*2048, *Xpsmp*2080, *Xpsmp*2013	*Xpsmp*2048, *Xpsmp*2013, *Xpsmp*2080,
$F_{in}=F_{out}= 3$	*Xpsmp*2048	*Xpsmp*2048, *Xpsmp*2018, *Xpsmp*3022	*Xpsmp*2048, *Xpsmp*2013	*Xpsmp*2048, *Xpsmp*2013
$F_{in}=F_{out}= 4$	***Xpsmp*2048**	***Xpsmp*2048, *Xpsmp*2018, *Xpsmp*3022**	***Xpsmp*2048**	***Xpsmp*2048**
$F_{in}=F_{out}= 5$	*Xpsmp*2048	*Xpsmp*2048	*Xpsmp*2048	*Xpsmp*2048
$F_{in}=F_{out}= 6$	*Xpsmp*2048	*Xpsmp*2048	*Xpsmp*2048	*Xpsmp*2048
$F_{in}=F_{out}= 7$	*Xpsmp*2048	*Xpsmp*2048	-	-
$F_{in}=F_{out}= 8$	*Xpsmp*2048	*Xpsmp*2048	-	-
$F_{in}=F_{out}= 9$	-	-	-	-
$F_{in}=F_{out}= 10$	-	-	-	-

5. DISCUSSION

5. DISCUSSION

Discovery of A_1 cytoplasmic-nuclear male-sterility (CMS) system and development of male-sterile line Tift 23A (Burton, 1958, 1965) is a landmark in hybrid cultivar development in pearl millet. The A_1 CMS system has provided a strong base for the development of many commercial hybrids in India, with the first hybrid (HB-1) released in 1965. Large scale utilization of the single A_1 CMS source in all the hybrids raised a concern regarding its potential vulnerability to disease and insect-pest epidemics like large scale use of Texas cytoplasm-based maize hybrids in the USA leading to southern leaf blight disease epidemic (Scheifele *et al.*, 1970). Guided by this concern, efforts were made to identify and develop alternative CMS systems for diversification of hybrid seed parents in pearl millet. As a result, several other cytoplasmic sources, like A_2 and A_3 (Burton and Athwal, 1967), A_v (Marchais and Pernes, 1985), A_4 (Hanna, 1989), A_{egp} (Sujata *et al.*, 1994) and A_5 (Rai, 1995) differing from each other and from the A_1 CMS system were identified. A few other sources have also been reported such as PT 732A (Appadurai *et al.*, 1982), ex-Bornu (Aken'Ova, 1985), and Ghana and Botswana sources (Appa Rao *et al.*, 1989). Genetic diversification of seed parental lines (A-lines) in pearl millet is now underway at ICRISAT by using the A_4 and A_5 CMS systems along with the A_1 CMS system (K.N. Rai, pers. comm.). Understanding the genetics of male-sterility and fertility restoration of these CMS systems can enhance the efficiency of selection of good restorer and maintainer parents to develop high-yielding heterotic hybrids based on diversified CMS seed parents. There are a few reports on the genetics of CMS systems in pearl millet involving A_1, A_2 and A_3 systems (Burton and Athwal, 1967; Siebert, 1982) and on the A_4 CMS system (Du *et al.* (1996). These studies used limited genetic material and segregating populations, and also the limited environments in which these populations were evaluated. Therefore, results of these studies have remained only preliminary in nature.

The isonuclear lines in more than one cytoplasmic background are unique in the sense that it is possible to study the expression of different cytoplasms in a common nuclear background (as the observed differences will be solely due to cytoplasms) and effects of diverse nuclear backgrounds across male-sterile lines (A-lines) in a common cytoplasm (as the observed effects will be solely due to nuclear genome). Development of isonuclear A-lines of five diverse cytoplasms (A_1, A_4, A_v, A_{egp} and A_5) in three

nuclear backgrounds (81B, 5054B and ICMB 88004) (Rai, 1995; Rai *et al.*, 1996; Rai *et al.*, 2001; K.N. Rai, unpubl.) and their single-, dual- and triple-fertility restorers that restore fertility to either one or two or three sterile cytoplasms, respectively, laid the foundation for launching a comprehensive study in pearl millet with the aim to determine the genetics of the above-mentioned five CMS systems, allelic relationship and linkage between fertility restorer genes of alternative CMS systems (A_1 and A_4 CMS systems in the present study) and identify molecular markers for fertility restorer genes of the A_1 and A_4 CMS systems.

In the present study, information on the inheritance of the five CMS systems (A_1, A_4, A_v, A_{egp} and A_5) was derived from segregation pattern of male-fertile (F) and male-sterile (S) plants in F_2, BC_1 [A × (A × R)] and BC_2 [R × (A × R)] populations produced from A × R crosses. Also, there were F_2s produced from [(A × R) × (B × R)] crosses and testcrosses obtained by crossing A-lines with individual plants of (B × R)-derived F_2 and BC_1 populations. The segregation for F and S plants observed in F_2 and BC_1 populations was tested for χ^2 goodness of fit for monogenic, digenic and trigenic ratios. The different segregating populations, 108 (A × R)-derived F_2 and their corresponding BC_1 and BC_2 populations (coming from 36 crosses), testcrosses derived from the individual plants of eight (B × R)-derived F_2 and BC_1 populations (coming from two crosses), and the testcrosses of three (R × R) F_1s on $81A_1$ and $81A_4$ CMS lines, along with their respective parents and F_1s were evaluated in two contrasting test environments during the summer and rainy seasons 2003 at ICRISAT- Patancheru.

The temperature and relative humidity were recorded from one week before the time of first flowering entry to one week after the time of the last flowering entry in each environment (Appendix 1). The mean maximum and minimum temperature during summer season was 36 °C (range 30.4-39.2) and 19.9 °C (range 15.7-23.6), respectively. The mean maximum and minimum temperature during rainy season was 30 °C (range 26.4-31.7) and 19.6 °C (range 18-21.4), respectively. During the summer season, the mean relative humidity at 0700 hours was 69.2% (range 40-95%) and at 1400 hours, it was 31% (range 13-66%). During the rainy season, the mean relative humidity at 0700 hours was 88.1% (range 80-98 %) and at 1400 hours, it was 60.7% (range 48-76%).

The F_1 hybrids and BC_2 populations produced from A × R crosses for all the five CMS systems had fully fertile plants across the two test environments of summer and rainy seasons at ICRISAT- Patancheru, indicating that male-fertility was dominant over

male-sterility. Thus, this result will not be repeated in the discussion of individual CMS systems.

5.1 Inheritance of A_1 CMS system

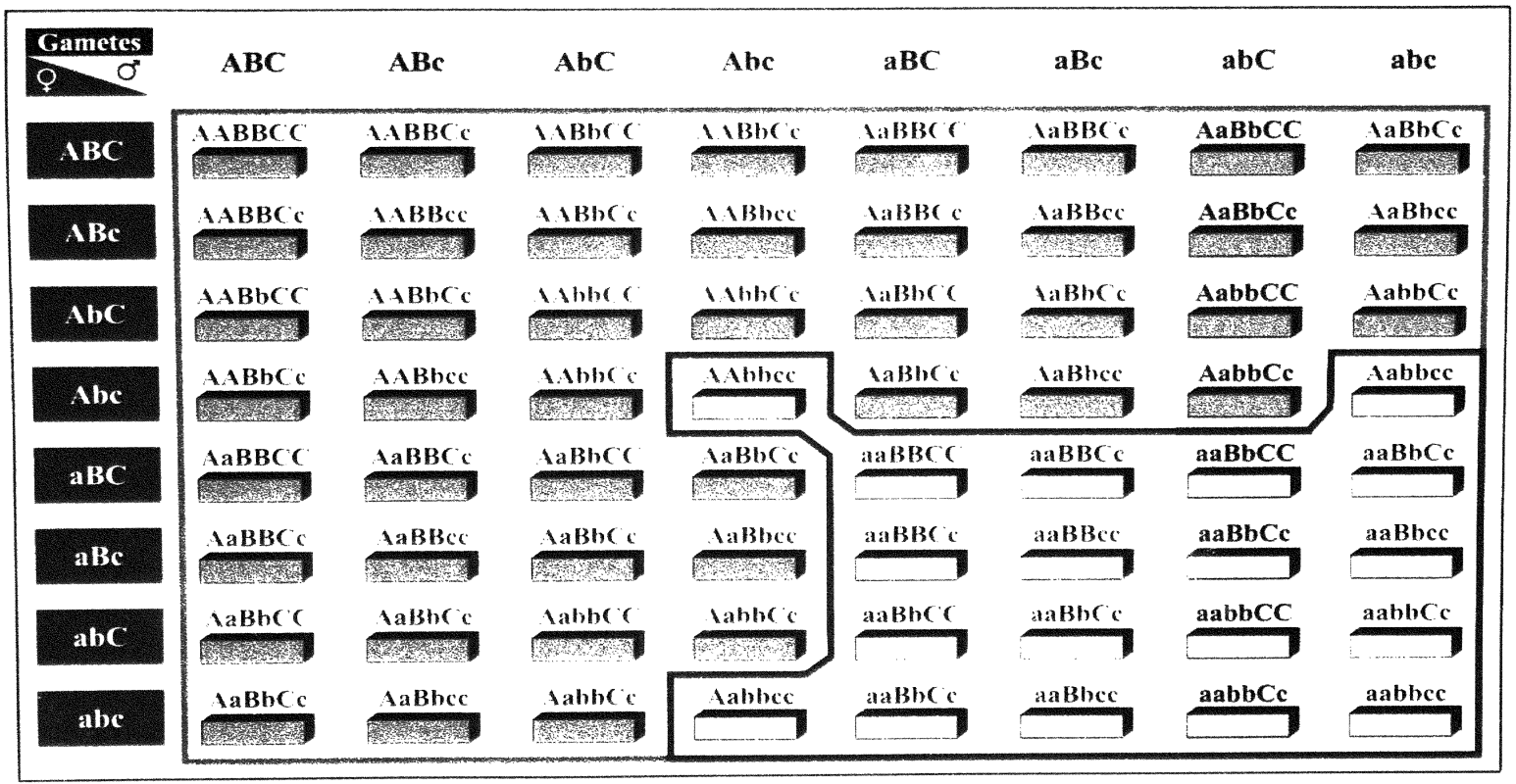
In 15 A × R crosses, out of 30 cases (15 each in summer and rainy season), 11 cases (6 in summer and 5 in rainy season) gave a good χ^2 fit to 45F:19S ratio in the F_2 and 1F:1S ratio in the BC_1 (Table 49) that is likely to result from a gene interaction involving one basic gene and two duplicate-complimentary genes. In another 10 cases (4 cases in summer and 6 in rainy season), the segregation pattern gave a good χ^2 fit to 54F:10S in the F_2 and 3F:1S in the BC_1 that is likely to result from a gene interaction involving any two of the three dominant duplicate-complimentary genes. To start with, let us consider 'A', 'B' and 'C' as the three genes involved in the fertility restoration of the A_1 CMS system. The postulated genotypic constitution of parents, F_2 and BC_1 with respect to the trigenic F_2 ratio of 45F:19S has been presented in Figure 13. The trigenic F_2 ratio of 45F:19S and the corresponding BC_1 ratio of 1F:1S is possible when the genotype of the female parent is 'aabbCC' possessing the dominant allele of one of the two duplicate-complimentary genes for fertility restoration (in this case 'C' gene), and the genotype of the restorer parent is 'AABBcc' with the dominant allele of the basic gene (A) and dominant allele of the other duplicate-complimentary gene (B). The F_1 of these parents will be fertile and heterozygous at all the three loci (AaBbCc). A plant in the F_2 will be fertile if it possesses dominant allele of the basic gene and dominant allele of any one or both of the duplicate-complimentary genes (A_B_C_, A_B_cc and A_bbC_). All other genotypes will produce sterile plants. The trigenic F_2 ratio of 54F:10S and the BC_1 ratio of 3F:1S is likely with the same genetic constitution of female parent 'aabbCC' and the male parent 'AABBcc' but with a gene interaction in which a plant will be fertile if it possesses dominant allele of at least two of the any three duplicate-complimentary (A_B_C_, A_B_cc, A_bbC_ and aaB_C_) (Figure 14). A close look at these two ratios in different crosses revealed that in crosses involving the restorer parents IPC 1518, IPC 511 and IPC 804 and A-lines in the genetic backgrounds of 81B and ICMB 88004, the segregating populations gave a good χ^2 fit to the trigenic F_2 ratio of 45F:19S and the corresponding BC_1 ratio of 1F:1S. However, the crosses involving the same restorer parents but with the 5054A gave a trigenic F_2 ratio of 54F:10S and the corresponding BC_1 ratio of 3F:1S. The trigenic F_2 ratio of 54F:10S could result from the shift of nine 'aaB_C_' genotypes from the sterile class in the 45F:19S ratio to the fertile class. The same may be applicable to BC_1 ratio as well. In the genetic background of 5054B, these

Table 49. Summary results of inheritance studies

Restorer parent	CMS system		A_1			A_4			A_{egp}			A_5			A_v		
	Nuclear background		81B	5054B	ICMB 88004	81B	5054B	ICMB 88004	81B	5054B	ICMB 88004	81B	5054B	ICMB 88004	81B	5054B	ICMB 88004
	Gen	Season	Observed ratio														
IPC 1518	F_2	Summer	45:19	54:10	45:19	54:10	54:10	54:10									
		Rainy	45:19	54:10	45:19	54:10	54:10	54:10									
	BC_1	Summer	1:1	3:1	1:1	3:1	3:1	3:1									
		Rainy	1:1	3:1	1:1	3:1	3:1	3:1									
IPC 804	F_2	Summer	45:19	45:19	45:19	45:19	45:19	9:7									
		Rainy	45:19	54:10	45:19	45:19	45:19	9:7									
	BC_1	Summer	1:1	1:1	1:1	1:1	1:1	1:3									
		Rainy	1:1	3:1	1:1	1:1	1:1	1:3									
IPC 511	F_2	Summer	45:19	54:10	45:19	3:1	3:1	3:1									
		Rainy	45:19	54:10	45:19	3:1	3:1	54:10									
	BC_1	Summer	1:1	3:1	1:1	1:1	1:1	1:1									
		Rainy	1:1	3:1	1:1	1:1	1:1	3:1									
IPC 382	F_2	Summer	54:10	63:1	54:10										63:1	57:7	57:7
		Rainy	54:10	63:1	54:10										63:1	57:7	57:7
	BC_1	Summer	3:1	7:1	3:1										7:1	3:1	3:1
		Rainy	3:1	7:1	3:1										7:1	3:1	3:1
L 67B	F_2	Summer	54:10	15:1	15:1				54:10	54:10	54:10				54:10	54:10	54:10
		Rainy	54:10	15:1	15:1				45:19	54:10	54:10				54:10	54:10	54:10
	BC_1	Summer	3:1	3:1	3:1				3:1	3:1	3:1				3:1	3:1	3:1
		Rainy	3:1	3:1	3:1				1:1	3:1	3:1				3:1	3:1	3:1
LSGP A_5	F_2	Summer										54:10	63:1	57:7			
		Rainy										54:10	63:1	57:7			
	BC_1	Summer										3:1	7:1	5:3			
		Rainy										3:1	7:1	5:3			

Ratio showing a poor fit (P= 0.01)

Figure 13. Segregation of male-fertile and male-sterile plants in F_2 (full punnet square) and BC_1 (only grey shaded column) from A × R cross for trigenic inheritance with one basic gene and two duplicate-complimentary genes responsible for fertility restoration



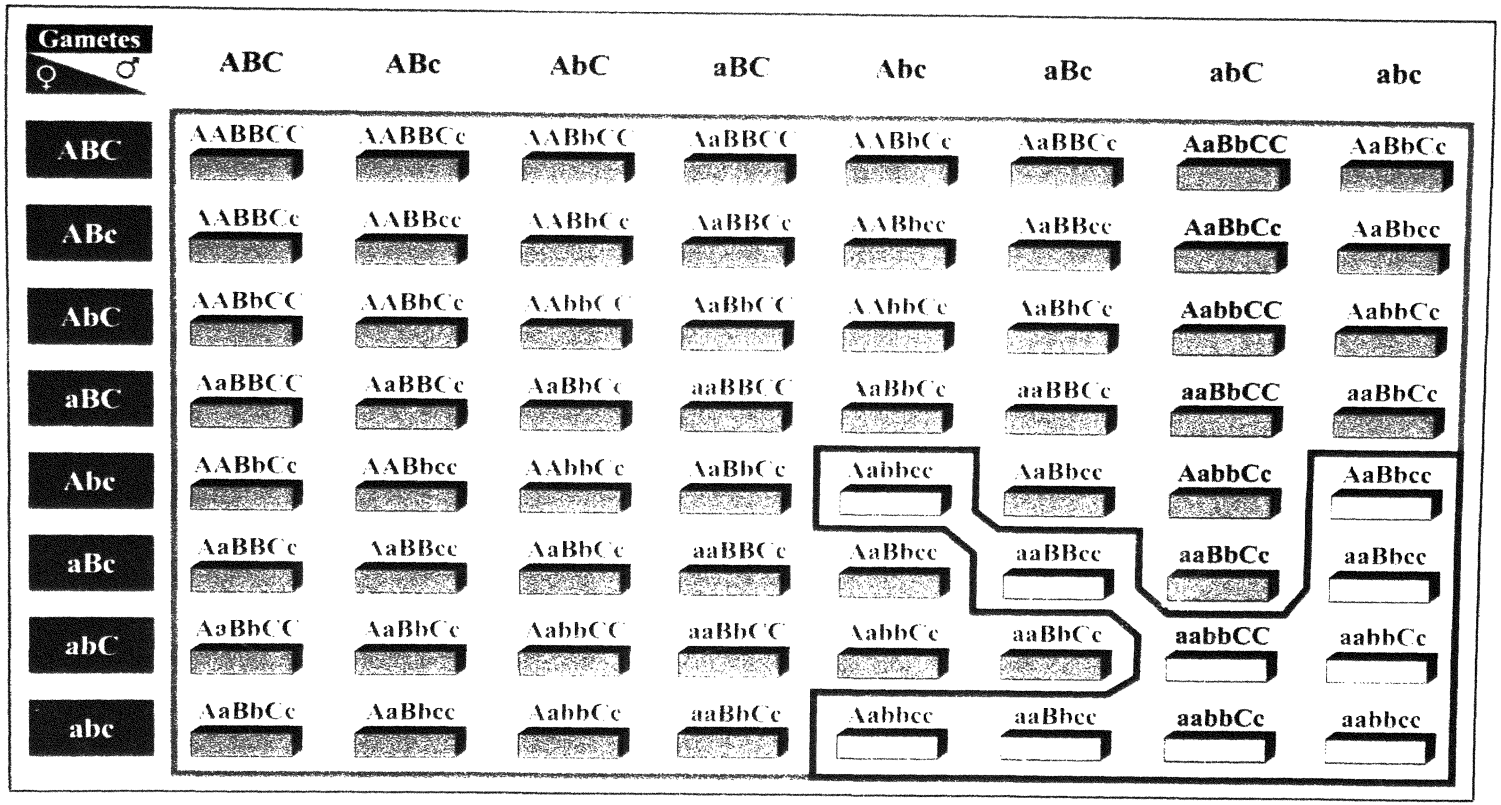
Parental genotype

Male parent: AABBCC
Female parent: aabbCC

Hypothetical segregation ratio

Generation	[illegible]	Sterile
F_2	45	19
BC_1	1	1

Figure 14. Segregation of male-fertile and male-sterile plants in F_2 (full punnet square) and BC_1 (only grey shaded column) from A × R cross for trigenic inheritance with any two of the three duplicate-complimentary genes responsible for fertility restoration



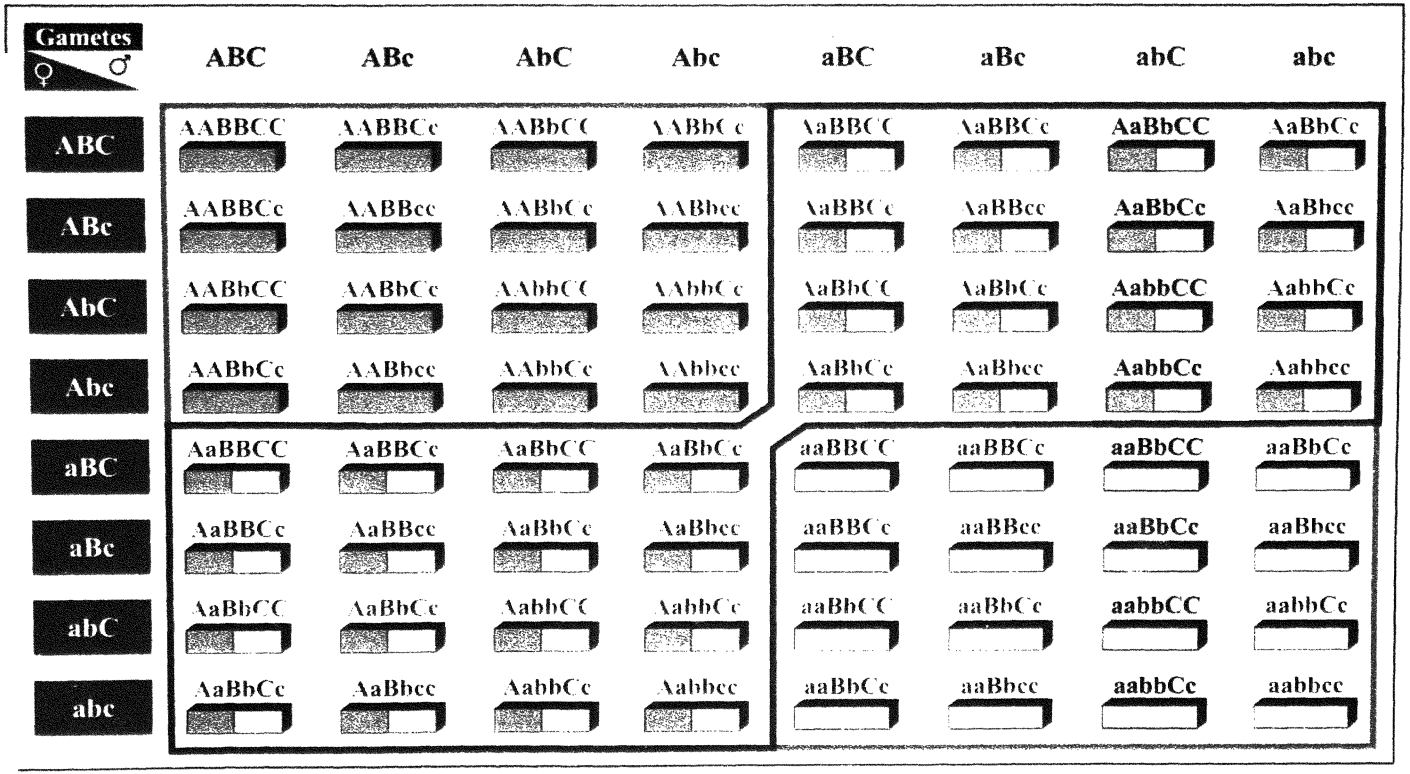
Parental genotype

Male parent: AABBCC
Female parent: aabbCC

Hypothetical segregation ratio

Generation		Sterile
F_2	54	10
BC_1	3	1

Figure 15. Segregation of male-fertile, segregating and male-sterile plants in F_2 (full punnet square) and BC_1 (only grey shaded column) testcrosses derived from B × R cross for trigenic inheritance with one basic gene and two duplicate-complimentary genes responsible for fertility restoration



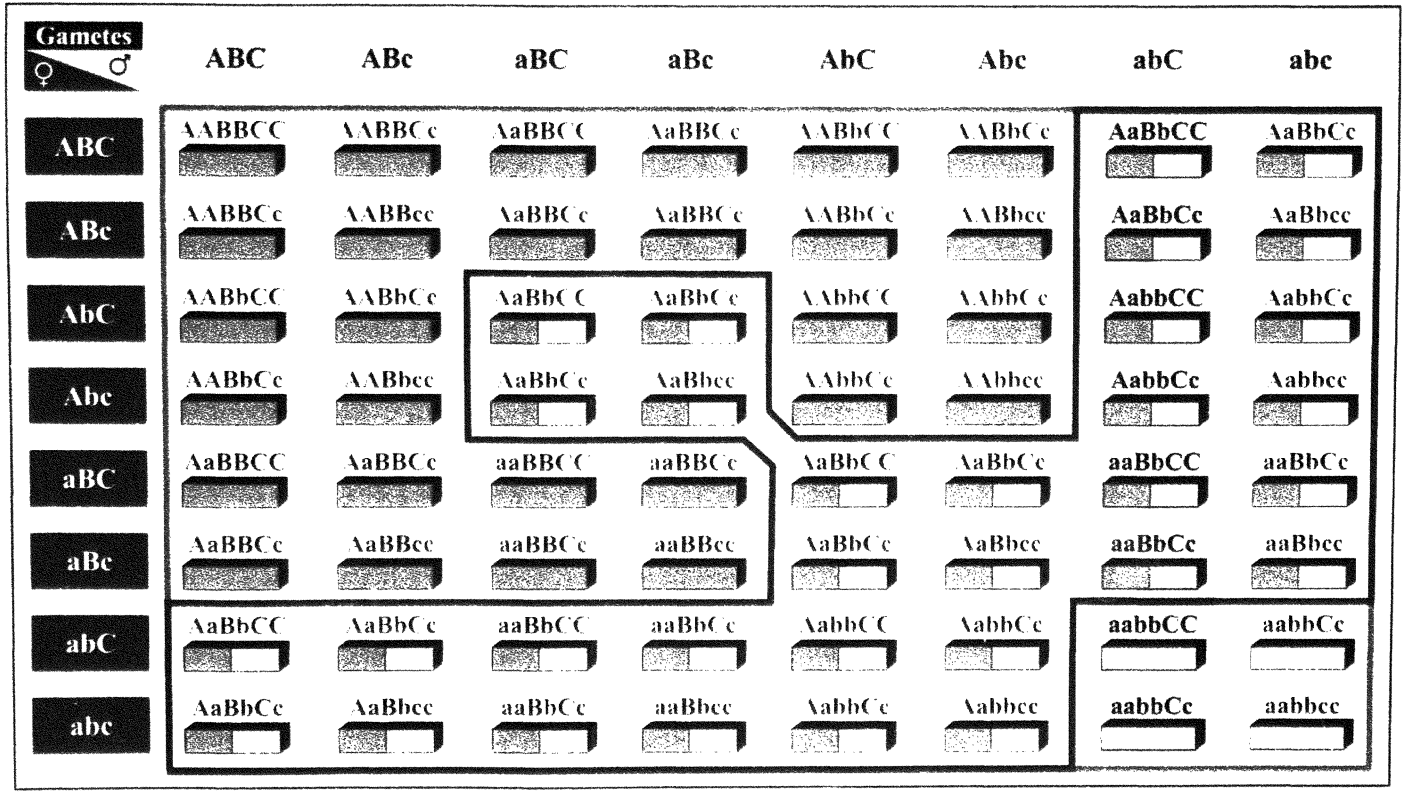
Parental genotype

Male parent: AABBCC
Female parent: aabbCC

Hypothetical segregation ratio

Generation		S	Sterile
F_2	1	2	1
BC_1	-	1	1

Figure 16. Segregation of male-fertile, segregating and male-sterile plants in F_2 (full punnet square) and BC_1 (only grey shaded column) testcrosses derived from B × R cross for trigenic inheritance with any two of the three duplicate-complimentary genes responsible for fertility restoration



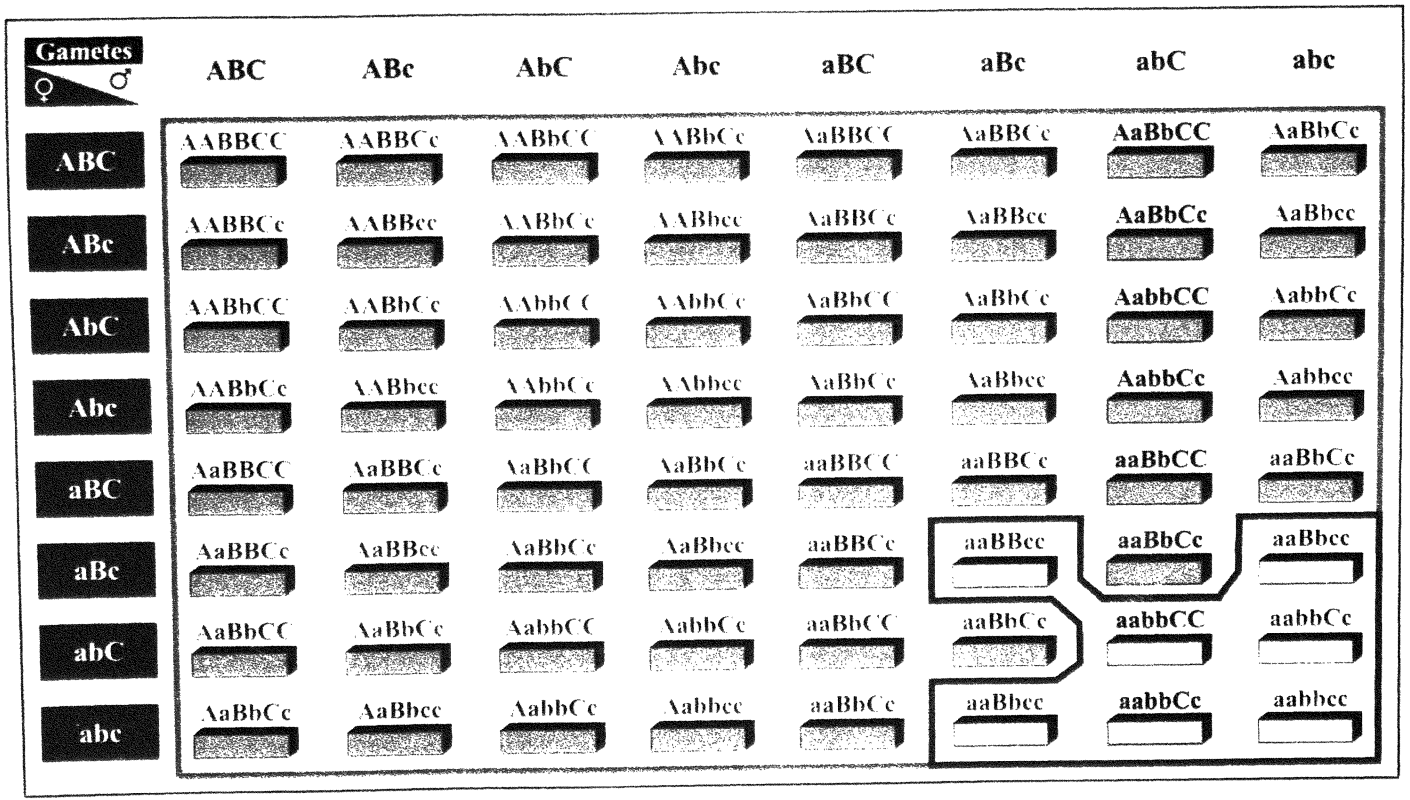
Parental genotype

Male parent : AABBCC
Female parent: aabbCC

Hypothetical segregation ratio

Generation		S	Sterile
F_2	7	8	1
BC_1	-	3	1

Figure 17. Segregation of male-fertile and male-sterile plants in F_2 (full punnet square) and BC_1 (only grey shaded column) from A × R cross for trigenic inheritance with either the basic gene alone and/or any two of the three duplicate-complimentary genes responsible for fertility restoration



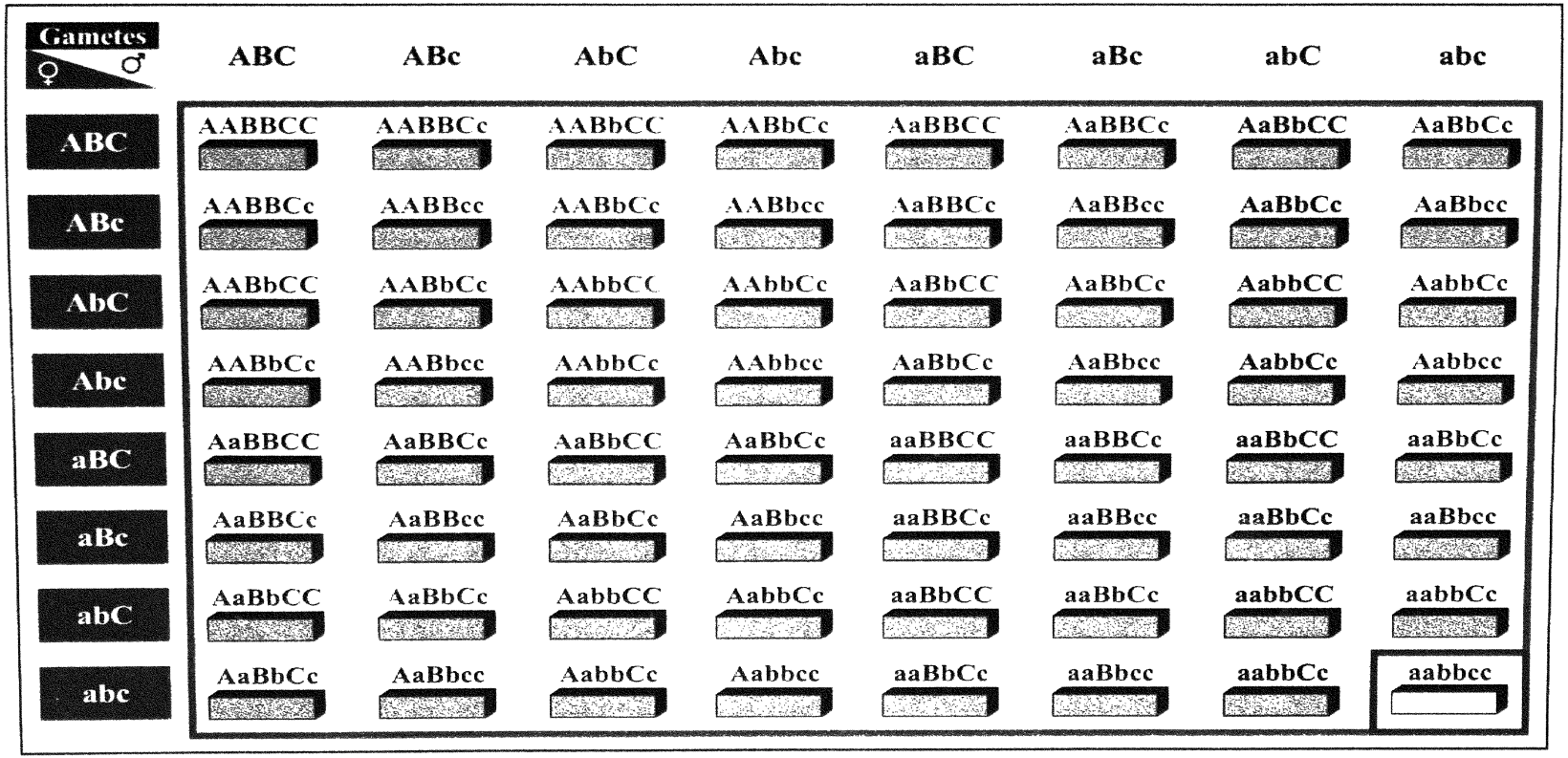
Parental genotype

Male parent: AABBCC
Female parent: aabbCC

Hypothetical segregation ratio

Generation		Sterile
F_2	57	7
BC_1	3	1

Figure 18. Segregation of male-fertile and male-sterile plants in F_2 (full punnet square) and BC_1 (only grey shaded column) from A × R cross for trigenic inheritance with three duplicate genes responsible for fertility restoration



Parental genotype

[redacted]
Female parent: aabbCC

Hypothetical segregation ratio

Generation	[redacted]	Sterile
F_2	63	1
BC_1	7	1

genotypes might behave as fertile in the absence of the dominant allele of the basic gene 'AA'. The presence of 54F:10S ratio in the F_2 and 3F:1S in the BC_1 in the genetic background of 5054B may also result from some of the crosses possibly made on pollen-shedding tiller panicles of 5054A. Although all the crosses made on 5054A were harvested only from those plants whose tiller panicle had been scored as fully sterile under selfing condition, the possibility of a pollen-shedding tiller panicle of the same plant involved in crosses can not be ruled out as these crosses were made during the rainy season. Pearl millet A-lines have been shown to produce a higher frequency of pollen-shedders in the rainy season than in the summer season, the more so in case of 5054A. (K.N. Rai, pers. comm.). Also, 54F:10S ratio in the F_2 was more frequent during the rainy season. This further supports the hypothesis of less stable male-sterility of 5054A producing a F_2 ratio of 54F:10S and BC_1 ratio of 3F:1S. It has been observed that even A-lines of the A_1 CMS system have low frequency of plants giving 0-5% selfed seed set under selfing and this happens more with the 5054A than with the $81A_1$ (K.N. Rai, unpubl.). Selfed seed-set (SSS) of F_2 and BC_1 plants had been recorded in some of the crosses. In case of the cross 5054A × IPC 511, it was observed that when pollen-fertile plants with 0-5% SSS were classified as sterile, the resulting trigenic F_2 ratio was 45F:19S instead of the ratio of 54F:10S observed on pollen-shedding basis. It is likely that the enhanced pollen shedding behavior in the 5054A genetic background is likely to be triggered in genotypes with the genetic constitution 'aaB_C_'. Hence, the observed trigenic ratio of 54F:10S could be considered as an escalation of 45F:19S ratio either due to pollen shedding behavior of 5054A or due to 'aaB_C_' behaving as fertile. In fact, segregation pattern in the F_2 produced from (5054A × IPC 511) × (5054B × IPC 511) did give a trigenic F_2 ratio of 45F:19S, which further supports the presence of one basic gene and two duplicate-complimentary genes for fertility restoration of A_1 CMS system in the genetic background of 5054B as well.

Individual plants of the F_2 and BC_1 populations from the cross 81B × IPC 804 had been testcrossed on the $81A_1$ and $81A_4$ for studying the linkage between the fertility restorer genes of the A_1 and A_4 CMS systems. Similarly, individual plants of the F_2 and BC_1 populations from the cross 5054B × IPC 511 had also been testcrossed on the $5054A_1$ and $5054A_4$ to have another set of material to study the linkage of fertility restorer genes of these two CMS systems. These testcrosses, classified as fully fertile (F), segregating for fertile and sterile (F+S) and fully sterile (S) provided ideal material to further probe into the genetics of each of these two CMS systems.

The testcross data of F_2 plants derived from the cross 81B × IPC 804 gave a good χ^2 fit to the expected ratio of 1 F:2 (F+S): 1S progenies in the rainy season but a poor fit in the summer season. However, the corresponding testcrosses from BC_1 plants gave a good fit to the expected ratio of 1(F+S):1S in both the seasons. These F_2 testcross ratio of 1F:2 (F+S):1S and BC_1 testcross ratio of 1(F+S):1S were supportive of the trigenic inheritance giving a F_2 ratio of 45F:19S and BC_1 ratio of 1F:1S (Figure 15).

The segregation pattern in testcrosses produced from the individual plants of the F_2 from the cross 5054B × IPC 511 did not give a good χ^2 fit to the 7F:8 (F+S):1S ratio expected for a F_2 ratio of 54F:10S (Figure 16) as there was an excess of (F+S) and S class, giving an observed ratio of 1.3F:3.7(F+S):1S ratio. The BC_1 testcrosses gave a good χ^2 fit in the summer season only to the 3 (F+S):1S ratio. This pattern, however, was reflective of a 45F:19S ratio in the F_2 as this should give a 1F:2 (F+S):1S ratio in the F_2 and 1 (F+S):1S ratio in the BC_1 testcrosses (Figure 15).

Another validation came in the form of the number of F_2 and BC_1 testcrosses segregating for the expected 1F:1S ratio within the segregating testcross progenies. Of the segregating testcrosses produced from F_2 plants on $81A_1$, 76.9% (180 out of 234) in the summer and 83.2% (188 out of 226) in the rainy season exhibited a 1F:1S ratio (Table 11). Of the segregating testcrosses produced from BC_1 plants on $81A_1$, 69.4% (43 out of 62) in the summer and 80.6% (50 out of 62) in the rainy season, segregated for the expected 1F:1S ratio. Further, individual χ^2 values for the expected 1F:1S ratio were calculated for each of the segregating testcrosses and summed up. The pooled χ^2 value was calculated by adding the fertile and sterile plants of the segregating testcrosses (Table 12). The pooled F_2 testcross data gave a good fit to the expected 1F:1S ratio in the summer season but poor fit in rainy season.

Of the segregating testcrosses produced from F_2 plants on $5054A_1$, 56.4% (136 out of 241) in the summer and 74.9% (182 out of 243) in the rainy season gave a 1F:1S ratio; and of the segregating testcrosses produced from BC_1 plants on $5054A_1$, 52.1% (49 out of 94) in the summer and 75.9% (63 out of 83) in the rainy season, segregated for the expected 1F:1S ratio (Table 11). Similarly, in F_2, 35.7% (86 out of 241) in the summer and 44.9% (109 out of 243) in the rainy season gave a 3F:1S ratio; and of the segregating testcrosses produced from BC_1 plants on $5054A_1$, 11.7% (11 out of 94) in the summer and 18.1% (15 out of 83) in the rainy season, segregated for the 3F:1S ratio (Table 11). Further, individual χ^2 values for the expected 1F:1S ratio for each of the segregating testcrosses was summed up and a pooled χ^2 value was also calculated by

adding the fertile and sterile plants of the segregating testcrosses (Table 12). The pooled F_2 and BC_1 data of the segregating testcrosses didn't fit the expected 1F:1S ratio in both the summer and in rainy season and the heterogeneity χ^2 was also significant across the segregating testcrosses.

From the intercrosses of three restorer parents (IPC 1518, IPC 511 and IPC 804) to test allelism, it was found that the F_1s of all the intercrosses when test crossed on $81A_1$ produced hybrids in which all plants were fully fertile, indicating the presence of same alleles of fertility restorer genes in all the restorer parents. Overall, these results suggested that more likely, one basic gene and two duplicate-complimentary genes are involved in fertility restoration. We propose genotype *rf*1a *rf*1a *rf*1b *rf*1b *Rf*1c *Rf*1c for A/B line and genotype *Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c for the restorers IPC 1518, IPC 511, IPC 804, and IPC 382 (Table 50).

In four segregating populations (2 F_2s and 2 BC_1s) from two crosses involving restorer line L 67B with A-lines 5054A and ICMA 88004, a digenic F_2 ratio of 15F:1S and the corresponding BC_1 ratio of 3F:1S was observed that results from two duplicate genes involved in fertility restoration of the A_1 CMS system, suggesting different inheritance pattern with different restorer parents. Although trigenic inheritance mechanism has been found to be operative for majority of the crosses of the A_1 CMS system, the manifestation of digenic (15F:1S) ratio reflects that in this case, only two genes out of the three segregate (Table 51). The female genotype being same (aabbCC), the digenic F_2 ratio of 15F:1S is possible with 'AABBCC' genotype of pollen parent. Hence it is likely that L 67B had varying genetic constitution for fertility restoration, where the plant of genotype *Rf*1a *Rf*1a *Rf*1b *Rf*1b *Rf*1c *Rf*1c gave digenic ratio, while the other one with genotype *Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c gave a trigenic ratio. The genetic variability within a line developed even after prolonged inbreeding cannot be ruled out in pearl millet.

In 10 segregating populations (3 F_2s and 7 BC_1s), deviation from expected ratio was observed mostly in one of the two seasons. Among the three populations (1 F_2 and 2 BC_1s) derived from the cross of $81A_1$ with IPC 1518 and IPC 804, giving poor χ^2 fit to the expected ratios, two were due to excess of fertile plants in the rainy season. Similarly, among the six segregating populations (1 F_2 and 5 BC_1s) derived from crosses of 5054A with all the five restorers, two had excess of sterile in summer season, and the remaining two had excess of fertile during rainy season. Among the three segregating populations (1 F_2 and 2 BC_1s) derived from crosses of ICMA 88004 with IPC 1518 and

Table 50. Proposed genotypes of A-lines and Restorer-lines

CMS system	Nuclear Background		
	81B	**5054B**	**ICMB 88004**
	A-lines		
A_1	*rf*1a *rf*1a *rf*1b *rf*1b *Rf*1c *Rf*1c	*rf*1a *rf*1a *rf*1b *rf*1b *Rf*1c *Rf*1c	*rf*1a *rf*1a *rf*1b *rf*1b *Rf*1c *Rf*1c
A_4	*rf*4a *rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c	*rf*4a *rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c	*rf*4a *rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c
A_v	*rfv*a *rfv*a *rfv*b *rfv*b *Rfv*c *Rfv*c	*rfv*a *rfv*a *rfv*b *rfv*b *Rfv*c *Rfv*c	*rfv*a *rfv*a *rfv*b *rfv*b *Rfv*c *Rfv*c
A_{egp}	*rfe*a *rfe*a *rfe*b *rfe*b *Rfe*c *Rfe*c	*rfe*a *rfe*a *rfe*b *rfe*b *Rfe*c *Rfe*c	*rfe*a *rfe*a *rfe*b *rfe*b *Rfe*c *Rfe*c
A_5	*rf*5a *rf*5a *rf*5b *rf*5b *Rf*5c *Rf*5c	*rf*5a *rf*5a *rf*5b *rf*5b rf5c *rf*5c	*rf*5a *rf*5a *rf*5b *rf*5b rf5c *rf*5c
	Restorer-lines		
	IPC 1518-P_3	**IPC 1518-P_1**	**IPC 1518-P_2**
A_1	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c
A_4	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c
	IPC 511-P_1	**IPC 511-P_3**	**IPC 511-P_2**
A_1	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c
A_4	*Rf*4a *Rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c	*Rf*4a *Rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c
	IPC 804-P_4	**IPC 804-P_3**	**IPC 804-P_1**
A_1	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c*
A_4	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c	*Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c
	IPC 382-P_1	**IPC 382-P_3**	**IPC 382-P_2**
A_1	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *Rf*1c *Rf*1c^	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c
A_v	*Rfv*a *Rfv*a *Rfv*b *Rfv*b *Rfv*c *Rfv*c#	*Rfv*a *Rfv*a *Rfv*b *Rfv*b *rfv*c *rfv*c	*Rfv*a *Rfv*a *Rfv*b *Rfv*b *rfv*c *rfv*c
	L 67B-P_3	**L 67B-P_2**	**L 67B-P_1**
A_1	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *Rf*1c *Rf*1c	*Rf*1a *Rf*1a *Rf*1b *Rf*1b *Rf*1c *Rf*1c
A_v	*Rfv*a *Rfv*a *Rfv*b *Rfv*b *rfv*c *rfv*c	*Rfv*a *Rfv*a *Rfv*b *Rfv*b *rfv*c *rfv*c	*Rfv*a *Rfv*a *Rfv*b *Rfv*b *rfv*c *rfv*c
A_{egp}	*Rfe*a *Rfe*a *Rfe*b *Rfe*b *rfe*c *rfe*c	*Rfe*a *Rfe*a *Rfe*b *Rfe*b *rfe*c *rfe*c	*Rfe*a *Rfe*a *Rfe*b *Rfe*b *rfe*c *rfe*c
	LSGP A_5-P_1	**LSGP A_5-P_1**	**LSGP A_5-P_1**
A_5	*Rf*5a *Rf*5a *Rf*5b *Rf*5b *rf*5c *rf*5c	*Rf*5a *Rf*5a *Rf*5b *Rf*5b *Rf*5c *Rf*5c	*Rf*5a *Rf*5a *Rf*5b *Rf*5b *Rf*5c *Rf*5c

* The female genotype is *rf*4a *rf*4a *rf*4b *rf*4b *rf*4c *rf*4c
^ The female genotype is *rf*1a *rf*1a *rf*1b *rf*1b *rf*1c *rf*1c
# The female genotype is *rfv*a *rfv*a *rfv*b *rfv*b *rfv*c *rfv*c

The designated symbol consists of:

Firstly, *Rf /rf* (written in italic) denoting fertility restorer genes in dominant/recessive condition followed by either a numeral (1, 4 or 5) or alphabet (e or v) referring to the CMS systems in pearl millet and, lastly the three genes symbolized with 'a', 'b' and 'c'

Table 51. Genetic constitution of male parents likely to give one-gene and two-gene inheritance for a three-gene mechanism of male-sterility and fertility restoration on male-sterile (S) lines with aabbCC genotype

Genotype of parents		F_1		Ratio (F:S)		Inheritance
Female	**Male**	**Genotype**	**F/S**	**F_2**	**BC_1**	
Three-gene mechanism: One basic gene; two duplicate-complimentary genes (F_2 ratio: 45F:19S; BC_1 ratio: 1F:1S)						
aabbCC	AABBCC	AaBbCC	F	3:1	1:1	Monogenic
	AAbbCC	AabbCC	F	3:1	1:1	Monogenic
	AAbbcc	AabbCc	F	9:7	1:1	Digenic
Three-gene mechanism: Any two of the three duplicate-complimentary genes (F_2 ratio: 54F:10S; BC_1 ratio: 3F:1S)						
aabbCC	AAbbCC	AabbCC	F	3:1	1:1	Monogenic
	aaBBCC	aaBbCC	F	3:1	1:1	Monogenic
	AAbbcc	AabbCc	F	9:7	1:1	Digenic
	aaBBcc	aaBbCc	F	9:7	1:1	Digenic
	AABBCC	AaBbCC	F	15:1	3:1	Digenic
Three-gene mechanism: Dominant allele of one of the three genes on its own restores fertility; alternatively, dominant alleles at both of the remaining loci would also restore fertility (F_2 ratio: 57F:7S; BC_1 ratio: 3F:1S)						
aabbCC	AAbbCC	AabbCC	F	3:1	1:1	Monogenic
	AAbbcc	AabbCc	F	3:1	1:1	Monogenic
	aaBBCC	aaBbCC	F	3:1	1:1	Monogenic
	aaBBcc	aaBbCc	F	9:7	1:1	Digenic
	AABBCC	AaBbCC	F	15:1	3:1	Digenic

IPC 382, one gave a poor χ^2 fit due to excess of sterile plants observed during summer and one was due to excess of fertile plants in the rainy season. This showed that in most of the cases, the poor χ^2 fit to the expected ratio was either due to excess of fertile plants in the rainy season or the excess of sterile plants in the summer season. Such deviations could be attributed to the effect of temperature and relative humidity on gene expression that could make an expected ratio giving poor χ^2 fit in one of the seasons. The maximum temperature of rainy season was 6 °C lesser than the summer along with 30% higher relative humidity during the flowering period. The expression of fertility restoration pattern of A_1 CMS system could be affected by such differences of temperature and relative humidity during the flowering period as observed by Rai *et al.* (1996, 2001) who showed that the frequency of pollen-shedders and the degree of selfed seed-set in A-lines were generally higher in the rainy season than in the dry summer season. The effect of temperature on fertility restoration has also been reported in wheat (Johnson and Paterson, 1973), rye (Scoles and Evans, 1979) and maize (Duvick, 1956).

5.2 Inheritance of the A_4 CMS system

The inheritance of the A_4 CMS system was investigated in nine A × R crosses involving three A-lines each in genetic background of 81B, 505B and ICMB 88004, and three restorer parents (IPC 1518, IPC 511 and IPC 804). The restorer parent IPC 1518 gave a good χ^2 fit to the trigenic F_2 ratio of 54F:10S across all the three genetic backgrounds and in both seasons. This segregation pattern could result from genetic interaction involving dominant alleles of any two of the three duplicate-complimentary genes. In BC_1, however, the 3F:1S ratio expected from such gene interaction gave a good χ^2 fit in the genetic background of 5054B in the summer season only (Table 49). In all the remaining BC_1 populations, poor χ^2 fit to the expected 3F:1S ratio was observed due to excess of sterile plants in both the seasons.

With the restorer IPC 804, the trigenic ratio of 45F:19S ratio in the F_2 and 1F:1S in the BC_1 was observed with good χ^2 fit across both the seasons in genetic backgrounds of 81B and 5054B. Such segregation can result from dominant allele of one basic gene and the dominant allele of at least one of the two duplicate-complimentary genes. But a digenic ratio of 9F:7S in the F_2 in the ICMB 88004 background (good χ^2 fit in the summer season) and 1F:3S in the BC_1 (good χ^2 fit in the rainy season) could result from dominant alleles of two complimentary genes. The χ^2 goodness of fit to the segregation ratio expected from this genetic interaction was poor in the F_2 during the rainy season

due to excess of fertile plants, and it was poor in BC_1 during the summer season due to excess of sterile plants.

The restorer IPC 511, in general, gave a good χ^2 fit to the monogenic ratio of 3F:1S in the F_2 and 1F:1S in BC_1 across all the three genetic backgrounds of A-line and in both seasons, except a poor χ^2 fit due to excess of fertile plants in the F_2 of $81A_4$ during the summer season and F_2 of $5054A_4$ during the rainy season. A major deviation from this postulated inheritance was the trigenic ratio of 54F:10S in the F_2 and the corresponding 3F:1S ratio in the BC_1 observed in the genetic background of $ICMA_4$ 88004 during the rainy season. This showed that the restorer parent as well as the genetic background of the female parent influenced the inheritance pattern of the A_4 CMS system. The genotypic constitution of F_2 and BC_1 populations with respect to the trigenic F_2 ratio of 45F:19S and 54F:10S has been previously explained for the A_1 system and presented in Figures 13 and 14. The trigenic F_2 ratios of 45F:19S and 54F:10S, and the corresponding BC_1 ratios of 1F:1S and 3F:1S, respectively, are possible when the genotype of the female parent is 'aabbCC' and the genotype of the restorer parent is 'AABBcc'. The digenic ratio of 9F:7S in the F_2 and 1F:3S in the BC_1 is also possible with 'AAbbcc' genotype of male parent. Similarly, the monogenic ratio of 3F:1S in the F_2 and 1F:1S in the BC_1 is possible with 'AAbbCC'genotype of male parent. But on the whole, it is likely that a trigenic inheritance mechanism in which either the dominant allele of any two of the three duplicate-complimentary genes and/or dominant allele of one basic gene alone might be involved in the fertility restoration of the A_4 CMS system.

The segregation pattern in the F_2 produced from ($5054A_4$ × IPC 511) × (5054B × IPC 511) also gave a trigenic F_2 ratio of 54F:10S, which further supports the presence of three-gene mechanism in which any two duplicate-complimentary genes restore fertility of the A_4 CMS system.

As explained in the A_1 CMS system, individual plants of the F_2 and BC_1 populations derived from the B × R crosses were testcrossed on the $81A_4$ and $5054A_4$, which provided ideal material to further probe into the genetics of each of these two CMS systems. The testcross data of F_2 and BC_1 plants derived from the cross 81B × IPC 804 gave a poor χ^2 fit to the 1F:2 (F+S):1S and 1 (F+S):1 S ratio, respectively (expected from either 3F:1S or 45F:19S ratio in the F_2 and 1F:1S in the BC_1), or the 1F:8 (F+S):7S F_2 and 1 (F+S):3 S BC_1 testcross ratio (expected from a 9F:7S ratio in the F_2 and 1F:3S in the BC_1), in both the seasons.

The segregation pattern of testcrosses produced from the individual F_2 plants of the cross 5054B × IPC 511 gave a good χ^2 fit to the 1F:2 (F+S):1S ratio and those produced from BC_1 plants gave a good χ^2 fit to the 1 (F+S):1 S ratio. This segregation pattern was reflective of a 3F:1S or 45F:19S ratio in the F_2 and 1F:1S in the BC_1 in the testcrosses.

The manifestation of monogenic and digenic ratio along with trigenic ratios in case of A_4 CMS system suggests the likely involvement of three genes in the inheritance of fertility restoration in A_4 CMS system. Even in a three-gene mechanism, there is a possibility of getting a one-gene or two-gene ratio depending on the number of genes segregating and the genetic background of the female and male parent (Table 51). Based on the Mendelian ratios obtained, the postulated genotypes of female parents could be *rf*4a *rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c or *rf*4a *rf*4a *rf*4b *rf*4b *rf*4c *rf*4c (with IPC 804 in case where digenic ratio fits) and male parents *Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c in cases where trigenic ratios were obtained *Rf*4a *Rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c (with IPC 511 where monogenic ratios were obtained) have been presented in the Table 50.

The (R × R) F_1 hybrids involving two cross combinations viz., IPC 511 × IPC 1518 and IPC 511 × IPC 804 produced completely fertile testcrosses made on $81A_4$ (Table 37). The testcross progenies produced from cross IPC 804 × IPC 1518 segregated into 3F:1S in both summer and rainy season. These results indicated triallelic situation where IPC 511 would have a genotype as $A^1A^1bbC^1C^1$, IPC 804 as $A^2A^2B^2B^2cc$ and IPC 1518 as $A^3A^3B^3B^3cc$ where $A^1 = A^2$ and $A^1 = A^3$, but $A^2B^2 \neq A^3\ B^3$, so A^2B^2 were likely to be dominant over $A^3\ B^3$. The F_1 of the cross IPC 804 × IPC 1518 gave a good χ^2 fit to the ratio of 3F:1S in the testcross progenies, the probable reasons for which have been explained in Figure 19. Similar allelic products of the same basic gene and its complimentary gene ($A^2\_B^2\_$ cc, $A^3_B^3_$cc) will produce fertile progenies in this cross. Further, $A^2_B^3_$cc is also likely to be fertile, as the allelic product of B^3 will complement allelic products of $A^2\_$. However, the progenies with genotypic constitution $A^3_B^2_$cc is likely to be sterile as allelic products of $A^3\_$ will not be complemented by the allelic product of B^2. Hence, based on the knowledge from inheritance pattern observed from the (A × R) crosses and test of allelism, the gene symbols for IPC 511 would be *Rf*4a^1 *Rf*4a^1 *rf*1b *rf*1b *Rf*1c^1 *Rf*1c^1, IPC 804 would be *Rf*4a^2 *Rf*4a^2 *Rf*4b^2 *Rf*4b^2 *rf*1c *rf*1c and IPC 1518 would be *Rf*4a^3 *Rf*4a^3 *Rf*4b^3 *Rf*4b^3 *rf*1c *rf*1c (Table 50). Similar phenomenon of allelism has been reported in maize (Duvick, 1956, Kheyr-Pour *et al.* 1981) and rice (Govinda Raj and Virmani, 1988; Ramalingam *et al.* 1995).

IPC 804 IPC 1518

$A^2A^2B^2B^2cc \times A^3A^3B^2B^2cc$

$\downarrow$

$A^2A^3B^2B^3\ cc$

F_1

Testcross progenies (F_1 crossed with $81A_4$)

F_1 ($A^2A^3B^2B^3$ cc) Gametes	$81A_4$ (aabbCC) abC	F/S	Ratio
A^2B^2c	A^2aB^2bcc	F	
A^2B^3c	A^2aB^3ccc	F	3F: 1S
A^3B^2c	A^3aB^2bcc	S	
A^3B^3c	A^3aB^3bcc	F	

B^2 will not complement the expression of A^3

Figures 19. Segregation pattern in the R x R test cross for the test of allelism in A_4 CMS System

5.3 Inheritance of the A_{egp} CMS system

The inheritance of A_{egp} CMS system was investigated in three A × R crosses involving A-lines in three genetic backgrounds of 81B, 5054B and ICMB 88004 and the restorer parent L 67B. In three A × R crosses, out of six cases (three each in summer and rainy season), five cases (three in summer and two in rainy season) gave a good χ^2 fit to 54F:10S ratio in the F_2 and 3F:1S ratio in the BC_1 (Table 49) that is likely to result from dominant alleles of any two of the three duplicate-complimentary genes. One case in the rainy season gave a good χ^2 fit to the trigenic ratio of 45F:19S ratio in the F_2 and 1F:1S ratio in the BC_1 that is likely to result from a gene action involving dominant allele of one basic gene and one or both dominant alleles of the two duplicate-complimentary genes. The same genetic constitution as given for the A_1 CMS system, both for A-lines (aabbCC) and the restorer parent (AABBcc) would give these two ratios each for the F_2 and BC_1 population (Figure 13, 14). Hence, A_{egp} CMS is likely to be controlled by trigenic inheritance mechanism.

Based on the Mendelian ratios obtained, the postulated genotype of female parents would be *rf*ea *rf*ea *rf*eb *rf*eb *Rf*ec *Rf*ec and male parent would be *Rf*ea *Rf*ea *Rf*eb *Rf*eb *rf*ec *rf*ec (Table 50). Since linkage studies of fertility restorers of A_1 and A_{egp} CMS systems were not done, it could be that one or more of the loci involved in the fertility restoration of A_{egp} system might be same as those in the A_1 system, but with different alleles. This comparison of the A_{egp} system specifically with A_1 CMS system is based on the observation that both have similar behaviour in fertility restoration pattern of their hybrids except that more inbreds produce sterile hybrids on A_{egp} than on the A_1 system A-lnes (K.N. Rai, unpubl.).

5.4 Inheritance of the A_5 CMS system

The inheritance of A_5 CMS system was investigated in three A × R crosses involving A-lines in three genetic backgrounds of 81B, 5054B and ICMB 88004 and the restorer parent LSGP A_5. Different inheritance mechanism exhibited by a good χ^2 fit to different ratios in the F_2 and BC_1 populations, indicated the likely influence of genetic background on fertility restoration pattern of the A_5 CMS system. In the 81B genetic background, the trigenic 54F:10S ratio in the F_2 and 3F:1S in the BC_1 was observed in both the seasons (Table 49) that is likely to result from dominant alleles of any two of the three duplicate-complimentary genes. In the 5054B genetic background, the trigenic 63F:1S ratio in the F_2 and 7F:1S in the BC_1 gave a good χ^2 fit in both the seasons that results from the dominant allele of any one, two or all three duplicate genes. In the

ICMB 88004 genetic background, the trigenic ratio of 57F:7S in the F_2 and 3F:1S in the BC_1 had a good χ^2 fit in both the seasons (Figure 17). Such segregation pattern could result from a gene interaction in which the fertility is restored by the dominant allele of one of the three genes on its own; alternatively, dominant allele at both of the remaining loci would also restore male-fertility.

Following the same three-gene system for the other CMS systems, the postulated genotypic constitution of the A-lines could be 'aabbCC' and that of the restorer line could be 'AABBcc'. The genotypic constitution of F_2 and BC_1 with respect to the above trigenic ratios has been presented in Figures 14, 17 and 18. The trigenic F_2 ratio of 54F:10S and the BC_1 ratio of 3F:1S is possible with a gene action in which a plant will be fertile with the genotypes 'A_B_C_', 'A_B_cc', 'A_bbC_' and 'aaB_C_'. The trigenic F_2 ratio of 57F:7S and the BC_1 ratio of 3F:1S is possible with a gene action in which a plant will be fertile with the genotypes 'A_B_C_', 'A_B_cc', 'A_bbC_', 'aaB_C_' and 'A_bbcc'. The trigenic F_2 ratio of 63F:1S and the BC_1 ratio of 7F:1S is possible with a gene action in which a plant will be fertile with the genotypes 'A_B_C_', 'A_B_cc', 'A_bbC_', 'aaB_C_', 'A_bbcc', 'aaB_cc' and 'aabbC_' (Figure 18).

What was most interesting with this CMS system was that the interaction of restorer genes varied depending on the genetic background of the A-line. But what was also most interesting with this CMS system was that the genetic ratios were consistent across the seasons for both F_2 and BC_1 populations and that the genetic hypothesis proposed on the basis of genetic ratios in the F_2s were fully supported by the corresponding genetic ratios in BC_1 populations for each of the A-lines.

Based on the Mendelian ratios obtained, the designated genotypes of $81A_5$/B could be *rf*5a *rf*5a *rf*5b *rf*5b *Rf*5c *Rf*5c and for $5054A_5$/B and $ICMA_5$/B 88004, it could be *rf*5a *rf*5a *rf*5b *rf*5b *rf*5c *rf*5c. Although the A_5 restorer was produced from prolonged inbreeding and single plant selection, genetic variability for fertility restorer genes in the line could have existed, leading to some genotypes being *Rf*5a *Rf*5a *Rf*5b *Rf*5b *rf*5c *rf*5c and others being *Rf*5a *Rf*5a *Rf*5b *Rf*5b *Rf*5c *Rf*5c (Table 50). Since linkage studies of fertility restorers of A_5 and one or more of the other CMS systems were not done, it could be that one or more of the loci involved in the fertility restoration of A_5 system might be same as those in the other systems, but with different alleles.

5.5 Inheritance of the A_v CMS system

The restorer parent IPC 382 gave a good χ^2 fit to the trigenic F_2 ratio of 63F:1S during the rainy season and to the BC_1 ratio of 7F:1S in both the seasons in 81B background that results from the dominant allele of any one, two or all three of the duplicate genes (Table 49). During the summer season, there was deviation from 63F:1S F_2 ratio due to excess of sterile plants. The same restorer line also gave good fit to the F_2 ratio of 57F:7S and to the BC_1 ratio of 3F:1S in 5054B and ICMB 88004 backgrounds during both rainy and summer season that results from a gene interaction in which the fertility is restored by the dominant allele of one of the three genes on its own. Alternatively, dominant allele at both of the remaining loci would also restore male-fertility. Another restorer line L 67B gave a good χ^2 fit to the trigenic F_2 ratio of 54F:10S in the genetic background of all three A-lines in both the seasons. The segregation in BC_1 also gave a good χ^2 fit to 3F:1S ratio in the genetic background of 81B in both the seasons and in the genetic background of ICMB 88004 in the summer season. In the remaining three cases, the segregation ratio of fertile and sterile plants was close to expected 3F:1S ratio, but the χ^2 was poor (χ^2= 10.25-32.36) due to excess of sterile plants in all the cases, irrespective of the growing season. Thus, although involvement of three genes in the fertility restoration of A_v CMS system was observed, the difference in the inheritance pattern of fertility restoration varied with the restorers and also with the genetic background of A-lines.

The trigenic F_2 ratio of 63F:1S and the BC_1 ratio of 7F:1S is possible with the genetic constitution of female parent ($81A_v$/B) 'aabbcc' and the male parent 'AABBCC' (IPC 382) as explained in the inheritance of A_5 CMS system and the trigenic F_2 ratio of 57F:7S and the BC_1 ratio of 3F:1S is possible with the genetic constitution of female parents ($5054A_v$/B and $ICMA_v$/B 88004) 'aabbCC' and the male parent 'AABBcc'. Further, the trigenic F_2 ratio of 54F:10S and the BC_1 ratio of 3F:1S is possible with the genetic constitution of female parent 'aabbCC' and the male parent 'AABBcc'as explained in the A_{cgp} CMS system. Hence, the inheritance of fertility restoration in A_v CMS system is likely to be controlled by trigenic inheritance mechanism.

Based on the genetic ratios obtained in the present study, the likely designated genotypes of the female parents could be *rf*va *rf*va *rf*vb *rf*vb *Rf*vc *Rf*vc for all three A/B lines except *rf*va *rf*va *rf*vb *rf*vb *rf*vc *rf*vc for $81A_v$/B when involved with IPC 382 with the genotype *Rf*va *Rf*va *Rf*vb *Rf*vb *Rf*vc *Rf*vc. In all other cases, the probable genotypes of the restorer parents involved could be *Rf*va *Rf*va *Rf*vb *Rf*vb *rf*vc *rf*vc (Table 50). Since

linkage studies of fertility restorers of A_v and one or more of the other CMS systems discussed earlier were not done, it could be that one or more of the loci involved in the fertility restoration of A_v system might be same as those in the other systems, but with different alleles.

5.6 Linkage between fertility restorer genes of A_1 and A_4 CMS systems

Linkage analysis was done between the fertility restorer genes of the A_1 and the A_4 CMS systems. Two (B × R)-derived F_2 and BC_1 populations from the crosses, 81B × IPC 804 and 5054B × IPC 511 were produced. The cross 81B × IPC 804 also served as the mapping population for fertility restorer genes of the A_1 and the A_4 CMS systems. The individual plants of F_2 and BC_1 populations of both the crosses were testcrossed onto respective A-lines of the A_1 CMS system ($81A_1$ and $5054A_1$) and A_4 CMS system ($81A_4$ and $5054A_4$). The testcrosses were evaluated in summer and rainy seasons 2003 at ICRISAT- Patancheru and data was collected on fertility (F) and sterility (S) reaction pattern of the testcross progenies. The testcross progenies were scored as fully fertile (F), segregating for fertile and sterile (F+S) plants and fully sterile (S).

In the cross 81B × IPC 804, the testcross segregation pattern for the A_1 CMS system gave a good χ^2 fit to the F_2 ratio of 1F:2 (F+S):1S and to the BC_1 ratio of 1(F+S):1S in both summer and rainy season, which was reflective of the trigenic ratio of 45F:19S in the F_2 and 1F:1S in the BC_1. However, for the A_4 CMS system, the segregation pattern didn't gave a good χ^2 fit to the 1F:2 (F+S):1S ratio in the F_2 and 1 (F+S):1S ratio in the BC_1. Joint segregation ratio in testcrosses of the F_2 plants produced nine classes (Table 39) and gave a significant χ^2 value in both summer and rainy seasons. Similarly, the joint segregation ratio in testcrosses of the BC_1 plants produced four classes and gave a significant χ^2 value in both summer and rainy seasons (Table 40). These results indicated the presence of linkage between fertility restorer genes of the A_1 and the A_4 CMS systems. But this indication of linkage could also likely result from the distortion in assessing the joint segregation because of a poor χ^2 fit to the segregation pattern of testcrosses in the A_4 CMS system (1F:2 (F+S):1S ratio in the F_2 and 1(F+S):1S) in the BC_1) that is expected from a trigenic F_2 ratio of 45F:19S and BC_1 ratio of 1F:1S.

In the cross 5054B × IPC 511, segregation pattern in the testcrosses of F_2 plants for the A_1 CMS system gave a poor χ^2 fit to the expected F_2 trigenic ratio of 7F:8 (F+S):1S and BC_1 ratio of 3 (F+S):1S in both the summer and rainy season (except BC_1

in summer season). The trend of the segregation was least reflective of trigenic F_2 ratio of 54F:10S and BC_1 ratio of 3F:1S but more indicative of the trigenic ratio of 45F:19S in the F_2 and 1F:1S in the BC_1. In contrast, for the A_4 CMS system, test cross segregation pattern gave a good χ^2 fit to the expected F_2 ratio of 1F:2 (F+S):1S ratio and BC_1 ratio of 1 (F+S):1S ratio in both the seasons. The expected joint segregation ratio in the testcrosses of the F_2 and BC_1 plants gave a poor χ^2 fit in both summer and rainy seasons (Tables 41 and 42). These distortions in the joint segregation ratios in the F_2 and BC_1 could be due to linkage between fertility restorer genes of A_1 and A_4 CMS systems or due to the poor χ^2 fit of the segregation pattern of A_1 CMS testcrosses which contributed to the overall distortion of the joint segregation ratio. But, it can be safely concluded from the results of the two crosses studied for linkage analysis that the fertility restorer genes of the A_1 and A_4 CMS systems were linked.

5.7 Molecular mapping of fertility restoration of the A_1 and A_4 CMS systems

For mapping the fertility restorer genes of A_1 and A_4 CMS systems, the 81B × IPC 804 mapping population (part of material produced for linkage studies) was used. Complete genotyping and phenotyping data from 397 F_2 plants was used to construct a linkage map using 36 markers (22 SSR, 11 RFLP and three morphological markers). The three morphological markers were d_2/non-d_2 plant type, bristling (*Br*) and leaf pubescence or hairiness (*hl*). The F/S reaction pattern of testcrosses was recorded on plot basis and data converted into % fertile class for marker analysis. Most of the markers used in the construction of linkage map have been mapped previously by Liu *et al.* (1994) and Qi *et al.* (2004). The level of polymorphism for SSR markers was 34% and for RFLP markers was 28%.

In the present study, 10 marker loci showed segregation distortion and of these five were SSR markers, three RFLP markers and two morphological markers. Most of the distorted markers were placed on LG 2, LG 4 and LG 7. LG 2 was most severely affected by segregation distortion as only one marker (*Xpsm*708.1) showed normal segregation out of five markers placed on it, followed by LG 4, where four out of seven marker loci exhibited significant distortion. Segregation distortion of marker loci is a common phenomenon in many crop species. In the present study a comparatively smaller proportion (26%) of distorted markers was observed compared to some earlier studies in pearl millet where up to 60% distortion was observed (Gulia, 2004). Segregation distortion is most commonly observed in inter-specific crosses; however,

previous studies have showed distortion phenomenon occurring in intra-specific pearl millet crosses (Liu et *al.* 1994; Busso *et al.* 1995).

Using a total of 36 marker loci, a genetic linkage map of 708.8 cM (Haldane) was constructed for the pearl millet F_2 mapping population based on cross 81B × IPC 804 (Figure 10, Table 52). The map length of individual linkage groups varied from a minimum of 20.5 cM (LG 5) to a maximum of 208.5 cM (LG 7) as shown in Figure 10. Three previously unmapped markers viz. *Xpsmp*2080, *Xpsmp*2068 and *Xicmp*3022 were mapped to LG 1, LG 3 and LG 6, respectively, whereas *Xpsmp*2225, which was mapped on LG 2 by previous workers, has been mapped on LG 4 in the present study. The average distance between markers came out to be 19.7 cM, which is near optimal for interval mapping provided that distribution of markers across the map is uniform.

The pearl millet consensus map given by Qi *et al.* (2004) that contains 242 loci and covers 473 cM, was used as a reference map for assigning linkage groups and confirming marker order. The pearl millet map published by Liu *et al.* (1994) from the cross LGD 1-B-10 × ICMP 85410 contained 181 loci, but spanned only 303 cM. The present linkage map is longer in map length than earlier maps but almost equal to some of the recent maps developed in pearl millet (Gulia, 2004). The mapping population size (397 individual) of the present study surpasses the size of earlier pearl millet mapping populations significantly (Table 52). Adequately large population with less markers is better than more markers but with a small population size (Doerge, 2002). Pearl millet maps all exhibit an interesting feature in the presence of large gaps suggesting high recombination rates in the distal chromosome regions and the present study is no exception to this phenomenon. Qi *et al.* (2004) further postulate that large gaps in the distal regions of the genetic map represent regions of high recombination rate rather than being caused by lack of polymorphic markers.

The complete data set available for 397 F_2 individuals was analysed for QTL detection and estimation using MAPMAKER/QTL version 1.1b (Lincoln *et al.* 1992b) and PLABQTL version 1.1 (Utz and Melchinger, 2000). The mapping population was developed with the twin objectives to identify molecular markers linked to fertility restoration genes of the A_1 and A_4 CMS systems. QTL mapping approach for fertility restoration has been adopted by few workers in crops such as sugar beet (Hjerdin-Panagopoulus *et al.* 2002), rice (Yao *et al.*, 1997; Tan *et al.*, 1998; Xie *et al.* 2002) and *Secale* (Stojalowski *et al.* 2004). The phenotypic distribution of the testcrosses in the F_2 population of cross 81B × IPC 804 for the A_1 CMS system (Figure 11) was W-shaped

Table 52. Total mapped genome and linkage group lengths for seven pearl millet mapping populations

Mapping populations	1	2	3	4	5	6	7	8**
Pedigree LG	LGD 1-B-10 × ICMP 85410	81B-P6 × ICMP 451-P_8	W 504-1-1 × P310-17-B	IP 18293 × Tift 238D_1	PT 732B-P_2 × P1449-2-P_1	ICMB 841-P_3 × ICMB 863-P_2	ICMB 89111-P_6 × ICMB 90111-P_6	**81B × IPC 804**
1	73.4	77.3	113.8	58.9	172.6	104.9	139.6	**28.6**
2	36.2	175.8	31.6	136.4	87.9	179.0	192.3	**165.7***
3	38.2	52.2	24.2	62.9	27.6	15.4	30.2	**89.1**
4	63.2	132.4	116.6	110.9	100.0	64.3	98.3	**140.3**
5	30.9	102.8	37.5	51.9	30.2	26.9	50.1	**20.5**
6	32.5	58.3	57.6	67.8	83.1	113.1	42.2	**56.1**
7	13.3	96.9	39.7	24.7	37.6	113.8	195.2	**208.5**
$Total mapped genome length	287.7	695.7	421.0	513.5	539.0	617.4	747.9	**708.8**
Mapped (F_2) population size	133	184	175	142	136	147	172	**397**
Reference	Liu et al., 1994	Devos *et al.*, 2000	Kolesnikova-Allen, 2001	Azhaguvel, 2001	Nepolean, 2003	Yadav *et al.*, 2004	(Gulia, 2004)	**Present study**

*Combined map length of LG 2a and LG 2b

**The presently studied cross 81B × IPC 804 is shown in bold font

$The map distances are shown in Haldane centimorgans (cM) except for mapping population 1 for which the Kosambi mapping function was used

with two extremes representing the almost equal proportions of completely sterile and completely fertile testcrosses. The segregating testcrosses formed a normal curve as expected. But the distribution pattern of the A_4 CMS system testcrosses (Figure 12) was highly skewed towards the sterile class with the majority of testcrosses falling in the 0-10% fertile class.

For the A_1 CMS system, MAPMARKER/QTL approach (SIM analysis) identified two QTL for fertility restoration (Table 44, Figure 10) in the summer season. Of these, one major QTL, designated as *Rf*1a, was located at a distance of 4 cM from RFLP marker *Xpsm*223 on bottom of LG 1 and flanked on other side by morphological marker *Br*. In the summer season, this QTL accounted for about 30% of the phenotypic variation for A_1 CMS system fertility restoration and had a high LOD value of 23.88. In the rainy season, this QTL explained 33.1% of phenotypic variation with an even higher LOD value of 26.34 indicating consistent expression of this major QTL across the two seasons. The second QTL, a minor one designated as *Rf*1b, was detected only in the summer season. It was positioned on LG 4 between marker loci *Xpsm*409.1 and *Xpsmp*2225 with a LOD of 2.03 and explained 5.1% of observed phenotypic variation in this season. The CIM approach in PLABQTL also detected only one QTL for this trait in both the summer and rainy seasons, positioned on LG 1 and flanked by marker loci *Xpsm*223 and *Br*.

For the A_4 CMS system, the SIM analysis in MAPMAKER/QTL revealed a single genomic region of large effect (Table 44, Figure 10) on LG 2a explaining 30% of the observed phenotypic variance in the summer season testcross male fertility restoration with a LOD score of 25.7. This putative major QTL is designated as *Rf*4a and is flanked by *Xpsmp*2072 and *Xpsmp*2077. The best two-QTL model explaining 56.1% of the phenotypic variance with a high LOD score of 36.1 (>25.7+2.0) was accepted as better than the single–QTL model for this trait. This two-QTL model included the single-QTL identified earlier and a second minor QTL, designated as *Rf*4b, on LG 2b flanked by marker loci *Xpsmp*2059 and *Xpsmp*2237. But as LG 2 is subdivided into LG 2a and LG 2b in the present study, it could be quite possible that this minor QTL is actually a part of the major QTL. In the rainy season, the best two-QTL model flanked by same markers as in the summer season and explaining 41.2% of the phenotypic variance with a LOD score of 34.1 (>28.7+2.0) was accepted as better than the single-QTL model.

The CIM approach in PLABQTL (Table 45) detected the same QTL as detected by the SIM analysis of MAPMAKER in the summer and rainy seasons but with a few

additional minor QTL in the rainy season. In summer, the major QTL, *Rf*4a, explained 51.3% of the phenotypic variance for testcross fertility restoration percentage with a LOD score of 36.55 and a significant additive effect of 4.87. In the rainy season, this major QTL explained 48.1% of phenotypic variance for this trait with a LOD score of 33.34 and significant additive effect of 4.60. The minor QTL, *Rf*4b, in summer season accounted for 5.1% and 3.7% of observed phenotypic variance in summer and rainy seasons, respectively. In the rainy season, two additional minor QTL were detected but only one with significant additive effect. This minor QTL, designated as *Rf*4c is located on LG 6 between marker loci *Xpsmp*2048 and *Xpsm*202 with a LOD score of 2.12 and explaining 4.3% of phenotypic variance. For the A_4 CMS system, QTL detected exhibited additive effect of about 5% but still explained about 50% of observed phenotypic variance in fertility restoration percentage. This might be due to the highly skewed phenotypic distribution of testcrosses of this system in both the summer and rainy seasons (Figure 12) and also due to lesser number of markers in the present study leading to large gaps on the linkage map.

Three map free approaches viz. single marker approach (SMA) using simple linear regression and two multiple marker approaches, namely step wise regression (SWR) and Bayesian information criterion (BIC) (Tables 46-48) were used to identify markers linked to putative QTL for fertility restoration in A_1 and A_4 CMS systems. For this analysis, only the 26 distortion-free markers were included and the genotypic data was converted into numeric codes. The most important marker selected by the SMA approaches and found to be linked with fertility restoration of the A_1 CMS system was *Xpsm*223 explaining 26.08% and 26.72% of phenotypic variance for the testcross fertility restoration percentage in summer and rainy seasons, respectively (Table 46). This marker was consistently selected by the more stringent SWR approach with the same level of contribution to phenotypic variance. The Bayesian information criterion also selected the marker *Xpsm*223 in both summer and rainy seasons with 22.46 and 24.80% contribution to phenotypic variance, respectively. Thus we found similarity in the map-based and map-free approaches in the identification of a common marker in both methods. Similar observations were also observed in chickpea by Chandra *et al.* (2004) and in rice by Tan *et al.* (1998). As marker *Xpsm*223 was linked to a putative QTL, *Rf*1a, for fertility restoration of the A_1 CMS system, it can be used for marker-assisted selection of restorer and maintainer lines with the supplement of marker *Xpsmp*2080 and the morphological marker, *Br*, governing panicle bristling. The SSR

marker *Xpsmp*2080 has also been identified by SMA to be an important marker significantly linked to fertility restoration of the A_1 CMS system. For the A_4 CMS system, the map-free approaches failed to identify any significantly linked marker to fertility restoration.

Thus, it can be inferred that the map-free approach seems to reliably identity important markers that would also tend to be identified by map-based approach, as seen in present study, at least for the A_1 CMS system. The phenotypic distribution pattern of A_4 CMS system testcrosses (Figure 12) is highly skewed towards the sterile class with majority of testcrosses falling in the 0-10% fertile class. This may be one of the reasons for not being able to identify markers linked to fertility restoration of A_4 CMS system by the map-free approach. A second explanation for this failure would be the inadequate genome coverage by the markers used in this study. Rectification of this second explanation, which would facilitate the map-based approach, would require identification of additional polymorphic markers that could fill current gaps or extend linkage groups.

5.8 General discussion

Overall, in 36 A × R crosses in the five CMS systems (A_1, A_4, A_v, A_{egp} and A_5), out of 72 cases (36 each in summer and rainy season), 30 cases (14 in summer and 16 in rainy season) gave a good χ^2 fit to the trigenic ratio of 54F:10S in the F_2 and the corresponding ratio of 3F:1S in the BC_1 (Table 49) that results from a gene action involving any two of the three dominant duplicate-complimentary genes. In another 16 cases (8 each in summer and rainy seasons) in three CMS systems (A_1, A_4 and A_{egp}), the segregation pattern gave a good fit to the trigenic ratio of 45F:19S in the F_2 and the corresponding ratio of 1F:1S in the BC_1 that results from a gene action involving dominant alleles of one basic gene and two duplicate-complimentary genes. In another 6 cases, the trigenic ratio of 57F:7S in the F_2 and 3F:1S in the BC_1 was observed to give a good χ^2 fit in the A_5 and A_v CMS systems. Such segregation pattern could result from a gene interaction in which the fertility is restored by the dominant allele of one of the three genes on its own; alternatively, by dominant allele at both of the remaining loci. The trigenic ratio of 63F:1S in the F_2 and the corresponding ratio of 7F:1S in the BC_1 was observed in five cases in the A_1, A_5 and A_v CMS systems.

Thus, in majority of crosses in the five CMS systems, a three-gene inheritance mechanism represented by trigenic ratios that result from different gene actions, was most likely to be operating. Among the different ratios, the two most prevalent trigenic

ratios were 54F:10S and 45F:19S in the F_2 and 3F:1S and 1F:1S in the BC_1. These trigenic F_2 ratios and their corresponding BC_1 ratios are possible when the genotype of the female parent is 'aabbCC', possessing the dominant allele of one of the two duplicate-complimentary genes for fertility restoration (in this case 'C' gene), and the genotype of the restorer parent is 'AABBcc', with the dominant allele of the basic gene (A) and dominant allele of the other duplicate-complimentary gene (B). In breeding for maintainer/sterile lines, the trigenic inheritance mechanism represented by dominant alleles of one basic gene and two duplicate-complimentary genes (45F:19S in the F_2 and 1F:1S in the BC_1) would give uniformly sterile lines in 80% of the cases as compared to 50% in the trigenic inheritance mechanism represented by dominant alleles of any two of the three duplicate-complimentary genes (54F:10S in the F_2 and 3F:1S in the BC_1) and only 30% in case of the trigenic inheritance mechanism represented by dominant allele of one of the three genes on its own or alternatively, dominant alleles at both of the remaining duplicate-complimentary genes restoring fertility (57F:7S in the F_2 and 3F:1S in the BC_1) (Table 53).

In the A_1 CMS system, the trigenic ratio of 54F:10S in the F_2 and 3F:1S in the BC_1 was exhibited mostly in the 5054B genetic background whereas the trigenic ratio of 45F:19S in the F_2 and 1F:1S in the BC_1 was present in the 81B and ICMB 88004 backgrounds. However, the inheritance pattern from the testcross data as well as information from the [(A × R) × (B × R)]-derived F_2 and from the selfed seed-set data in the 5054B background suggested that the observed trigenic ratio of 54F:10S could be considered as an escalation of 45F:19S ratio either due to pollen shedding behavior of 5054A or due to plants with 'aaB_C_' genotypes behaving as fertile. The presence of 54F:10S ratio in the F_2 and 3F:1S in the BC_1 in the genetic background of 5054B may also result from some of the crosses possibly made on pollen-shedding tiller panicles of 5054A. Although all the crosses made on 5054A were harvested only from those plants whose tiller panicle had been scored as fully sterile under selfing condition, the possibility of a pollen-shedding tiller panicle of the same plant involved in crosses can not be ruled out as these crosses were made during the rainy season. Pearl millet A-lines have been shown to produce a higher frequency of pollen-shedders in the rainy season than in the summer season, the more so in case of 5054A. (K.N. Rai, pers. comm.).

Deviation from expected ratio was observed mostly in one of the two seasons in 21 segregating populations (7 F_2s and 14 BC_1s) in all the CMS systems except the A_5 system. The 5054B background had a maximum of nine segregating populations (2 F_2s

Table 53. Potential maintainer (B-line) genotypes of three-gene mechanism for male-sterility in pearl millet with aabbCC genotype of A-line

Genotype		F_1		Ratio (F:S)		
A-line	**Maintainer**	**Genotype**	**F/S**	**F_2**	**BC_1**	**BC_1 (F_1 × maintainer)**
Three-gene mechanism: One basic gene; two duplicate-complimentary genes (F_2 ratio: 45F:19S; BC_1 ratio: 1F:1S)						
aabbCC	AAbbcc	AabbCc	F	9:7	1:1	1:1
	aaBBCC	aaBbCC	S	0:1	0:1	0:1
	aaBBcc	aaBbCc	S	0:1	0:1	0:1
	aabbCC	aabbCC	S	0:1	0:1	0:1
	aabbcc	aabbCc	S	0:1	0:1	0:1
Three-gene mechanism: Any two of the three duplicate-complimentary genes (F_2 ratio: 54F:10S; BC_1 ratio: 3F:1S)						
aabbCC	AAbbcc	AabbCc	F	9:7	1:1	1:1
	aaBBcc	aaBbCc	F	9:7	1:1	1:0
	aabbCC	aabbCC	S	0:1	0:1	0:1
	aabbcc	aabbCc	S	0:1	0:1	0:1
Three-gene mechanism: Dominant allele of one of the three genes on its own restores fertility; alternatively, dominant alleles at both of the remaining loci would also restore fertility (F_2 ratio: 57F:7S; BC_1 ratio: 3F:1S)						
aabbCC	aaBBcc	aaBbCc	F	9:7	1:1	1:1
	aabbCC	aabbCC	S	0:1	0:1	0:1
	aabbcc	aabbCc	S	0:1	0:1	0:1

and 7 BC_1s) followed by six populations each in the 81B (3 F_2s and 3 BC_1s) and ICMB 88004 (2 F_2s and 4 BC_1s) backgrounds that exhibited deviation from the expected ratios. The overall results indicated that the inheritance pattern for all the CMS systems is likely to be governed by a trigenic mechanism represented in a majority of cases either by a 54F:10S ratio in the F_2 and 3F:1S in the BC_1 or a 45F:19S ratio in the F_2 and 1F:1S in the BC_1. Three-gene control of fertility restoration has been reported for A_1 and A_2 CMS systems in sorghum (Lonkar and Borikar, 1994), the CMS-D_{2-2} system of cotton (da Silva, 1981; Maranhao et al., 1984), ogu CMS of Raphanus (Bett and Lydiate, 2004) and G-type CMS system of rye (Melz and Adolf, 1991).

Although trigenic inheritance mechanism has been found to be operative for majority of the crosses of all the CMS systems, the manifestation of digenic ratio of 15F:1S in the F_2 and 3F:1S in the BC_1 in crosses involving the restorer L 67B and the F_2 ratio of 9F:7S and BC_1 ratio of 1F:3S in the BC_1 in cross involving IPC 804 suggests that in these cases, only two of the three genes segregate (Table 51). It is interesting to note that these ratios could result with the same female genotype of 'aabbCC' but a different male parent genotype than the one giving trigenic ratios. There are a good number of studies that report a digenic inheritance mechanism such as in pearl millet for the A_1 and A_2 CMS systems (Siebert, 1982), sorghum for A_1 CMS (Miller and Pickett, 1964; Schertz *et al.*, 1989; Lonkar and Borikar, 1994), A_2 CMS (Murty and Gangadhar, 1990), A_3 CMS (Tang *et al.*, 1998; Pring *et al.*, 1999), and for 9E and A_4 CMS (Elkonin *et al.*, 1998), in the cotton for CMS-D_{2-2} system (Meyer, 1975) and in maize for CMS-T system (Laughnan and Gabay-Laughnan, 1983).

Similarly, in a three-gene mechanism, there is also a possibility of getting a one-gene ratio (Table 51). Monogenic ratio of 3F:1S in the F_2 and 1F:1S in the BC_1 was observed in the A_4 CMS system crosses involving the restorer IPC 511. This ratio is also possible with the genotype of female parent as 'aabbCC' and male parent as 'AAbbCC' as hybrid of these would segregate only for one loci. Several reports indicating the monogenic mode of inheritance of fertility restoration have been reported in pearl millet for the A_1, A_2 and A_3 (Burton, 1966; Burton and Athwal, 1967) and A_4 (Du *et al.*, 1996) CMS systems; in sorghum for the A_1 (milo) CMS system (Schertz *et al.*, 1989; Murty and Gangadhar 1990), and for the 9E and A_4 CMS systems (Elkonin *et al.*, 1998); in maize for CMS-T (Blickenstaff, 1958), CMS-S (Kheyr-Pour *et al.*, 1981), and CMS-C (Laughnan and Gabay, 1978); in wheat for CMS-*timopheevi* (Tahir and Tsunewaki, 1967); in rice for CMS-bo (Teng and Shen, 1994), CMS-BT (Komori *et al.*, 2003),

CMS-HL (Huang *et al.*, 2000), and CMS-Dian Type-1 (Tan *et al.*, 2004) and in cotton for CMS-D_8 and CMS-$D_{2\text{-}2}$ (Zhang and Stewart, 2001a, b). Therefore, it is quite possible that the monogenic and digenic inheritance mechanisms reported in majority of earlier studies could have resulted from variable genetic constitution of parents actually being a part of the trigenic inheritance mechanism.

The linkage analysis revealed that the fertility restorer genes of A_1 and A_4 CMS system could be linked to each other as exhibited by significant χ^2 values in the joint segregation analysis. Since linkage studies of fertility restorers of other CMS systems were not done, it could be that one or more of the loci involved in the fertility restoration of these systems might be same, but with different alleles. This is more likely to be so for the A_{egp} and the A_1 CMS systems have similar behaviour in fertility restoration pattern of their hybrids except that more inbreds produce sterile hybrids on A_{egp} than on the A_1 system A-lnes (K.N. Rai, unpubl.).

The precise linkage relationship among the fertility restorer genes of the two CMS systems can be better worked out using molecular marker technology. The molecular marker analysis of the F_2 mapping population based on the cross 81B × IPC 804 detected two putative QTL (*Rf1a* and *Rf1b*), which are likely to be involved in the fertility restoration of A_1 CMS system. One of these QTL, *Rf1a* is a major QTL and *Rf1b* is a minor QTL. The markers linked to QTL identified with fertility restoration of A_1 CMS system were the RFLP marker *Xpsm*223 and the panicle bristling marker (*Br*) located on LG 1. Similarly, for the A_4 CMS system, three putative QTL were identified by QTL mapping approach (one major QTL, *Rf4b* and two minor QTL, *Rf4b* and *Rf4c*). The markers linked to QTL identified with fertility restoration of A_4 CMS system were the SSR marker *Xpsmp*2072 and *Xpsmp*2072, mapped on LG 2.

It was interesting to note that the QTL for the A_1 and A_4 CMS systems were located on different linkage groups and no common marker linked with fertility restoration of these CMS systems was identified.

The present molecular study represented only a beginning in identifying QTL of fertility restorer genes as less number of polymorphic markers still presented large gaps on the linkage map. This might be the reason for not being able to detect the third QTL. It is quite possible that one of the two QTL could be the basic gene and another QTL is the same as one of the two duplicate-complimentary genes as observed in the trigenic inheritance mechanism represented by the Mendelian ratio (45F:19S F_2 ratio) obtained from the A × R crosses. There is a need to add more markers to saturate the linkage map

with reasonably placed markers at 10-15 cM distance from each other to identify the third QTL and to estimate the realistic effect and location of these QTL. The reasons for not being able to identify markers that are closely linked to fertility restoration of these CMS systems might be due to the inadequate genome coverage by the markers used in this study. Rectification of this second explanation could be achieved by placing polymorphic markers at a desired regular interval of 10-15 cM as this would facilitate the precise identification of genomic regions responsible for fertility restoration with closely spaced markers. This would further enable marker-assisted classification of lines as either maintainer (B-line) or restorer (R-line) without the need for field evaluation of testcrosses; and it would also permit their rapid backcross transfer of fertility restorer genes in elite inbred lines.

6. SUMMARY

6. SUMMARY

The present investigation was undertaken to determine the inheritance of five diverse CMS systems (A_1, A_4, A_{egp}, A_5 and A_v), allelic relationship of fertility restorer genes in A_1 and A_4 CMS systems, genetic linkage between the fertility restorer genes of the A_1 and A_4 CMS systems, and identification of molecular markers linked to fertility restoration of these two CMS systems in pearl millet. The plant material for the study consisted of isonuclear A-lines of the five diverse male-sterility-inducing cytoplasm in three diverse nuclear genetic backgrounds (81B, 5054B and ICMB 88004) and six pollen parents restoring the male fertility of hybrids based on any one, two or three male-sterile cytoplasm. The segregation patterns of male-fertile (F) and male-sterile (S) plants were studied in F_2, BC_1 [A × (A × R)] and BC_2 [R × (A × R)] populations produced from A × R crosses, F_2s produced from some of the (A × R) × (B × R) crosses, and testcrosses obtained by crossing A-lines with individual plants of (B × R)-derived F_2 and BC_1 populations. The F_1 hybrids and BC_2 populations produced from A × R crosses for all the five CMS systems had all plants fully fertile across the two test environments of summer and rainy seasons, indicating that male-fertility is dominant over male-sterility. The segregation patterns for F and S plants observed in the F_2 and BC_1 populations were tested for χ^2 goodness of fit for monogenic, digenic and trigenic ratios to determine the number of genes involved in the fertility restoration of each of the five cytoplasmic-genic male-sterility (CMS) systems. A summary of the results obtained in segregating populations and testcrosses included in the present study conducted at ICRISAT-Patancheru is given below.

For the A_1 CMS system, the segregation pattern in crosses involving three restorer parents (IPC 1518, IPC 511 and IPC 804) and A-lines in the genetic backgrounds of 81B and ICMB 88004 generally gave a good χ^2 fit to the expected trigenic ratios of 45F:19S in the F_2 and 1F:1S in the BC_1, that may result from a gene action involving one basic gene and two duplicate-complimentary genes. The goodness of fit in the crosses involving the same restorer parents with the A-line in the 5054B genetic background gave generally a good fit to the expected ratio of 54F:10S in the F_2 and 3F:1S in the BC_1, that may result from a gene action involving any two of the three dominant duplicate-complimentary genes. The A-lines, especially $5054A_1$, have been observed to have a low frequency of plants giving upto

5% seed set when selfed. Thus, when male-fertile plants with 0-5% selfed seed set were classified as sterile in the segregating populations (F_2 and BC_1) derived from cross 5054A_1 × IPC 511, the resulting segregation gave a good χ^2 fit to the expected trigenic ratios of 45F:19S in the F_2 and 1F:1S in the BC_1.

F_2 and BC_1 populations derived from two B × R crosses (81B × IPC 804 and 5054B × IPC 511) were used for linkage analysis between fertility restorer genes of the A_1 and A_4 CMS systems. Individual plants from the F_2 and BC_1 populations from the cross 81B × IPC 804 were crossed both on 81A_1 and 81A_4 to study the co-segregation of restorer genes of both CMS systems. Similarly, individual plants of the F_2 and BC_1 populations from the cross 5054B × IPC 511 were crossed both on 5054A_1 and 5054A_4. The testcross progenies were scored as fully fertile (F), segregating for fertile and sterile (F+S) plants and fully sterile (S). These progeny classes gave a good χ^2 fit to 1F:2 (F+S):1S ratio where F_2 plants were involved in testcrosses and 1(F+S):1S where BC_1 plants were involved in the testcrosses with respect to the A_1 CMS system. These segregation patterns were supportive of trigenic inheritance that had given a ratio of 45F:19S in the F_2 population and 1F:1S in BC_1 population in the cross 81A_1 × IPC 804. However, these patterns were as well reflective of monogenic inheritance that gives 3F:1S ratio in the F_2 and 1F:1S in the BC_1. The segregation patterns in the testcrosses made with F_2 and BC_1 plants derived from the cross 5054B × IPC 511 were not supportive of the trigenic inheritance that had given a ratio of 54F:10S in the F_2 population and 3F:1S in BC_1 population.

The test of allelism determined from the fertility/sterility reaction of three-way hybrids obtained by crossing A-lines of A_1 CMS system (81A_1) with the F_1s of inter-crosses among three R-lines (IPC 1518, IPC 511 and IPC 804) indicated the presence of the same alleles at all the three loci in these lines as all the three-way hybrids were completely fertile.

Most of the evidences in the A_1 CMS system supported the likely involvement of the dominant alleles of one basic gene and at least any one of the two duplicate-complimentary genes in the fertility restoration. The likely genetic constitution is suggested to be *rf*1a *rf*1a *rf*1b *rf*1b *Rf*1c *Rf*1c for A-lines and *Rf*1a *Rf*1a *Rf*1b *Rf*1b *rf*1c *rf*1c for the restorer parents.

Although trigenic inheritance mechanism has been found to be operative in majority of the crosses of the A_1 CMS system, in four segregating populations (2 F_2s and 2 BC_1s) derived from two crosses involving L 67B restorer parent and 5054A and

ICMA 88004 seed parents, a digenic F_2 ratio of 15F:1S and the corresponding BC_1 ratio of 3F:1S that results from dominant alleles of either one or both of the duplicate genes, was observed. This segregation pattern could have resulted from the same gene action that gives a three-gene ratio. In this digenic case, the restorer parent would have dominant alleles at all three loci (*Rf*1a *Rf*1a *Rf*1b *Rf*1b *Rf*1c *Rf*1c), which makes the F_1 heterozygous at only two loci as the A-line has *rf*1a *rf*1a *rf*1b *rf*1b *Rf*1c *Rf*1c genotype. The segregating generations from this F_1 would obviously give a 2-gene ratio as observed.

In the A_4 CMS system, the two trigenic ratios 45F:19S in F_2 and 1F:1S in the BC_1; or 54F:10S in the F_2 and 3F:1S in the BC_1, like those observed for the A_1 CMS system, were more prevalent, which would result from gene interactions suggested before.

Individual plants from the F_2 and BC_1 populations from the crosses 81B × IPC 804 and 5054B × IPC 511 were testcrossed onto $81A_4$ and $5054A_4$ A-lines of the A_4 CMS system, respectively. The testcross progenies gave a good χ^2 fit to 1F:2 (F+S):1S ratio where F_2 plants were involved in the testcrosses and 1(F+S):1S where BC_1 plants were involved in the testcrosses with respect to the A_4 CMS system. These segregation patterns were supportive of monogenic inheritance with the observed 3F:1S ratio in the F_2 and 1F:1S ratio in the BC_1 in the cross $5054A_4$ × IPC 511. However, these testcross segregation patterns were not supportive of the trigenic inheritance that gives a ratio of 45F:19S in the F_2 population and 1F:1S in BC_1 population in the cross $81A_4$ × IPC 804.

In addition, monogenic ratio of 3F:1S in the F_2 and 1F:1S in BC_1 and digenic ratio of 9F:7S in the F_2 and 1F:3S in BC_1 that results from parental differences at one or two loci, respectively, were also observed in some crosses. Even in a three-gene mechanism, there is a possibility of getting a one-gene or two-gene ratio depending on the genotype of the male and female parents and hence the of number genes segregating in the F_2 and BC_1 populations. Hence, based on the Mendelian ratios obtained, the postulated genotypes of female parents would be rf4a *rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c or *rf*4a *rf*4a *rf*4b *rf*4b *rf*4c *rf*4c. The postulated genotype of the male parents would be *Rf*4a *Rf*4a *Rf*4b *Rf*4b *rf*4c *rf*4c that would give a digenic or trigenic ratio, depending on which of the above two genotypes of the female parent applies. The male parent with *Rf*4a *Rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c genotype will actually give a monogenic ratio when crossed with a female parent that has rf4a *rf*4a *rf*4b *rf*4b *Rf*4c *Rf*4c genotype.

Two (R × R) F_1 hybrids (IPC 511 × IPC 1518 and IPC 511 × IPC 804) produced completely fertile three-way hybrids when crossed onto $81A_4$. The testcross progenies

produced from the cross IPC 804 and IPC 1518 segregated into 3F:1S in both summer and rainy season. These results indicated that IPC 511 most likely possessed the same alleles of the restorer genes as those present in both IPC 1518 and IPC 804 for the A_4 CMS system, whereas IPC 804 and IPC 1518 perhaps carried different alleles.

The joint segregation ratio in the testcrosses made on $81A_1$ and $81A_4$ from the individual plants of F_2 and BC_1 populations derived from the cross 81B × IPC 804 gave a poor χ^2 fit in both summer and rainy season. This might be due to the presence of linkage between the fertility restorer genes of A_1 and A_4 CMS systems or due to the distortion in the joint segregation ratio that results from a poor χ^2 fit of the individual segregation pattern of testcrosses in the A_4 CMS system (1F:2 (F+S):1S ratio in the F_2 and 1(F+S):1S) in the BC_1) expected from the F_2 ratio of 45F:19S and BC_1 ratio of 1F:1S.

Similarly, the joint segregation ratio in the testcrosses made on $5054A_1$ and $5054A_4$ from the individual plants of F_2 and BC_1 populations derived from the cross 5054B x IPC 511 also gave a poor χ^2 fit in both summer and rainy seasons either due to the presence of linkage or distortion in the joint segregation ratio that results from a poor χ^2 fit of the individual segregation pattern of testcrosses in the A_1 CMS system (7F:8 (F+S):1S ratio in the F_2 and 3(F+S):1S) in the BC_1) expected from the F_2 ratio of 54F:10S and BC_1 ratio of 1F:1S.

In the A_{egp} CMS system, the segregation pattern in the crosses involving restorer parent L 67B with A-lines in all the three genetic backgrounds gave a good χ^2 fit to trigenic ratio of 54F:10S in the F_2 and 3F:1S ratio in the BC_1 that results from dominant alleles of any two of the three duplicate-complimentary genes. Hence, the postulated genotype of female parents is *rf*ea *rf*ea *rf*eb *rf*eb *Rf*ec *Rf*ec and male parent is *Rf*ea *Rf*ea *Rf*eb *Rf*eb *rf*ec *rf*ec.

Three different trigenic inheritance mechanisms were found to operate in the fertility restoration patterns of the A_5 and A_v CMS systems as exhibited by a good χ^2 fit to different trigenic ratios in the F_2 (54F:10S, 63F:1S and 57F:7S) and the corresponding BC_1 populations (3F:1S, 7F:1S and 5F:3S), indicating varying genetic constitution or the likely influence of genetic background on segregation of fertile and sterile plants in the A_5 and A_v CMS systems. The trigenic ratio of 63F:1S in the F_2 and 7F:1S in the BC_1 could result from the dominant allele at any one, two or all three loci. The trigenic ratio of 57F:7S in the F_2 and 3F:1S in the BC_1 could result from a gene interaction in which the fertility is restored by the dominant allele of one of the three

genes on its own; alternatively, dominant allele at both of the remaining loci would also restore male-fertility. Hence, the postulated genotype for $81A_5$/B would be *rf*5a *rf*5a *rf*5b *rf*5b *Rf*5c *Rf*5c and for $5054A_5$/B and $ICMA_5$/B 88004 would be *rf*5a *rf*5a *rf*5b *rf*5b rf5c *rf*5c. The postulated genotypes for restorer parent LSGP A_5 would be *Rf*5a *Rf*5a *Rf*5b *Rf*5b *rf*5c *rf*5c which gives a 54F:10S F_2 and 3F:1S BC_1 ratio or *Rf*5a *Rf*5a *Rf*5b *Rf*5b *Rf*5c *Rf*5c that gives a 63F:1S F_2 and 7F:1S BC_1 or 57F:7S F_2 and 5F:3S BC_1 ratio, depending on the plant of the R-line involved in the crosses, in which probably different alleles of the fertility restorer genes might have been fixed. Similarly, based on the genetic ratios obtained in the inheritance of A_v CMS system, the likely genotype of the female parents would be *rf*va *rf*va *rf*vb *rf*vb *Rf*vc *Rf*vc for all three A/B lines or *rf*va *rf*va *rf*vb *rf*vb *rf*vc *rf*vc for $81A_v$/B. The postulated genotype of the restorer parents involved could be *Rf*va *Rf*va *Rf*vb *Rf*vb *rf*vc *rf*vc, which would give a ratio of 54F:10S in the F_2 and 3F:1S in the BC_1 or 57F:7S in the F_2 and 3F:1S in the BC_1 when crossed with a female of genotype *rf*va *rf*va *rf*vb *rf*vb *Rf*vc *Rf*vc. The probable genotype of IPC 382 could be *Rf*va *Rf*va *Rf*vb *Rf*vb *Rf*vc *Rf*vc, which when crossed with a female of genotype *rf*va *rf*va *rf*vb *rf*vb *rf*vc *rf*vc would give a trigenic ratio of 63F: 1S in F_2 and 7F:1S in BC_1 as observed.

The molecular marker analysis of the F_2 mapping population based on the cross 81B × IPC 804 detected two putative QTL (a major one, say *Rf1a*; and a minor one, say *Rf1b*) involved in the fertility restoration of A_1 CMS system. Three putative QTL (one major, say *Rf4a*; and two minor, say *Rf4b* and *Rf4c*) were detected to be involved in the fertility restoration of the A_4 CMS system. Further, all these five QTL for the A_1 and A_4 CMS systems put together were located on different linkage groups as there was no common marker identified with these five QTL. The QTL for other probable loci involved in the inheritance of fertility restoration could not be identified because the pearl millet marker map is still not well saturated; but more importantly, the number of polymorphic markers available for this study was simply not adequate. In view of this, since linkage studies of fertility restorers of A_1 and other CMS systems were either incomplete (as in case of A_4) or not done (as in case of other CMS systems), it could not be ascertained as to which fertility restorer genes were common across two or more CMS systems. The linkage relationships among fertility restorer genes of different CMS systems can be more precisely assessed through intensive application of molecular marker technology.

7. BIBLIOGRAPHY

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APPENDICES

Appendix 1. Weather parameters during 35th to 70th day after planting period in two test environments at ICRISAT-Patancheru center

Environment	Planting date	Flowering period	Daily temperature				Relative humidity (%)			
			Maximum (°C)		Minimum (°C)		0700 h		1400 h	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range
Summer 2003	31st Jan	17 Mar-8 Apr	36.0	30.4-39.2	19.9	15.7-23.6	69.2	40-95	31.0	13-66
	7th Feb	18 Mar-8 Apr								
Rainy 2003	23rd Jul	30 Aug-10 Sep	30.0	26.4-31.7	19.6	18.0-21.4	88.1	80-98	60.7	48-76
	25th Jul	30 Aug-10 Sep								
	26th Jul	1 Sep-14 Sep								

Appendix 2. Genomic DNA isolation protocol using S-buffer

Following Sharp *et al.* (1988) with modifications (Mace *et al.*, 2004)

First day

1. Aliquot 15 mL of S-buffer into sterile polypropylene tubes (plastic with conical base) and incubate in 65^0C water bath. Grind 5g leaf tissue in a pre-cooled pestle and mortar under liquid N_2 to a fine powder and transfer ground tissue to pre-heated S-buffer (65^0C) containing tubes, make sure clumps are suspended by mixing thoroughly on a rotor for 5-10 minutes and incubate samples at 65^0C water bath for 30 minutes.
2. Bring the samples to room temperature (RT) and add 60µL proteinase K (10mg mL^{-1}) per sample, mix thoroughly and keep at 55^0C water bath (stirrer on) for 1-1.5 hours and in between keep mixing manually also
3. Bring the samples to room temperature (RT) and add equal volume (15 mL) of freshly prepared Phenol: Chloroform (1:1) per sample, mix well by gently inverting tubes and transfer to new tubes (plastic with round base) for centrifuge, balance the tubes and if unbalanced add extra phenol:chloroform
4. Centrifuge at 2000-3000 rpm for 20 minutes at 4^0C
5. Transfer the supernatant to clean plastic tubes (with conical base), add 0.6 volume or 9 mL of cold Isopropanol
6. Mix gently by inversion and keep at -20^0C for 10-15 minutes (if required), at this stage DNA precipitates, spool out DNA with the help of glass rod in fresh 15 mL glass tubes having 3-4 mL of 70 % ethanol
7. Centrifuge at 3000 rpm at 4^0C for 5 minutes, pour off the supernatant and wash again with 70 % ethanol, pour off the ethanol and air dry the pellet for 20-25 minutes
8. Add 2 mL of $T_{50}E_{10}$ and 10-15 µL of RNAse (10mg mL^{-1}) per sample disturb the pellet after some time for easy dissolving, keep overnight at RT.

2nd day

9. Keep the samples at 37^0C for 1 hour and mix in between every 15-20 minutes
10. In the same tubes, add 2 mL of phenol:chloroform and mix gently by inversion
11. Centrifuge at 2000 rpm for 5 minutes at 4^0C after balancing the tubes
12. Transfer the supernatant to clean 15 mL glass tubes with the help of Pasteur pipette and be careful to avoid transferring the precipitate and then add 2mL of chloroform per sample

13. Mix gently by inversion to form an emulsion and then centrifuge at 2000 rpm for 10 minutes at 4^0C, transfer supernatant to clean 15 mL glass tubes with the help of Pasteur pipette
14. Add $1/10^{th}$ volume or 200 µL of 3M Sodium Acetate
15. Add 3-4 mL of 100 % ethanol and mix gently by inversion and if required keep at – 20^0C for 10-15 minutes for better precipitation
16. Spool out precipitates with a glass rod and put in eppendorf tube having 1 mL of 70% ethanol and repeat washing with centrifuge after each wash at 8000 rpm for 5 minutes
17. Pour off excess ethanol and air dry for 20-25 minutes or till the ethanol smell disappears
18. Suspend pellet in appropriate volume of $T_{10}E_1$ buffer and disturb the pellet after some time for easy mixing and keep the samples at 4^0C

Appendix 3. Plasmid DNA extraction and PCR amplification

1. LB media preparation (500 mL)

Take 5g NaCl, 5g trypton and 2.5g yeast extract in sterile distilled water, mix and make volume upto 500 mL by adding sterile dH_2O, adjust pH to 7.2 with 1N NaOH, autoclave

2. Preparation of LB+ ampicillin plates (for culturing of probes)

Add 1.5g agar in 100 mL of LB media, autoclave and add 2 µL ampicillin per 1 mL of LB+Agar solution, mix and pour 20 mL per petridish plates. Allow solidifying at room temperature and cover the plates with parafilm and store at 4^0C

3. Inoculation and extraction of plasmid DNA

1. Take 5mL of LB and 10µL ampicillin per culture tube
2. Take a small amount from glycerol stock with the help of a tooth-pick or if inoculated plates are available, take a small amount with a loop and inoculate in the culture tubes
3. Mix and keep in 37^0C incubator shaker at 250-300 rpm for overnight (16 hrs) incubation
4. Next day centrifuge the tubes at 6000 rpm for 10 minutes
5. Decant and add 200 µL of solution A (GTE solution) (1mL GTE solution and 4 mg lysozyme per sample), pellet forms

6. Disturb the pellet by pipetting and transfer to fresh 1.5 mL eppendorf tubes
7. Add 300 µL of freshly prepared solution B (1N NaOH-2mL, 10% SDS-1mL, sterile distilled H_2O-7mL; mix well) in the tubes
8. Add 300 µL of solution C (7.5M Ammonium Acetate)
9. Mix well and keep in ice for 10 minutes
10. Centrifuge at 10000 rpm for 10 minutes
11. Collect supernatant in fresh eppendorf tubes
12. Add 10-15 µL RNAse per tube, mix well and keep ät 37^0C for 1hour
13. Add 700 µL of phenol:chloroform per tube, mix well and centrifuge at 5000 rpm for 5 minutes
14. Collect supernatant in fresh tubes and add 700µL chloroform per tube, mix gently and centrifuge at 5000 rpm for 5 min.
15. Collect supernatant carefully and transfer in fresh tubes and add 700µL of cold isopropanol, mix gently and keep in –20°C for 30 min.
16. Centrifuge at 10000 rpm for 10 min, decant supernatant and wash the pellet twice by adding 1mL of 70% ethanol, with centrifuge each time at 5000 rpm for 5 min.
17. Air-dry the pellet and add 30µL $T_{10}E_1$ and store at 4^0C
18. Check the plasmid DNA quality and concentration on 0.8% agarose gel.

4. PCR for plasmid DNA amplification

Plasmid DNA-3 µL, 10x Buffer-5 µL, 2mM dNTP-2 µL, M13F-1 µL, M13R-1 µL, Taq DNA Polymerase (2U/µL)- 0.4 µL, Mg^{++}-6 µL, Rest dH_2O

PCR Program

1. 94°C for 2 min
2. 94°C for 1 min
3. 58°C for 45 s
4. 72°C for 1.2 min
5. Go to step 2 and repeat 29 times
6. 72°C for 5 min
7. 4°C forever
8. End

Appendix 4. Southern Transfer based on Reed and Mann, 1985

1. Nylon membranes are cut according to the size of the gel and pre-washed in sterile distilled water.
2. Take a large square petri-dish and pour 500 mL of 0.4 M NaOH.
3. Place a piece of glass on top, soak three sheets of Whatman 3 mm paper wicks in 0.4 M NaOH and place on the glass.
4. Starting with one of the gel edges, gradually slide the gel from the gel tank on to the petry-dish. Air-bubbles trapped in between the gel and Whatman sheets are removed.
5. Place the nylon membrane (Amersham Hybond-N^+) on top of the gel. Remove the trapped air-bubbles between the gel and the membrane.
6. Wet a piece of Whatman 3 mm paper cut to the size of the gel and place on top of the nylon membrane. Remove the trapped air-bubbles.
7. Place two dry Whatman paper sheets and 500 g weight on top.
8. Leave overnight.

Appendix 5. Preparation of buffers and other chemicals

0.5 M EDTA

186.1 g of Na_2.EDTA in 800 mL sterile dH_2O

adjust to pH 8.0 with NaOH pellets

make the volume 1 litter with SDW

autoclave

1M Tris-Cl

dissolve 121.1 g of Trizma base in 800 mL of dH_2O

adjust to pH 8.5 with conc. HCl

make the volume 1 liter with SDW

autoclave

5M NaCl

dissolve 292.2 g of NaCl in 750 mL of dH_2O

make the volume 1 liter with SDW

autoclave

20% SDS

slowly add 400 g of SDS to 2 liters of warm water

stir until dissolved

store warm

Buffer S (100 mM Tris-Cl, 100 mM NaCl, 50 mM EDTA, 2% SDS)

add together:

200 mL of 1 M Tris-Cl, pH 8.5,

40 mL of 5 M NaCl,

200 mL of 0.5 M EDTA, pH 8.0,

200 mL of 20% SDS

make the volume 2 liters

store warm

Proteinase K (10 mg/mL)

dissolve 100 mg of Proteinase K in 10 mL of SDW

stir thoroughly

dispense in 1 mL aliquots

store at -20°C

0.5 M Tris-Cl

dissolve 60.507 g of Trizma base in 800 mL of dH_2O

adjust pH with 6 N HCl to 8.0

make the volume 1 liter with SDW

autoclave

$T_{50}E_{10}$ buffer

add 100 mL of 0.5 M Tris-Cl, pH 8.0 and

20 mL of 0.5 M EDTA, pH 8.0 to 600 mL of dH_2O

make the volume 1 liter with dH_2O

$T_{10}E_1$ buffer

add 20 mL of 0.5 M Tris-Cl, pH 8.0 and

2.0 mL of 0.5 M EDTA, pH 8.0 to 600 mL of dH_2O

make the volume 1 liter with dH_2O

Chloroform (24:1)

add 10 mL of isoamyl alcohol in 240 mL of chloroform

mix thoroughly

70% Ethanol

add 300 mL of dH_2O per 700 mL of 100% ethanol

RNase (10mg/mL)

dissolve 100 mg of RNase in 10 mL of dH_2O

place in boiling water for 20 min

cool slowly

dispense into 1 mL aliquots

store at -20°C

3 M Sodium acetate

dissolve 408.24 g of sodium acetate in 600 mL of dH_2O

adjust to pH 5.2 with glacial acetic acid

make the volume 1 liter with dH_2O

autoclave

10x TBE

dissolve 108 g of Trizma base in 500 mL of dH_2O

add 55 g boric acid and 40 mL of 0.5 M EDTA

adjust pH 8.4 with 6N HCl

make the volume 1 liter with dH_2O

50x TAE

dissolve 242 g of Trizma base in 500 mL of dH_2O

add 100 mL of 0.5 M EDTA pH 8.0

add 57.1 mL of glacial acetic acid

make the volume 1 liter with dH_2O

1x TAE

add 20 mL of 50x TAE per 980 mL of water

Kesara's loading buffer

in a beaker take:

0.10 g of Bromophenol blue,

0.10 g of Xylene cyanol,

10 mL of Glycerol,

0.372 g of $Na_2EDTA.2H_2O$

make the volume 20 mL with 1x TAE

stir until dissolved

dispense into 2 mL screw-cap tubes

store at 4°C

0.25M HCl

add 43 mL of conc. HCl (sg = 1.18) per 1957 mL of dH_2O

4M NaOH

dissolve 160 g of NaOH pellets in 800 mL of dH_2O

make the volume 1 liter with dH_2O

0.4 M NaOH

dissolve 96 g of NaOH pellets in 2 liters of dH_2O on a stirrer

make the volume 6 liters with dH_2O

20x SSC

dissolve 877 g of NaCl and 441 g of sodium citrate in 4 liters of dH_2O

make the volume 5 liters with dH_2O

2x SSC

add 200 mL of 20x SSC to 1800 mL of dH_2O

Stripping solution (0.1x SSC, 0.5%SDS)

add 50 mL of 20% SDS and

10 mL of 20x SSC to 1940 mL of dH_2O

Carrier DNA

dissolve 5 g of salmon sperm DNA in 1 liter of dH_2O

autoclave

dispense into 50 mL aliquots

store at -20°C

^{32}P Blots wash solutions

Wash 1 (2x SSC, 1% SDS)

1700 mL of dH_2O

200 mL of 20x SSC

100 mL of 20% SDS

Wash 2 (0.2x SSC, 1% SDS)

1880 mL of dH_2O

20 mL of 20x SSC

100 mL of 20% SDS

Prehybridization solution / 7% SDS phosphate solution

dissolve in 300 mL of dH_2O:

35.5 g of disodium hydrogen phosphate (Na_2HPO_4),

5 g BSA,

35 g SDS

adjust pH with phosphoric acid (H_3PO_4)

make the volume 500 mL with dH_2O

Developer

warm 700 mL of SDW up to 52°C and

slowly add 157 g of D-19

make the volume 1 lit

Rapid fixer

to 700 mL of SDW slowly add

250 mL of solution A and

28 mL of solution B at room temperature

make the volume 1 liter with SDW

Loading buffer

in 5 mL of dH_2O dissolve:

4 g of Sucrose,

25 mg of bromophenol blue,

400 µL of 0.5 M EDTA pH 8

make the volume 10 mL

ABSTRACT

Title of Thesis	:	**Genetics of cytoplasmic-nuclear male sterility and identification of molecular markers of fertility restorer genes in pearl millet [*Pennisetum glaucum* (L.) R. Br.]**
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Degree Awarding University	:	CCS Haryana Agricultural University Hisar-125004 (Haryana), India.
Year of Award of Degree	:	2005
Major Subject	:	Plant Breeding
No. of words in the abstract	:	501
Total No. of Pages in the Thesis	:	175 + i-xxx + I-IX

Key words: Pearl millet, *Pennisetum glaucum*, Cytoplasmic-nuclear male-sterility, CMS systems, Isonuclear A-lines, Fertility restoration, Genetics, Allelism, Linkage, Molecular markers

Genetics of fertility restoration of diverse cytoplasmic-nuclear male sterility (CMS) systems in pearl millet was studied in segregating F_2, BC_1 and BC_2 populations of the crosses involving isonuclear A-lines of the five diverse CMS systems (A_1, A_4, A_{egp}, A_5 and A_v) in three diverse nuclear genetic backgrounds (81B, 5054B and ICMB 88004) and six pollen parents restoring the male fertility of hybrids based on any one, two or three male-sterile cytoplasm. Linkage between the fertility restorer genes of the A_1 and A_4 CMS systems, allelism among the fertility restorer genes of these CMS systems and molecular markers linked to fertility restorer genes of the A_1 and A_4 CMS systems were also studied. In a majority of crosses across the CMS systems, fertility restoration was governed by a trigenic inheritance mechanism represented either by dominant alleles of one basic gene and two duplicate-complimentary genes (F_2 ratio 45:19 and BC_1 ratio 1:1) or dominant alleles of any two of the three duplicate-complimentary genes (F_2 ratio 54:10 and BC_1 ratio 3:1). In few other crosses, different trigenic mechanisms with F_2 ratio of 57F:7S and 63F:1S and corresponding BC_1 ratio of 3F:1S and 7F:1S, respectively, were

also observed. Although monogenic and digenic (F_2 ratio 15F:1S and 9F:7S and BC_1 ratio 3F:1S and 1F:3S, respectively) inheritance ratios were also observed in a few crosses, these resulted from the segregation of one or two genes out of the three involved in the trigenic inheritance. Segregation patterns of testcrosses from individual plants of F_2 and BC_1 populations derived from two B × R crosses were broadly supportive of the trigenic inheritance mechanism. Test of allelism studied from the fertility/sterility reaction of the three-way hybrids obtained by crossing A-lines with the F_1s of inter-crosses among three restorer lines (IPC 1518, IPC 511 and IPC 804) indicated the presence of same alleles of all the fertility restorer genes for the A_1 CMS system, whereas different alleles for the A_4 system. Joint segregation analysis revealed the presence of linkage between the fertility restorer genes of A_1 and A_4 CMS systems. A linkage map of 708.8 cM was constructed using 397 individuals and 36 molecular (SSR and RFLP) and morphological markers in the F_2 mapping population derived from the cross 81B × IPC 804. For the A_1 CMS system, two QTL (*Rf1a* and *Rf1b*) and for the A_4 system, three QTL (*Rf4a, Rf4b* and *Rf4c*) were identified with different unlinked genomic regions involved in the fertility restoration of these CMS systems. Based on the overall inheritance pattern observed, possible genotypes of the A-lines irrespective of CMS background were assigned as *rf*_a *rf*_a *rf*_b *rf*_b *Rf*_c *Rf*_c or *rf*_a *rf*_a *rf*_b *rf*_b *rf*_c *rf*_c and of the restorer lines as *Rf*_a *Rf*_a *Rf*_b *Rf*_b *rf*_c *rf*_c or *Rf*_a *Rf*_a *Rf*_b *Rf*_b *Rf*_c *Rf*_c (underscore to be replaced with the numbers '1', '4' or '5' or alphabet 'e' or 'v' denoting the CMS systems). The information emanating from the study has implications in the breeding of maintainer and restorer lines of diverse CMS systems.

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