MARKER-ASSISTED IMPROVEMENT OF PEARL MILLET (*Pennisetum glaucum*) DOWNY MILDEW RESISTANCE IN ELITE HYBRID PARENTAL LINE H 77/833-2

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

2001

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CERTIFICATE I

This is to certify that this dissertation entitled, **"Marker-assisted improvement of pearl millet (*Pennisetum glaucum*) downy mildew resistance in elite hybrid parental line H 77/833-2"**, submitted for the degree of Doctor of Philosophy in the subject of Plant Breeding, of Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Arun Sharma** 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.

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Acknowledgements

Fervently and modestly, I extol the genuine cooperation and inspiration offered to me by the Chairman of my Advisory committee, Dr. D.C. Nijhawan, Professor & Head, Oilseeds section, Department of Plant Breeding, CCS HAU, Hisar, for his valuable counsel and meticulous guidance during my study at HAU and kind encouragement during the process of achieving the final form of this thesis.

It gives me immense pleasure in extending my deep sense of reverence and gratitude to Dr. C.T. Hash, Principal Scientist, Molecular Breeding, ICRISAT, Patancheru and Co-chairman of my Advisory committee, for providing research facilities, suggestion of the research problem, immensely valuable guidance and constructive criticism throughout the period of this investigation and preparation of the manuscript.

I am sincerely obliged and indebted to Dr. R.K. Behl, Professor and Dr. Ashok Chhabra, Associate Professor, Department of Plant Breeding, for their invaluable consultations, sagacious advice and able direction throughout the course of study and bringing out the manuscript.

I am also thankful to other members of my advisory committee, Dr. (Mrs.) Santosh Dhillon, Professor, Department of Chemistry & Biochemistry, Dr. R.K. Jain, Professor, Department of Biotechnology & Molecular Biology and Dr. K.K. Saxena, Senior Scientist, Department of Mathematics & Statistics, for constructive suggestions and guidance for completion of this research project.

I am grateful to Dr. B.P.S. Lather, Professor & Head, Department of Plant Breeding and also to the former Head, Dr. G.P. Lodhi for extending all possible help and support during the course of my study.

It gives me pleasure in expressing my sincere thanks to Dr. S. Sivaramakrishnan, Senior Scientist (Radio Isotope Laboratory), Dr. N. Seetharama, Senior Scientist (Molecular Biology), Dr. R.P. Thakur, Senior Scientist (Pathology), Dr. K.N. Rai, Principal Scientist (Pearl millet Breeding), Dr. F.R. Bidinger, Principal Scientist (Cereal Physiology) and Dr. H.D. Upadhyaya, Scientist (Genetic Resources Division) for their kind help which enabled me to accomplish this task with ease.

With respectful regards and immense pleasure, I wish to acknowledge and express my thanks to Dr. S. Chandra (Senior Statistician) and Mr. Hari (Scientific Officer, Statistics unit) for valuable advice and help rendered during analyses.

I express my sincere gratitude to Dr. S. N. Nigam, Principal Scientist, Groundnut Breeding, for boosting my morale and giving me time for scientific discussions.

Dr. C.J. Howarth, Scientist, IGER, Aberystwyth, UK, deserves special mention to permit me to reproduce linkage and QTL maps used in the present study. I am also grateful to Dr. Rattan Yadav, Molecular Geneticist, IGER, Aberystwyth, UK, for being supportive and encouraging throughout this research project.

Assistance rendered by Ms. Seetha and Ms. Santosh helped me in completing my lab work efficiently and smoothly.

I feel privileged to express heartfelt words of appreciation to all PMB staff especially Mr. Bhaskar Raj, Mr. Ganapathi, Mr. OmParkash, Mr. Murali and Mr. Izaz Ahmed for their kind cooperation and help rendered.

I thank Mr. K. Prabhakar, Senior Secretary (PMB) and former secretary Mr. S.B. Stanley for being very nice to me by promptly completing any assignment given to them.

Special thanks are extended to Library staff, Learning Systems Unit staff especially Mr. Prasad Rao and Mr. Vidya Sagar (Photographer) for their excellent assistance during my research work at ICRISAT.

It was with the utmost blessings and good wishes of my parents and affection of my brothers Siddhartha and Rajeshwar, sister Sujata and brother-in-law Ashwini that I could attain academic heights by undergoing doctorate studies.

I shall be failing in my duty if I do not express my cordial thanks to all my colleagues and friends, Maria, Azhagu, Ranjana, Lava, Gopala, Devvart, Kulkarni, Sarosh, Raghu, Naveen, Gaurav, Atul, AMK Raju, Dinesh, Manoj, Rupesh, Anil and Deepak for their untiring help, whole-hearted cooperation and nice company during my stay both, at ICRISAT and Hisar.

I take this opportunity to thank Dr. C.L.L. Gowda, Program Leader, Learning Systems Unit, ICRISAT and Dr. B. Diwaker, former Program Leader, for their kind cooperation to work in collaboration with PMB division of ICRISAT on Memorandum of Understanding.

Financial assistance received in form of Merit stipend awarded by CCS HAU and the grant of Research Scholarship by ICRISAT is also acknowledged.

Special thanks is also extended to the Department for International Development (DFID), Plant Sciences, UK, for providing funds to carry out this investigation.

Arun Sharma

HISAR
March 07, 2001

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1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food crop of semi-arid tropical regions of India and West Africa and is better adapted than any other cereal to regions of low rainfall, low soil fertility and hot sandy soils. Pearl millet is widely cultivated in different parts of the world. It is a multi-purpose cereal grown for grain, stover and green fodder on about 27 million hectares, primarily in Asia and Africa (ICRISAT and FAO, 1996). However, its importance in Latin America is expanding rapidly (Hash et al., 1999). In terms of annual production, pearl millet is the sixth most important cereal crop in the world, following wheat, rice, maize, barley and sorghum. In India it is important in the states of Rajasthan, Maharashtra, Gujarat and Haryana but it is also grown in other parts of the country where the rainfall is 150-750 mm/annum, primarily during the south-west monsoon from June to September (Kumar, 1989).

Pearl millet is an excellent organism for genetic research because of its low chromosome number (2n = 14), short life cycle, high multiplication ratio (up to 1:1000), ratooning ability and the ease with which cross pollination can be done due to protogyny. It has also been found very suitable for molecular genetic studies.

Breeding for resistance to diseases of current and potential economic importance contributes to increased productivity and stability of pearl millet grain, stover and forage yields. Disease resistance is a major concern in pearl millet improvement programs, and has been the subject of several reviews (Louvel, 1982; Williams and Andrews, 1983; Williams, 1984a; Andrews *et al.*, 1985; Talukdar *et al.*, 1994; Rai and Anand Kumar, 1994; Hash *et al.*, 1997; Hash *et al.*, 1999). In breeding improved pearl millet cultivars, it is necessary to maintain moderate levels of resistance to many potential pathogens currently of minor importance in the breeders' target environments (Mohan *et al.*, 1978; Singh *et al.*, 1993b). This helps ensure that these constraints do not become actual problems later.

Sclerospora graminicola (Sacc.) J. Schröt. is an obligate biotrophic fungus that causes downy mildew in pearl millet, often resulting in devastating yield losses under epiphytotic conditions. *S. graminicola* was first reported on pearl millet in India by Butler (1907). Althouth it is established throughout most pearl millet growing areas in Asia and Africa, higher disease incidence and losses were initially reported only in poorly drained low lying areas (Butler, 1918; Mitra and Tandon, 1930). However, since the introduction of high yielding single-cross hybrids in India, in the late 1960's, downy mildew has been a major production constraint and a major focus of pearl millet improvement research both by ICRISAT and the Indian National Program (Nene and Singh, 1976; Safeeulla, 1976; Williams, 1984b; Andrews, 1987; Dave, 1987; Rai and Singh, 1987; Shetty, 1987; Singh *et al.*, 1987, Singh 1995 and Singh *et al.*, 1993a).

The symptoms of downy mildew may appear at any stage of plant growth. Leaf symptoms begin as chlorosis (yellowing) at the base of the first infected leaf and subsequent leaves show progressively greater chlorotic symptoms. The infected chlorotic areas produce massive numbers of asexual spores (sporangia), generally on lower surface of leaves, giving them a 'downy' appearance. Severely infected plants remain stunted and do not produce panicles. However, the most typical symptom of this disease is the transformation of infected floral parts into leafy structures (Pinard, 1989). Therefore, the disease is also known as the green ear disease.

ICRISAT has developed highly effective field (Williams *et al.*, 1981) and greenhouse (Singh and Gopinath, 1985; Singh *et al.*, 1993a; Weltzien and King, 1995) screening techniques that can easily differentiate between resistant and susceptible progenies.

In the present scenario, incorporation of a diverse range of downy mildew resistance genes into the parental lines of elite pearl millet hybrids is major priority in order to achieve grain and stover yield stability. It has been found in most previous studies on genetics of resistance to downy mildew that host resistance was continuously distributed

in the progeny (Singh *et al.* 1980; Basavaraju *et al.,* 1981a; Dass *et al.,* 1984; Shinde *et al.,* 1984). However, this does not necessarily imply that the inheritance is complex and that many genes are segregating (Basavaraju *et al.,* 1981b). Polygenic system of resistance being most sensitive to environmental variation and vertifolia effects of oligogenes in the "genetic environment", if any, the expression of resistance is often inconsistent. Accumulation of maximum number of favourable alleles controlling disease resistance is a general strategy when host plant resistance mechanisms are under polygenic control. This warrants for the use of techniques that have maximum resolution. Use of molecular markers, like restriction fragment length polymorphisms (RFLP), linked to resistance genes enhances both the effectiveness and rate of progress of breeding improved hybrid parental lines. Also these markers are independent of variation caused by the genetic, biotic and/or abiotic environment and this offers the advantage of permitting selection for resistance in absence of the pathogen, or of specific variants of the pathogen that are otherwise required to identify segregants with pyramided resistance genes.

Marker-assisted selection has been possible, if not always practical, for a wide range of crop plant traits since relatively early in the 20th century (e.g. Sax, 1923; Hash and Blake, 1981; Burton and Werner, 1991). With the development of molecular tools and the first molecular genetic linkage maps for plants, marker-assisted selection (MAS) has become much more broadly applicable. During the past decade, the developing ability to transfer target genomic regions using DNA markers resulted in extensive mapping experiments aimed at development of MAS (Dudley, 1993; Lee, 1995; Mohan *et al.,* 1997). The molecular marker based genetic linkage map of pearl millet (Liu et al., 1994) has permitted identification of at least 16 quantitative trait loci (QTLs) for downy mildew resistance (Hash *et al.,* 1997; 1999; Hash and Witcombe, in press). The essential requirements for MAS in a plant breeding programme are:

a) marker(s) should co-segregate with the desired trait; molecular markers should be closely linked (with no crossovers or very low frequency of crossovers) with the

gene(s) governing the target trait. In other words, the linkage should be stable across generations and populations;

b) an efficient means of screening large populations for the molecular marker(s) should be available; at present, this means, relatively easy analysis based on PCR technology; and

c) the screening technique should have high reproducibility across laboratories, be economical to use, and should be user-friendly.

A backcross breeding program is aimed at gene introgression from a donor line into the genomic background of a recipient line. The potential utilization of molecular markers in such programs has received considerable attention in the recent past. Markers could be used to assess the presence of the introgressed gene ("foreground selection") when direct phenotypic evaluation is not possible, or too expensive, or only possible late in the development. This was proposed by Tanksley (1983). Markers could also be used to accelerate the return to the recipient parent genotype at other loci ("background selection"). This was first proposed by Hillel et al. (1990).

Another major limiting factor in the cultivation of pearl millet is bird damage. In spite of several well known devices used for scaring off the birds, the loss in grain yield may be as high as 25 to 100 per cent (Lal and Singh, 1971). The presence of long panicle bristles are said to protect the filling grains from bird damage and from untimely rain at flowering periods, the later resulting in damage to stigmas and washing down of the pollen-producing anthers and consequently empty panicles. In varieties of pearl millet commonly grown in India, the bristles surrounding each spikelet on the ear do not extrude beyond the level of the grain surface. Some African introductions, however, possess long, well-developed bristles that have been found to give at least some protection against bird damage.

Two populations from planned crosses, intended to map QTLs contributing to seedling heat tolerance, have been produced by the pearl millet breeding unit of ICRISAT (Hash

and Witcombe, 1994). Both mapping populations involve inbred H 77/833-2, the pollinator parent of three high-tillering and high yielding single-cross grain hybrids: HHB 60, HHB 67 and HHB 68 (Kapoor *et al.*, 1989a, b, c). H 77/833-2 is non-bristled and is susceptible to some strains of pearl millet downy mildew present in India. Downy mildew QTLs have been mapped in both of the populations involving H 77/833-2. Therefore, the proposed study was planned with following objectives:

Objectives :

I. Marker-assisted backcross transfer of downy mildew resistance gene(s) to H 77/833-2 from resistance source ICMP 451.

II. Transfer of bristling gene(s) from ICMP 451 to H 77/833-2 through conventional backcrossing.

III. Field and greenhouse evaluation of plants, and their hybrids having the transferred genes.

2. REVIEW OF LITERATURE

The objective of this review is to present all information relevant to the objectives outlined for the study (i.e. marker-assisted backcross transfer of downy mildew resistance gene(s), transfer of bristling gene(s) through conventional backcrossing, and field and greenhouse evaluation of plants having the transferred genes). While doing so we shall draw not only on the published research work on pearl millet, which is quite meager, but also on other crops related to pearl millet.

2.1 Inheritance of downy mildew resistance

Literature on the inheritance of host plant resistance to downy mildew of pearl millet is briefly reviewed here to better understand the subject.

Since shortly after the onset of the hybrid era for pearl millet in India, downy mildew has been a major research focus by scientists of both ICRISAT and the Indian national program involved in improvement of this crop (Singh *et al.*, 1993a; Hash, 1997; Hash *et al.*, 1997, 1999). There are several published papers on the inheritance on downy mildew resistance. However, most such studies have been hampered because both the pathogen and host are allogamous and highly variable (Thakur *et al.*, 1992; Jones *et al.*, 1995) and segregation of host plant resistance generally shows continuous variation (Singh *et al.*, 1980; Basavaraju *et al.*, 1981a, b; Dass *et al.*, 1984; Shinde *et al.*, 1984). In addition, regional variability in pathogen populations used and difficulties maintaining high and uniform disease pressure have led to conflicting conclusions from earlier studies (Jones *et al.*, 1995). However, a meaningful summary is still possible. The literature on inheritance of downy mildew resistance has been adequately discussed in several fairly recent reviews (Koduru and Krishna Rao, 1983; Hash *et al.*, 1997, 1999).

Appadurai *et al.* (1975) reported that resistance to *Sclerospora graminicola* (causal organism downy mildew) is governed by one or two dominant genes, while Gill *et al.* (1978) reported two dominant duplicate factors conferring resistance to downy mildew

and proposed the gene symbols DM_1DM_2, DM_1dm_2, and dm_1DM_2 for resistant and dm_1dm_2 for susceptible genotypes. Dass *et al.* (1984); Thakur *et al.* (1992); Singh (1995) reported resistance to be dominant over susceptibility and probably controlled by one or a few genes. Except in one case where resistance was reported to be recessive (Singh *et al.*, 1978) resistance is generally observed to be dominant and variation in segregating populations is continuous (Singh *et al.*, 1993a).

The quantitative nature of inheritance to downy mildew was reported by Singh *et al.* (1978) with significant additive and non-additive genetic variance. Basavaraju (1978); and Basavaraju *et al.* (1980) concluded that resistance to downy mildew is not simply inherited, but is due to a series of non-allelic interactions. Many authors (e.g., Tyagi and Iqbal Singh, 1989; Deswal and Govila, 1994; Kataria *et al.*, 1994) have concluded that non-additive gene action is responsible for much of the heritable variability for host plant reaction of downy mildew, agreeing with simpler studies that show resistance to be dominant or partially dominant.

Weltzien and King (1995) subjected one population of pearl millet highly susceptible to downy mildew to two cycles of recurrent selection for downy mildew resistance and demonstrated that even in a susceptible population, recurrent selection effectively increased the level of resistance to this disease. However, progress in the second cycle of selection was much less than that in the first suggesting fixation had occurred after the first selection cycle at the loci contributing most to disease reaction in this population.

2.2 Applications of RFLP technique

In recent years, developments in DNA cloning and the use of restriction endonucleases have enabled scientists to more quickly and effectively construct genetic linkage maps by studying directly the segregation of DNA fragments. Advances in molecular biology during the past several decades have provided a new class of genetic markers at the level of DNA, termed restriction fragment length polymorphisms (RFLPs). RFLPs often

occur in sufficient quantities to generate detailed genetic maps (Botstein *et al.*, 1980; Soller and Beckmann, 1983). Investigation in maize (*Zea mays*) (Helentjaris *et al.*, 1986; Burr *et al.*, 1988), rice (*Oryza sativa*) (McCouch *et al.*, 1988), soyabean (*Glycine max*) (Apuya *et al.*, 1988), tomato (*Lycopersicon esculentum*) (Bernatzky and Tanksley, 1986), and brassicas (*Brassica* spp.) (Figdore *et al.*, 1988) have demonstrated that a potentially unlimited number of RFLPs exist, which should enable plant geneticists to establish well-saturated genetic linkage maps for any species.

RFLP differences between plants are inherited in the same fashion as conventional Mendelian genes, thus genetic linkage maps of RFLPs can be constructed using conventional methods. Such RFLP maps indicate the location of specific restriction fragments of chromosomal DNA relative to one another. Ellis (1986) reported that simple consideration of RFLP mapping as a method of analyzing the inheritance of quantitative characters suggests that there are several limitations to the utility of this approach.

Gale and Witcombe (1992) and Hash (1991) emphasized the opportunities for potential use of RFLPs in pearl millet breeding with particular reference to downy mildew resistance. Markers are most useful when their map position is known (Hospital *et al.*, 1992). A number of recent papers suggest that use of RFLPs as markers offers a clear advantage in breeding for improvement in quantitative traits (Arunachalam and Chandrashekaran, 1993; Mohan *et al.*, 1997; Paterson *et al.*, 1991).

The two primary advantages of RFLP markers over morphological markers are codominance and absence of pleiotropic effects. Since RFLP markers have no known effect on the phenotype of the plant, they are ideal for studying quantitative traits (Stuber, 1992).

RFLP and morphological markers have been used in practical plant breeding programs to map quantitative trait loci (QTLs) (Tanksley *et al.*, 1982; Edwards *et al.*, 1987a, b; Stuber *et al.*, 1987; Weller *et al.*, 1988) and to monitor response to recurrent selection

(Stuber *et al.*, 1980, 1982). Morphological markers have also been studied for possible use in backcross improvement of yield potential of elite pearl millet forage hybrids (Burton and Werner, 1991).

Costs of applying RFLPs to genetic improvement were assessed by Beckmann and Soller (1983) in terms of individuals and number of polymorphisms per individual that are scored for various applications including varietal identification, identification and mapping of quantitative trait loci and their marker-assisted introgression from resource strain to commercial variety.

Liu *et al.* (1994) analyzed a sample of 19 diverse pearl millet inbred genotypes with 200 homologous genomic DNA probes and found this crop species to be extremely polymorphic as 85% of probes detected polymorphism using only two restriction enzymes.

2.3 Genetic linkage mapping

Scientists are constructing genetic linkage maps of DNA markers for many plant species today (Helentjaris, 1987; McCouch *et al.*, 1988; Huen *et al.*, 1991; Tanksley, 1993; Mohan *et al.*, 1997). Two types of DNA markers have been widely used, RFLP markers (Botstein *et al.*, 1980) and random amplified polymorphic DNA markers (RAPDs) (Williams *et al.*, 1990). Both detect DNA polymorphisms and monitor the segregation of a DNA sequence among progeny of a genetic cross permitting construction of a linkage map. However codominant RFLP markers are more robust and repeatable than RAPD markers, which are generally dominant (or presence/absence) and very sensitive to protocol variation.

The first true RFLP-based genetic linkage map in a crop plant (tomato) was constructed in 1986 with only 44 F_2 plants and 57 marker loci (Bernacchi and Tanksley, 1986). A detailed linkage map of lettuce (*Lactuca sativa*) was constructed by Landry *et al.* (1987) using 53 genetic markers including 41 RFLP loci, 5 downy mildew resistance genes, 4

isozyme loci and 3 morphological markers. The genetic markers were distributed into nine linkage groups covering 404 cM, which may represent 25-30% of the lettuce genome. Using RFLPs as genetic markers, Helentjaris *et al.* (1988), constructed linkage maps for maize and tomato. A subsequent comparison of the RFLP inheritance patterns in F_2 populations of maize and tomato permitted arrangement of the loci detected into genetic linkage groups for both species.

McCouch *et al.*, (1988) reported the construction of an RFLP-based genetic linkage map of rice chromosomes. The map was comprised of 135 loci corresponding to clones selected from a *Pst*I genomic library covering 1,389 cM of the rice genome. Chao *et al.* (1989) attempted RFLP mapping in wheat (*Triticum aestivum*) using 18 cDNA clones, 14 anonymous and 4 of known function. The loci identified by these probes were mapped on one or more of wheat homeologus group 7 chromosomes. Graner *et al.* (1991) analyzed two populations to construct an RFLP-based genetic linkage map of barley using 250 genomic and cDNA markers. Maps of chromosomes 3A, 3B and 3D of wheat and 3R of rye were developed by Devos *et al.* (1992) using 22 DNA probes and 2 enzyme marker systems.

Liu *et al.* (1992) constructed an RFLP-based genetic linkage map in pearl millet using 180 probes from a *Pst*I genomic library. Later Liu *et al.* (1994) published the first linkage map using 200 genomic DNA probes employing two crosses. The total length of this map, which comprised seven linkage groups, was 303 cM. On this map 181 loci were placed by studying segregation (RFLP banding pattern) in a F_2 population derived from a single F_1 plant. The average map distance between RFLP marker loci was 2 cM.

Nearly every agronomic trait imaginable has been subjected to DNA marker mapping and QTL analyses e.g., drought tolerance (Martin, 1989), seed hardness (Keim *et al.*, 1990), seed size (Fatokun *et al.*, 1992), maturity and plant height (Lin *et al.*, 1995), disease resistance (reviewed, Young, 1996), oil and protein content (Diers *et al.*, 1992), soluble solids (Paterson *et al.*, 1988), and, of course, yield (Stuber *et al.*, 1987).

2.4 Quantitative trait loci (QTL) mapping

The conflict between the Mendelian theory of particulate inheritance and the observation that most trait in nature exhibit continuous variation was eventually resolved by the concept that quantitative inheritance can result from segregation of multiple genetic factors, modified by environmental effects (Johannsen, 1909; Nilsson-Ehle, 1909; East, 1916). Breeding studies confirmed numerous predictions of this theory (East, 1916) and pioneering genetic mapping studies (Sax, 1923; Rasmusson, 1933; Thoday, 1961; Tanksley *et al.*, 1982; Edwards *et al.*, 1987a, b) showed that it was even possible occasionally to detect linkage to the putative quantitative trait loci (QTL). Recently such studies have become practically possible in principle with the advent of RFLPs as genetic markers (Botstein *et al.*, 1980) and the increasing availability of nearly complete RFLP maps in many organisms.

The theoretical basis of interpreting the association of marker loci with quantitative trait loci (QTLs) has been outlined by Mather and Jinks (1971), Tanksley *et al.* (1982), Soller and Beckmann (1983) and Edwards *et al.* (1987a, b). The theoretical basis for identification of QTL associated with individual marker loci have been studied by several authors (Jayakar, 1970; McMillan and Robertson, 1974; Soller and Beckmann, 1983; Edwards *et al.*, 1987a, b; Cowen, 1988). Likewise, the use of flanking marker loci for QTL identification has been suggested by Lander and Botstien (1989) and Knapp *et al.* (1990). Experimental studies (Law, 1967; Tanksley *et al.*, 1982; Osborn *et al.*, 1987; Stuber *et al.*, 1987) have shown that marker genes are in fact linked to genes controlling quantitative characters in several crop species like tomato, wheat and maize.

Experimental designs for determination of linkage between marker loci and QTL have been widely described (Elston and Stewart, 1971; Geldermann, 1975; Hill, 1975; Jensen, 1989; Knapp *et al.*, 1990; Lander and Botstein, 1989; Soller and Beckmann, 1983, 1990), and a number of successful experimental studies have been carried out

(Beevar *et al.*, 1989; Edwards *et al.*, 1987a, b; Gelderman *et al.*, 1985, Paterson *et al.*, 1988; Sax, 1923; Tanksley *et al.*, 1982; Weller, 1987; Weller *et al.*, 1988).

Jaykar (1970) suggested methods for the detection and estimation of linkage between marker gene and a locus influencing a quantitative character. First use of a reasonably complete RFLP-based genetic linkage map was reported by Paterson *et al.* (1988) in resolving quantitative traits to discrete Mendelian factors in an inter-specific backcross of tomato. They mapped at least six QTLs controlling fruit mass and four QTLs controlling soluble solids.

Detecting marker-QTL associations can be carried out through *t*-tests based on single markers (Soller *et al.*, 1976) or by means of likelihood ratio tests that involve the use of a pair of markers bracketing a QTL, a procedure termed "interval mapping" (Jensen, 1989; Knapp *et al.*, 1990; Lander and Botstein, 1989; Weller, 1987), although simpler approaches are possible (Haley and Knott, 1992; Thoday, 1961; Weller, 1987).

Lander and Botstein (1989) described set of analytical methods that modify and extend the classical theory for mapping QTLs and that are implemented in the computer software package MapMaker/QTL. They provided explicit graphs that allow experimental geneticists to estimate, in any particular case, the number of progeny required to map QTL underlying a quantitative trait.

Selective genotyping can markedly decrease the number of individuals genotyped for a given power at the expense of an increase in the number of individuals phenotyped, Darvasi and Soller (1992). They showed that the observed differences in quantitative trait values associated with alternative marker genotypes in the selected population can be much greater than the actual gene effect at quantitative locus when the entire population is considered. This is an result of the smaller effective population size used in such selective marker genotyping studies. Chandra *et al.* (2000) suggested a more

economic way, Bootstrap method, that could allow using real experimental data to quantify the bias in and to obtain realistic estimates of QTL parameters.

Michelmore *et al.* (1991) used a modification of "conventional QTL mapping" to detect QTLs for downy mildew resistance in lettuce, in a procedure they called bulk segregant analysis, which is remarkably similar to that previously described by Burton and Wells (1981) for assessing the value of a trait in near-isogenic F_3 populations.

Edwards *et al.* (1992) explained that the availability of numerous marker loci in some genomic regions allowed

- more accurate localization of QTLs,
- resolution of linkage between QTLs affecting the same trait, and
- determination that when some chromosome regions are found to affect a number of traits, this is likely to be due to linkage.

Effective utilization of molecular marker technology to manipulate loci controlling quantitative traits is considered to be dependent on tight linkage between the marker (s) and the QTL (Dudley, 1993). However, Darvasi *et al.* (1993) showed that power of detecting a QTL was virtually the same for a marker spacing of 10 cM as for an infinite number of markers and was only slightly decreased for marker spacings of 20 cM or 50 cM. However, a very important consideration is the confidence interval for the QTL position on the linkage group.

As reported by Kearsey and Farquhar (1998) the analytical methods locate QTL with poor precision unless the heritability of particular trait is high. Also the estimates of the QTL effects, particularly the dominance effects, tend to be inflated because only large estimates are detected as being statistically significant.

QTLs affecting testcross performance of maize were mapped and characterized by Schon *et al.* (1994). They discussed the consistency of these QTLs across environments

and testers. Jones *et al.* (1995) mapped QTLs for resistance to several pathogen populations of *Sclerospora graminicola* in F_2 derived F_4 self bulks from a cross of resistant and susceptible pearl millet inbreds. Independent inheritance of resistance to pathogen populations from India and Senegal and populations from Niger and Nigeria were shown. Four QTL were identified by Romagosa *et al.* (1996) in barley (*Hordeum vulgare*) that accounted for most of the differential genotypic expression for grain yield across environments. Four QTLs were mapped to barley chromosomes 2, 3, 6 and 7 at regions that also were identified using the MQTL software package (Tinker and Mather, 1995a; Tinker and Mather, 1995b).

Kearsey (1998) gave a non-mathematical explanation of the principles underlying QTL analyses, to discuss their potential.

Prioul *et al.* (1997) described the genetical methods required to analyze possible associations between traits that are inherited in a quantitative manner using QTL analysis. Advantages, and some limitations, of QTL analysis over other methods currently in use by physiologists to test associations between traits were also discussed.

Yadav *et al.* (1999, 2000) have identified a number of QTLs associated with terminal drought tolerance of grain yield in pearl millet. Some of the identified QTLs were common across water-stress environments and genetic backgrounds of two mapping populations while others were specific to a particular population and/or environment.

2.4.1 Reliability of QTL estimates

For marker-assisted selection (MAS) to be effective, reliable estimates of QTL positions and effects are required. Adequate power, precision and accuracy of QTL analyses can only be expected from large, well-suited mapping populations, using a marker set with good genome coverage, and phenotypic values based on multi-environment trials (Van Ooijen, 1992; Utz and Melchinger, 1994; Beavis, 1998). From a recent literature review of Kearsey and Farquhar (1998), updated by Lynch and Walsh (1998), it is

evident that in most QTL studies the number of QTLs is considerably underestimated and the percentage of genetic variation explained by markers is highly erratic and often over-estimated. In verification studies with maize, Melchinger et *al.* (1998) found that 50% or less of variance attributable to markers in the calibration experiment could be recovered in an independent sample of progenies of the same initial F_2 population. Such uncertainties of QTL analyses have the potential to seriously reduce the efficiency of MAS. Verification of individual QTLs, e.g. by re-estimation in advanced generations or by evaluating near-isogenic backcross-derived lines (NILs) contrasting for genome segments of interest (Romagosa *et al.*, 1999), is therefore imperative. An additional need is to verify estimated QTL effects and the possible epistatic interactions of QTL alleles with the genetic background of the material to be improved (Phillips, 1999; Kerns *et al.*, 1999).

2.5 Marker-assisted selection (MAS)

An important area in which molecular biology is being applied to plant disease resistance is that of marker-assisted selection (MAS) (Dudley, 1993; Jones *et al.*, 1997; Lee, 1995; Malyshev and Kartel, 1997; Michelmore, 1995; Mohan *et al.*, 1997; Young, 1996, 1999). MAS has been advocated as a useful tool for rapid genetic advance in case of quantitative traits (Lande and Thompson, 1990; Knapp, 1994, 1998). Gimelfarb and Lande (1995) presented detailed analysis of the relationships between genetic markers and QTLs in the process of MAS.

Mohan *et al.* (1997) concluded that MAS can be used to pyramid major genes including disease and insect resistance genes, with the ultimate goal of producing crop cultivars with more desirable traits. Thus with MAS it is now possible for plant breeders to conduct many rounds of selection in a year. A study conducted by Eathington *et al.* (1997) assessed the usefulness of marker-associated effects estimated from early generation testcross data for predicting later generation testcross performance.

Many forms of plant disease resistance are simply or oligo-genically inherited (Agrios, 1997). In addition, resistance QTLs can be inherited as a quantitative trait. In both cases, marker-assisted selection offers very strong potential for future resistance breeding (Dudley, 1993; Michelmore, 1995; Tanksley, 1993; Young, 1996). Use of marker-assisted selection in breeding for disease resistance has been reviewed (Michelmore, 1995; Mohan *et al.*, 1997; Young, 1996).

Hash *et al.* (1997, 1999), Witcombe and Hash (2000), and Hash and Witcombe (in press) proposed that MAS will permit breeding of modified three-way hybrid cultivars of pearl millet that are uniform for agronomic characters but heterogeneous for their resistance gene complements. Such hybrids are expected to be less vulnerable to epidemics of new pathogen strains that have so often evolved when genetically uniform single-cross pearl millet hybrids have been widely or repeatedly cultivated (in India; pathogen = downy mildew, and in USA; pathogen = rust, caused by *Puccinia substriata* Ell. & Barth. var. *indica* Ramachar & Cummins).

MAS can be used to pyramid several resistance-genes into a single host genotype. Where hybrid cultivars are possible, Witcombe and Hash (2000) have described how multiple resistance gene pyramids can be used practically to strategically deploy resistance genes in a potentially more durable manner than has been previously practiced. The frequency of genotypes having resistance-alleles at several loci increases greatly in both seed parent and hybrid when the overall frequency of resistance-alleles in maintainer lines increases.

2.5.1 Theoretical studies on the efficiency of MAS

While most researchers involved in QTL mapping are optimistic about the usefulness of the MAS, little research has been done to evaluate its practical effectiveness. MAS for QTL have the potential to make traditional breeding strategies for variety improvement more efficient. The effectiveness and efficiency, and strategies of MAS for QTL have been evaluated and proposed with both experimental and actual breeding populations

(Gimelfarb and Lande, 1995; Lindhout *et al.*, 1994; Monforte *et al.*, 1996; Ribaut *et al.*, 1997; Van Berloo and Stam, 1998). Results from a few studies have suggested that MAS is at least as effective in identifying superior genotypes as phenotype selection, and is more predictable across years and locations (Stuber, 1992, 1994, 1995). Schneider *et al.* (1997) have reported that MAS improved drought tolerance performance by 11% under stress and 8% under non-stress in common bean (*Phaseolus vulgaris*).

Using the model developed by Hanson (1959), using some simplifying assumptions, Tanksley and Rick (1980) predicted that the proportion of recurrent parent genome expected in the first backcross generation after selection for twelve markers (one per chromosome in tomato) was nearly same as in the third backcross without selection for recurrent parent phenotype.

Lande and Thompson (1990) studied the efficiency of MAS in the improvement of quantitative traits and concluded that molecular genetics can be integrated with traditional methods of artificial selection on phenotypes by applying MAS. The increase in selection efficiency from the use of marker loci, and sample size necessary to achieve them, depends on the genetic parameters and the selection scheme.

While investigating the use of markers to hasten recovery of the elite parent genome during an introgression breeding program, Hospital *et al.* (1992) showed that MAS may lead to a gain in time of about two generations.

Computer simulations were used to evaluate responses to MAS by Edwards and Page (1994). They compared MAS responses with those typical of phenotypic recurrent selection in an allogamous annual crop species, such as maize or pearl millet, and concluded that MAS may offer a primary advantage of enabling two selection cycles per year versus the 2 years per cycle.

That the higher efficiency of MAS on QTLs with large effects in early generation is balanced by a higher rate of fixation of unfavorable alleles at QTLs with small effects in later generations was reported by Hospital *et al.* (1997). This explains why MAS may become less efficient than phenotypic selection in the long term. MAS efficiency therefore depends on genetic determinism.

Knapp (1998) presented estimates of the probability of selecting one or more superior genotypes by MAS to estimate its cost-efficiency relative to phenotypic selection. The frequency of superior genotypes among selected progeny increases as selection intensity increases. Effectiveness of MAS compared to phenotypic selection was assessed by Van Berloo and Stam (1998) showing that MAS appears particularly promising when dominant alleles are present at QTLs and linked in coupling phase. Uncertainty in estimated QTL map positions reduces the benefits of MAS.

Based on his studies Young (1999) pointed that despite innovations like better marker systems and improved genetic mapping strategies, most marker associations are not sufficiently robust for successful MAS. Romagosa *et al.* (1999) verified the value of four QTLs for selection and compared the efficiency of alternative MAS strategies using these QTLs vs. conventional phenotypic selection for grain yield. Genotypic (MAS) and tandem genotypic and phenotypic selections were at least as good as phenotypic selection. Studies of Charmet *et al.* (1999) showed that the accuracy of QTL location determination greatly affects selection efficiency.

In rice, several authors have demonstrated the efficiency of MAS for the successful transfer of major genes for blast resistance (Inukai *et al.*, 1996; Hittalmani *et al.*, 2000) and for bacterial blight resistance (Huang *et al.*, 1997). MAS for QTLs has recently started to be applied to the genetic improvement of quantitative characters in several crops such as tomato (Lawson *et al.*, 1997; Bernacchi *et al.*, 1998), maize (Graham *et al.*, 1997) and barley (Han *et al.*,1997; Toojinda *et al.*, 1998). Useful guidelines have been provided for methodological choices (Visscher *et al.*, 1996a; Hospital and Charcosset, 1997), and overall breeding strategies have been proposed (Tanksley and Nelson, 1995; Tuinstra *et al.*, 1997).

2.5.2 Integration of MAS in to breeding program

As genomic molecular markers become available in certain species, questions are being raised about the practicality and economic efficiency of their use in breeding programs. In case of selection for a quantitative trait, marker-assisted selection programs can be undertaken (Lande and Thompson 1990).

For the introgression of qualitative traits such as pathotype-specific disease resistances, which are typically controlled by single, dominant genes, backcross breeding has been used for a long time (Allard, 1960). It allows the transfer of one or a few genes from a – often agronomically inferior – donor genotype into an elite recipient genotype, the recurrent parent.

Stam and Zeven (1980) estimated the length of chromosome segment with the desired marker gene introgressed from a donor by backcrossing in to recurrent parent and found that, for instance, for a chromosome with length of 100 cM the length of the introgressed segment wil average 32 cM in the BC_6 generation. MAS has the potential to considerably reduce the linkage drag that is associated with conventional backcross breeding programmes. Young and Tanksley (1989) estimated that, to transfer a gene with only 5 cM of donor DNA into the recipient parent, the number of backcross generations could be reduced from 100 to 2 using MAS. At the same time the heterozygotes at each resistance locus could be eliminated so that the plant breeder could rapidly select for genes in the homozygous state.

Lee (1995) suggested the utility of MAS for achieving and improving genetic gain through backcross breeding depends upon the current and potential role of that breeding method. Backcross breeding has been widely used for introducing monogenic characters and less so for polygenic traits. Perhaps the utility of this method could be made more broadly applicable through QTL mapping.

Markers were efficient in introgression backcross programs for simultaneously introgressing an allele and selecting for the desired genomic background Visscher *et al.* (1996a). Using a marker spacing of 10-20 cM gave an advantage of one to two backcross generations selection relative to random or phenotypic selection for recurrent parent phenotype controlled by alleles in non-target areas of the genome. When the position of the gene to be introgressed is uncertain, a chromosome segment should be introgressed that is likely to include the allele of interest.

Hospital and Charcosset (1997) demonstrated that using at least three markers per target QTL allows a good control over several generations and background selection is even more efficient in a pyramidal backcrossing program where QTLs are first monitored one by one.

Frisch *et al.* (1999) conducted computer simulations to compare selection strategies with regard to (i) proportion of recurrent parent genome recovered and (ii) the number of marker data points required in a backcross program designed for introgression of one target allele from a donor line into a recipient line. Again Frisch *et al.*, (1999) reported that molecular markers can accelerate recovery of recurrent parent genome when (i) the distance between the flanking markers and target locus is optimized and (ii) the minimum number of individuals required to obtain individuals that carry the donor allele at the target locus and have minimum proportion of donor genome on the carrier chromosome are taken into consideration.

Hash *et al.* (2000) described several alternative marker-assisted backcrossing (MABC) procedures that can be used for transferring QTL from a donor to a elite recurrent parent when these two lines have been used in forming the base mapping population. Charmet *et al.* (1999) advocated that a recurrent selection scheme is highly preferable for pyramiding many QTLs.

An approach was suggested by Ribaut and Betran (1999) that conducting a single large scale marker-assisted selection (SLA-MAS) to select plants at an early generation with a fixed, favorable genetic background at specific loci, while maintaining as much as possible the allelic segregation in the rest of the genome.

2.6 Inheritance of bristling in pearl millet

Rangaswami Ayyangar and Hariharan (1936) mentioned that an African race, *Pennisetum echinurus*, which has bristled panicles, when crosses with *P. leonis* without bristles showed an F_2 segregation with a wide range of bristled and non-bristled forms. Grouping all the bristly forms together, they obtained a ratio of 3 bristled : 1 non-bristled types. Kadam *et al.* (1940) reported sterility characterized by panicles bearing bristles. Ahluwalia and Shankar (1964) reported that bristling is governed by a single dominant gene (*Br*) and variation in density of bristling is possibly through the influence of modifying factors. Inheritance studies by Athwal and Gill (1966) have shown that bristling of panicles in pearl millet is a simply inherited dominant character and can thus be easily incorporated in inbred lines and varieties. Several other authors reported identical results (Krishnaswamy, 1962, quoted by Ahluwalia and Shankar, 1964; 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 (1974) noted monogenic incomplete dominance for bristling.

In crosses between long- and short-bristled plants, however, the bristle length was intermediate in F_1 and continuous variation was observed in F_2, indicating the additive action of more than one gene (Appa Rao *et al.*, 1988).

Athwal and Luthra (1964) advocated that bristling of pearl millet panicles is a useful economic character as it confers reduced vulnerability to bird damage and showed a monogenic mode of inheritance. Also they showed that there is no association between bristling and grain shedding and thus grain density in the panicle is not influenced by the presence of bristling. Athwal and Luthra (1964), Ahluwalia and Shankar (1964), and

Joshi (1968) observed that bristled lines are agronomically superior, as they confer resistance to bird damage, and the grain is not shed loosely. Beri *et al.* (1969) also noticed that bristling acted as a deterrent to grain-feeding birds.

Literature on inheritance of panicle bristling in pearl millet has been discussed in detail in several reviews of inheritance of morphological marker traits in this crop (Koduru and Krishna Rao, 1983; Anand Kumar and Andrews, 1983; Poncet, *et al.*, 1998, 2000).

3. MATERIALS AND METHODS

The present investigation was carried out during the period from August, 1998 to December, 2000 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The details of the experiments, conducted in the lab and field, are given below.

3.1 RFLP analysis

Table 1. Details of target traits and probe-enzyme combinations used for marker-assisted downy mildew resistance improvement of elite pearl millet pollinator H 77/833-2

Donor parent	Linkage group	Probe	Enzyme	Target trait
ICMP 451	LG 1	PgPSM 513	*Hind*III	Downy mildew resistance
		PgPSM 858	*Dra*I	
		PgPSM 565	*Hind*III	
		PgPSM 757	*Eco*RI	
ICMP 451	LG 4	PgPSM 464	*Dra*I	Downy mildew resistance
		PgPSM 716	*Dra*I	
		PgPSM 265	*Hind*III	
		PgPSM 416	*Dra*I	

3.1.1 Genomic DNA isolation

Basic steps involved in any DNA isolation procedure include:

Dark-grown, young seedlings (etiolated) or soft, non-green, stem internode tissues are generally used to isolate genomic DNA as they yield better DNA with better digestibility with restriction enzymes because of lower concentrations of phenolics and other

adhering compounds as compared to green tissues. Further, grinding of the plant material in a coffee grinder with dry ice or liquid nitrogen is done. The procedure of DNA isolation must be able to lyse the cell walls and cell membranes and release the DNA in the soluble media with the use of extraction buffer having SDS (sodium dodecyle sulphate), EDTA and proteinase K. This is followed by differential centrifugation to isolate genomic DNA from cell debris; precipitation of SDS-protein-carbohydrate complexes with sodium acetate-isopropanol precipitation, phenol and phenol-chloroform extractions; and a second precipitation of DNA with absolute alcohol.

Several procedures for genomic DNA isolation have been reported, but results obtained by the protocol given by Sharp et al. (1988) were most satisfactory. Therefore, the procedure given by Sharp et al. (1988) was adopted in the present study.

According to this protocol, DNA was isolated from 5 grams of etiolated seedlings, 10-14 days after emergence. These were quick frozen in liquid nitrogen and ground to a fine powder using either a coffee grinder with dry ice or a pre-chilled mortar and pestle with liquid nitrogen. The ground tissue were transferred to a 50 mL plastic centrifuge tube with 20 mL of extraction buffer containing 100 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCL, 2% SDS. Incubated in 65°C water bath for half an hour with mixing and then 50 µL of protenase K (10 mg/mL) was added. Again mixed and incubated for an hour in 55°C water bath. Samples were then extracted with equal volumes of phenol-chloroform-isoamyl alcohol (24:24:1 v/v) mixture and the emultion was separated by centrifugation at 5,000 rpm for 20 min at 4°C in a Sorvall HB7 rotor. The upper aqueous phase was re-extracted with equal volumes of chloroform-isoamly alcohol (24:1 v/v) and the emultion separated by centrifugation at 5,000 rpm for 20 min at 4°C. To the aqueous phase equal volumes of isopropanol was added and gently mixed and held at -20°C for 30 min. Precipitated DNA was spooled with a hook and dissolved in 2 mL of RNase-$T_{50}E_{10}$ (50 mM Tris-HCl pH 8.0 and 10 mM EDTA pH 8.0) buffer and incubated overnight (O/N) at room temperature. To inactivate RNase, samples were re-extracted with equal volumes of phenol-chloroform as described above. To the aqueous phase 2.5 volumes

of ethanol was added and held at -20°C for 1 h to precipitate DNA. DNA was spooled and washed with 70% ethanol (twice) and pellets were air-dried. The DNA was finally resuspended in appropriate volumes of $T_{10}E_1$ (10 mM Tris-HCl and 1 mM EDTA pH 8.0) according to the size of pellet and stored at 4°C.

DNA was quantified based on spectrophotometer measurements of UV absorption at 260 nm, assuming 1 OD at 260 nm is equal to 50 μg of DNA (Maniatis et al., 1982). The ratio of OD_{260} to OD_{280} was calculated to check the purity of DNA sample. Pure DNA preparation shows an OD_{260} to OD_{280} ratio ideally between 1.7 and 1.8 (Maniatis *et al.*, 1982).

DNA was analysed in 0.8% TAE-agarose gel to test the integrity as described in Maniatis *et al.* (1982). Gels were stained in ethidium bromide and viewed on UV-transillumninator before photographing with a camera fitted with a UV filter.

3.1.2 Restriction enzyme digestion

Twenty μg of DNA with sterile distilled water (SDW) was digested with *Dra*I, *Eco*RI, *Eco*RV and *Hind*III restriction endonucleases following the endonuclease supplier's instructions (Amersham Pharmacia Biotech, Ltd.). The digestion was carried out in a total volume of 30 μL and the reaction was terminated by addition of 3μLof loading buffer (25% sucrose, 0.1% bromophenol-blue and 20 mM EDTA) in each 30μL sample.

3.1.3 Electrophoresis

Fragments of digested DNA obtained after enzyme digestion were separated by electrophoresis in a 0.8% TAE-agarose horizontal slab gels (Bio-Rad DNA Sub Cell™) electrophoresis 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 molecular size marker. Gels were stained in 0.5 μg/mL ethidium bromide for 15 min, destained for 30 min in distilled water, viewed on a UV-transilluminator and photographed to assess the quality of digestion.

3.1.4 Southern blot hybridization

3.1.4.1 Preparation of southern blots

DNA fragments obtained after digestion were transferred from agarose gel on to Nucleic Acid Nylon Transfer Membrane (Hybond-N^+, Amersham Pharmacia Biotech, Ltd.) following the procedure of Reed and Mann (1985) (Appendix i). Transferred membranes were soaked in 2x SSC for 2 min to neutralize the alkali, air dried, and wrapped with cling film and stored at -20°C for future use.

3.1.4.2 Labelling of probes

The random-primed method of Feinberg and Vogelstein (1983) was used for labelling DNA with α-^{32}P. Purified insert DNA was denatured by heating at 95°C for 10 min, quenched on ice for 5 min before the labelling reaction mixture was added and incubated at 37°C for 3 hours. The reaction was terminated by adding 2.5 µL of 3 M NaOH to use in the hybridization step.

Labelling reaction mixture: 5 µL of oligo-labelling buffer (Amersham Pharmacia Biotech, 2 µL equimolar concentrations of dCTP, dGTP and dTTP, 2 µL (10 mg/ml) acetylated BSA, 5 µL of 50 µCi ^{32}P-dCTP, and 2 units of Klenow enzyme.

3.1.5 Hybridization to labeled probe

3.1.5.1 Prehybridization

Southern blots were prehybridized at 65°C with 5 mL of prehybridization solution (3 ml of 5x HSB, 1.5 mL of denatured salmon sperm DNA and 1.5 mL of Denhardt's solution and sterile distilled water to 15 mL) for 6 hours in case of new blots and 1 hour for stripped blot. Prehybridization was performed in a Techne Hybridizer (HB-1D).

3.1.5.2 Hybridization

Labelled probe was added to prehybridization mixture and incubated at 65°C in hybridization oven for at least 16 h (O/N). Care was taken to remove air bubbles present in between the blot and the hybridization bottle.

3.1.6 Washing of blots

Following hybridization, the blots were washed following four changes of 50 mL each of ^{32}P-wash solutions. Each wash was carried out for 15 min at 65°C in hybridization bottles using hybridization oven. First two washes were done using wash 1 solution (100 ml 20x SSC, 25 mL 20% SDS and distilled water to 1 liter) followed by two washes with wash 2 solution (10 mL 20x SSC, 25 ml 20% SDS and distilled water to 1 liter). Membranes were air dried and enclosed in cling films.

3.1.7 Autoradiography

Autoradiography was conducted at -70°C by exposing the membrane to photographic film (Kodak, X-OMAT™, XK-5) using Kodak intensifying screens in a cassette for various exposure times depending on counts. The X-ray films were developed with Kodak developer for 2-5 min followed by a stop bath (1% acetic acid) treatment for 1 min, fixed with Kodak fixer for 2 min, washed in running tap water and air dried. The autoradiograms were photographed using Kodak 100 ASA color films.

3.1.7.1 Scoring RFLP bands

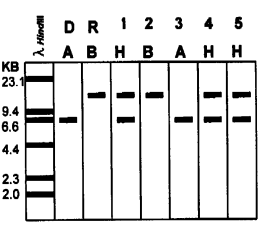
The banding patterns obtained from RFLP procedure were scored as follows

Donor parent genotype = A (D)

Recurrent parent genotype = B (R)

Heterozygotes = H

1 to 5 represent progenies



3.2 Mapping population and QTL mapping

Scientists at ICRISAT, Patancheru, India and the Institute of Grassland and Environmental Research (IGER), UK, jointly produced two pearl millet mapping populations to tag genes that control seedling heat tolerance of elite inbred pollinator H 77/833-2 (Howarth et al., 1994). The restriction fragment length polymorphism (RFLP)-based skeleton map for a population derived from a cross between H 77/833-2 and ICMP 451 was completed in 1994 at IGER. Field data on downy mildew (DM) incidence (Patancheru field population of *Sclerospora graminicola*), flowering time and 1000-grain mass were collected in the 1994 and 1996 dry season DM nurseries at ICRISAT, Patancheru, using F_4 self bulks derived from 94 of 154 mapped F_2 plants. Combining these two data sets using interval mapping procedures with Mapmaker/QTL 1.1 (Lincoln et al., 1992) permitted evaluation of the ability of the map to detect quantitative trait loci (QTLs). The basic pearl millet genetic linkage map and DM resistance QTL map for this cross are given in Fig. 1. and Fig. 2, respectively. The QTL map for this cross for other agronomically important traits is given in Fig. 3.

3.3 Choice of target segments and markers analyzed

Based on QTL mapping results, two segments were chosen for introgression of DM resistance from ICMP 451. The targeted regions for DM resistance in this cross were on linkage group 1 and 4 (Fig. 1 and Fig. 2).

The marker loci flanking the targeted regions were used for genotyping work. They are listed below:

-Target region of linkage group 1: *Xpsm* 513, *Xpsm* 858, *Xpsm* 565, *Xpsm* 757

-Target region of linkage group 4: *Xpsm* 464, *Xpsm* 716, *Xpsm* 265, *Xpsm* 416

In certain cases, where we had problems in getting useable results with any of above listed markers, we used other closely-linked markers to save time. (*Xpsm* 513 in place of *Xpsm* 280; *Xpsm* 265in between *Xpsm* 716 and *Xpsm* 416.

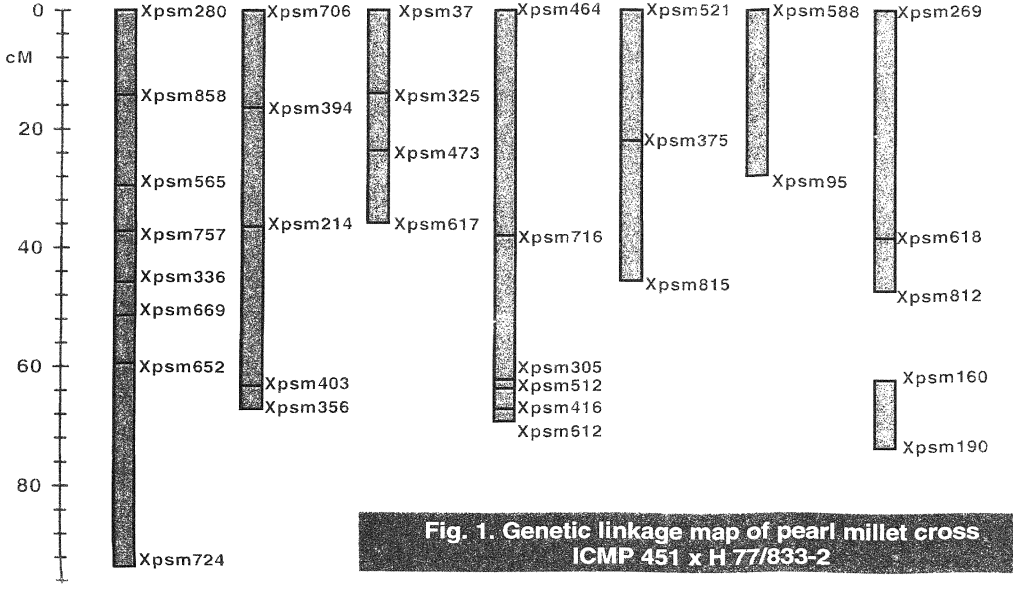
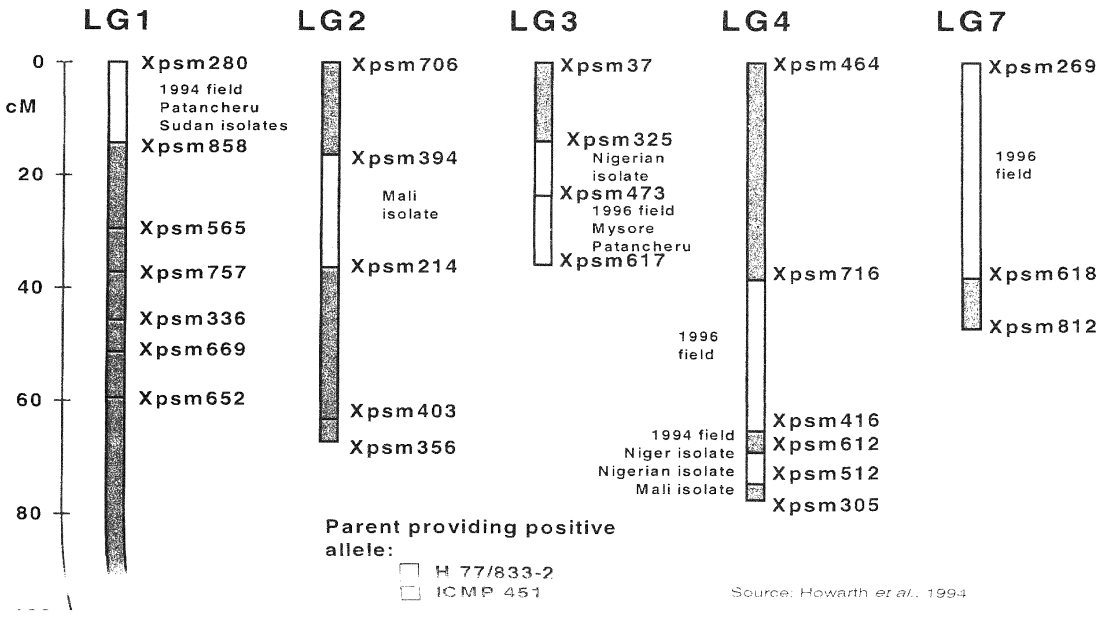
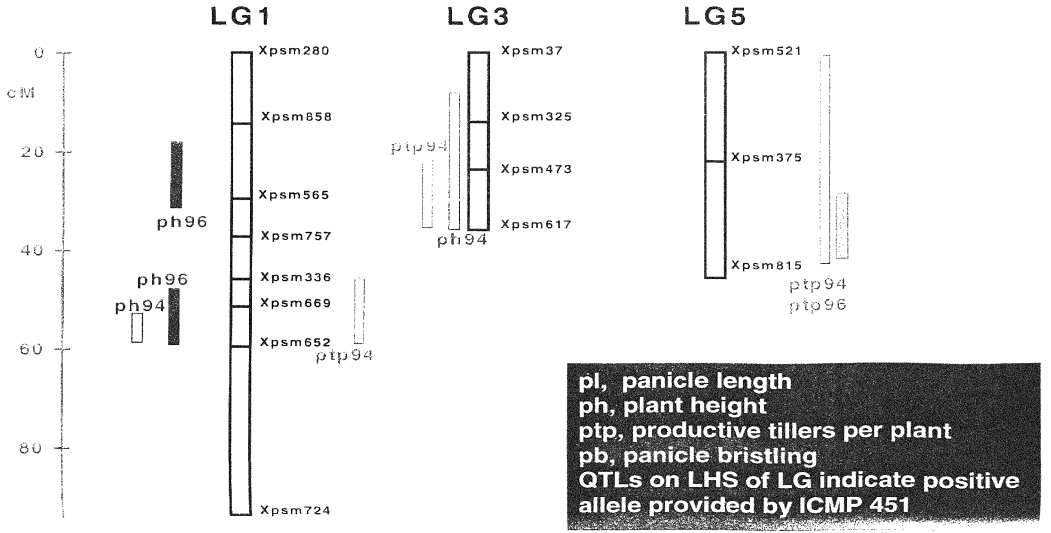


Fig. 1. Genetic linkage map of pearl millet cross ICMP 451 x H 77/833-2



Source: Howarth *et al.*, 1994

Fig. 3. QTLs associated with morphological characters in cross [illegible]



3.4 Parental lines used in crossing program

3.4.1 Donor parent: ICMP 451 (IPC 000107) (Anand Kumar et al., 1995) is a near-inbred line, LCSN 71-1-2-1-1, derived by selfing from the ICRISAT Late Composite (Fig. 4). It is the pollinator parent of hybrid ICMH 451 = MH 179 = 81A x ICMP 451, released for cultivation in India in 1986 (ICRISAT, 1988). It is tall, has long-bristled, semi-compact panicles, globular seeds of medium size, and is moderately resistant to the Patancheru isolate of pearl millet downy mildew. The subselection of ICMP 451 used as parent in this study, unlike the base population of ICMP 451, continues to have a relatively high level of resistance to downy mildew strains from across India.

3.4.2 Recurrent parent: H 77/833-2, the pollinator parent of three single-cross grain hybrids bred by Dr. R.L. Kapoor and his co-workers (Fig. 5) at Department of Plant Breeding, Haryana Agriculture University, Hisar, Haryana, India (Kapoor et al., 1989a, b, c). H 77/833-2 was bred by selfing and selection within a Rajasthani landrace population. It is early to flower, has medium height, tillers profusely at both base and nodes and has very small seed size. It has small-diameter panicles without bristling and is susceptible to the Patancheru isolate of pearl millet downy mildew. Compared to its base population, the subselection of H 77/833-2 used producing the mapping population study is about 2 days earlier to flower, has conical rather than cylindrical panicle shape, and has slightly larger grain. However, a more typical version of H 77/833-2 was used as the recurrent parent in the marker-assisted backcrossing program described in this thesis.

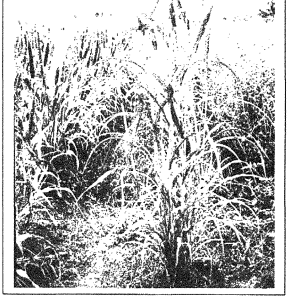
Phenotypes of parental lines grown at ICRISAT, Patancheru

Fig. 4.

Donor parent ICMP 451-P_6

X

Fig. 5.



Recurrent parent H 77/833-2

Fig. 6.

MAS-improved DMR version of H 77/833-2

3.5 Marker-assisted selection of backcrossed progenies for DMR

The sequence of operations is presented in Table 2. ICMP 451 was the donor of alleles increasing downy mildew resistance; H 77/833-2 was the recipient and recurrent parent. Marker-assisted selection (MAS) was used to select plants carrying ICMP 451 alleles at markers flanking the target regions in the BC_1F_1, BC_2F_1, BC_3F_1, BC_4F_1 and BC_4F_2 generations. The schematic representation of the development of the BC_4F_3 near-isogenic lines (NILs) using MAS at different generations is presented in Fig. 7. In each generation up to BC_4F_1, progenies with the desired genotype profile were selected before heading and used as female parent in crosses with H 77/833-2 in order to reduce the frequency of ICMP 451 alleles at non-target regions. The BC_4F_2 plants were selfed and screened for plants homozygous for the ICMP 451 allele at the target regions. In the BC_4F_2 generation plants were selected if they fit one of three categories:

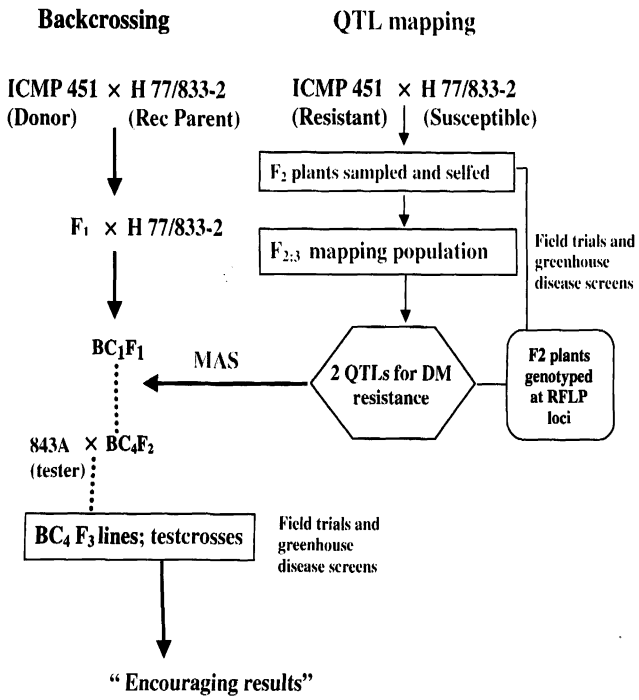
a. Homozygous for ICMP 451 alleles at markers flanking target regions of both linkage groups 1 and 4.
b. Homozygous for ICMP 451 alleles at markers flanking target regions of either linkage group 1 or 4.
c. Homozygous for ICMP 451 alleles at different and overlapping chromosome segments around the target segment on one of the linkage groups 1 and 4. These plants are intended for fine mapping the DM resistance QTLs.

Five or more progenies were advanced in each of the backcross generation. Selfed seed from all the backcross generations was used for tissue sampling for DNA isolation and RFLP analysis. BC_4F_3 rows derived from BC_4F_2 plants, homozygous for donor marker genotype in genomic regions immediately flanking target QTL, were selected.

Table 2. MAS operations and results in each generation

Backcross generation	Season	Selection for target trait	Type of plants selected	Product	Status of the product	Plants genotyped	Plants selected for further backcrossing or selfing
Hybridization ICMP 451 × H 77/833-2	1997 *Rabi*	No	None	F_1 seeds	50% H 77/833-2 50% ICMP 451		
BC_1F_1	1998 Summer	Yes	Heterozygous	BC_1F_1 seeds	75% H 77/833-2 25% ICMP 451	10	3
BC_2F_1	1998 *Kharif*	Yes	Heterozygous	BC_2F_1 seeds	87.5% H 77/833-2 12.5% ICMP 451	15	5
BC_3F_1	1999 Summer	Yes	Heterozygous	BC_3F_1 seeds	93.7% H 77/833-2 6.3% ICMP 451	25	6
BC_4F_1	1999 *Kharif*	Yes	Heterozygous	BC_4F_1 seeds	96.9% H 77/833-2 3.1% ICMP 451	100	7
Selfing	1999 Late *Kharif*	Yes	Homozygous	BC_4F_2 seeds	96.9% H 77/833-2 3.1% ICMP 451	178	9

Fig. 7. Schematic for transfer of downy mildew (DM) resistance by marker-assisted backcrossing in pearl millet



3.6 Conventional backcross introgression for bristling

Another experiment for the transfer of panicle bristling, from donor parent ICMP 451 to recipient and recurrent parent H 77/833-2, was also conducted. Using panicle bristling itself as a morphological marker, plants with bristled panicles were selected in BC_1F_1 through BC_4F_2 generations. We scored the panicle bristling (phenotypic judgement) as:

a. No bristling = 1
b. Small bristles = 2
c. Medium bristles = 3
d. Long bristles = 4

3.7 Field trials

Two field trials (DMR and panicle bristling) were conducted at ICRISAT, Patancheru in the genetic background of HHB 67 (843A x H 77/833-2). Material was sown in a Randomized Complete Block Design (RCBD) in three replications during *kharif* 2000 in the RP 8A (Fig. 8, 9, 10). Each entry was accommodated in two rows of four-meter length in each replication. Row to row distance was kept 30 cm and a plant to plant distance of 15 cm was maintained. Fertilizer was applied as per usual recommendation and recommended package of practices of the crop were followed for raising a good pearl millet crop.

In one trial, 178 BC_4F_2 plants were crossed on to 843A and the hybrids were compared with the original HHB 67. Here marker-assisted backcrossing had been used to transfer two additional downy mildew resistance genes from donor parent ICMP 451 to the genetic background of H 77/833-2.

In another trial, hybrids produced on 843A with 15 BC_4F_3 progenies derived by conventional backcrossing of the long panicle bristling trait from ICMP 451 into the genetic background of pollinator H 77/833-2 were compared with the original HHB 67. Observations were recorded on 14 phenotypic traits in both the trials during *kharif* season 2000.

Fig. 8.

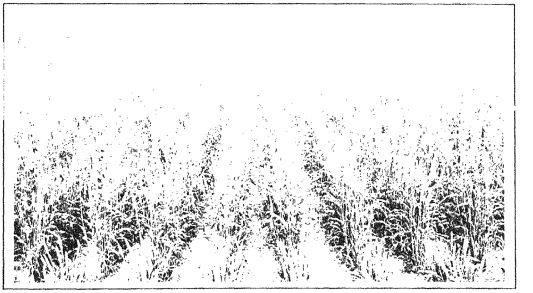
Testcross trial at flowering stage

Fig. 9.

Testcross trial post-anthesis measurement of plant height

Fig. 10.

Testcross trial at harvesting stage



3.8 Observations and measurements in trial

The observation and measurements taken during the trial were as follows

1. Time to bloom (TB): Time to 50% flowering was recorded as the number of days from sowing until 50% of the plants in each entry produced stigmas on their main stem panicles.
2. Plant height (PH): Plant height (cm) was measured from the base of the stem to the tip of the panicle at maturity. Data was recorded on 5 random plants from the middle of each row.
3. Panicle length (PL): Length of the panicle (cm) was measured for same plants considered for plant height in each plot.
4. Panicle girth (PG): Panicle girth (mm) was measured using vernier calipers on all those panicles for which panicle length was recorded.
5. Plant count (PC): Number of plants in the middle 3 m of two rows of each plot were counted for all the entries.
6. Head count (HC): Panicles from middle 3 m of two rows of each plot were harvested and counted for all the entries.
7. Effective tillers (ET): Number of productive tillers per plant were calculated by dividing PC by HC.
8. Panicle yield (PY): After harvesting was completed, panicles were put in an oven for 24 hours and dried at a temperature of 60°C. The dry weight of the panicles was then recorded before threshing.
9. Grain yield (GY): Panicles were threshed and their grain cleaned. The weight of the grains from each plot was recorded.
10. Fresh stover yield (FSY): After panicles were harvested, the stems and the tillers were cut for biomass analyses from the middle 3 m area of two rows for all the entries.

11. Sub sample fresh stover weight (SWS): Samples of fresh stover were then collected from each entry and chopped and fresh weights of these samples were taken.
12. Sub sample dry weight (SDS): The chopped samples were kept in a drier for two days at temperature of 60°C and their dry weights were then recorded.
13. 1000-grain mass (TGM): One thousand grains were counted and their weight was the recorded for each entry.
14. Bristling (BR): Based on presence or absence and length of panicle bristles the data was recorded for each entry as a score from 1-4 (1 = no bristles; 2 = small bristles; 3 = medium bristles; 4 = long bristles).
15. Biomass yield: Above-ground biomass yield was calculated for each plot as the sum of PY and the product of FSY * (SDS/SWS).

3.9 Statistical analyses

All the analyses were performed using Genstat version 5 from Rothamsted, UK. Analysis was performed using the data recorded from testcross trials for DMR and bristling. For each trait measured on individual plants, the phenotypic data was analysed as means of ten individual plants from each plot.

3.9.1 Statistical methods

The data obtained from *kharif* 2000 for different phenotypic traits were statistically analysed on the basis of model described by Panse and Sukhatme (1967).

$Y_{ij} = \mu + a_i + b_j + e_{ij}$

where,

Y_{ij} = observation in the i^{th} treatment and j^{th} block

μ = general mean

a_i = i^{th} treatment effect

b_j = j^{th} block effect, and

e_{ij} = random error associated with the i^{th} treatment and the j^{th} block.

The assumptions of the model are:

a. All the observations should be independent
b. Different effects in the model should be additive
c. Error involved in the population should be normally and independently distributed with mean zero and variance σ_e^2

3.9.2 Analysis of variance (ANOVA)

Analysis of variance for all characters under study were carried out, separately, as follows

Source	d.f.	M.S.	Expected M.S.	F ratio
Replication	(r-1)	M_{rii}	$\sigma^2_{eii} + t\sigma^2_{rii}$	Mt_{ii}/Me_{ii}
Treatment	(t-1)	Mt_{ii}	$\sigma^2_{eii} + r\sigma^2_{gii}$	
Error	(r-1) (t-1)	Me_{ii}	σ^2_{eii}	

where,

r = number of replications;

t = number of treatments or genotypes;

M_{rii}, Mt_{ii} and Me_{ii} stands for mean sum of squares due to replication, treatment and error, respectively;

σ^2_{gii} = genotypic variance of character x_i; and

σ^2_{eii} = error variance of character x_i.

3.9.3 The genotypic and phenotypic variances were calculated as follows

$$\text{Genotypic variance of character } x_i = \sigma^2_{gii} = \frac{Mt_{ii} - Me_{ii}}{r}$$

$$\text{Phenotypic variance of character } x_i = \sigma^2_{pii} = \sigma^2_{gii} + \sigma^2_{eii}$$

3.9.4 Parameters of variability

(i) Mean :

Mean value ($\bar{x}$) of each character was worked out dividing the sum of the observed values by the corresponding number of observations:

$$\bar{x} = \frac{\Sigma X_{ij}}{N}$$

where,

X_{ij} = any observation in the i^{th} and j^{th} replication, and

N = total number of observations.

(ii) Range:

Lowest and highest values for each character were recorded

(iii) Standard error:

Standard errors of means were calculated for each character from the corresponding mean square error values from the analysis of variance tables as:

$$\text{S.E. (m)} : \sqrt{\frac{\sigma^2_e}{r}}$$

where,

σ^2_e is estimated mean sum of squares

S.E. is the standard error of the mean, and

r is the number of replications.

(iv) Honestly significant difference (hsd):

For all the characters, hsd was calculated to compare treatment means as suggested by Tukey (1953), using the equation:

$$W = q_\alpha (p, n_2) s_{\bar{x}}$$

where,

q_α is obtained from Table for $\alpha = .05$ or .01 (Steel and Torrie, 1960; Appendix vi)

p is the number of treatments,

n_2 is the error degrees of freedom,

$s_{\bar{x}}$ is the standard error of mean, and

w is used to judge the significance of each of the observed pair-wise differences between treatment means.

(v) Coefficient of Variation:

Genotypic and phenotypic coefficients of variation were estimated by the formula suggested by Burton (1952) for each character as:

$$\text{Genotypic coefficient of variation (G.C.V.)} = \frac{\sqrt{\sigma^2_{gii}}}{\bar{x}} \times 100$$

$$\text{Phenotypic coefficient of variation (P.C.V.)} = \frac{\sqrt{\sigma^2_{pii}}}{\bar{x}} \times 100$$

where $\bar{x}$ is the mean of that particular trait.

(vi) Heritability (in broad sense):

Heritability in broad sense was calculated according to the formula suggested by Hanson et al. (1956) for each character as given below

$$H = \frac{\sigma^2_{gii}}{\sigma^2_{pii}} \times 100$$

(vii) Genetic advance expressed as percentage of mean:

Estimates of appropriate variance components were substituted for the parameters to predict expected genetic gain as suggested by Lush (1949) and Johnson et al. (1955). The expected genetic advance was calculated at 5% selection intensity for each character as :

$$\text{Genetic advance (\% of mean)} = \frac{K\,\sigma_p . H}{\bar{x}} \times 100$$

where, $K\,\sigma_p$ is the selection differential expressed in terms of phenotypic standard variations (using 5% selection in a large sample from a normally and independently distributed population, the value of selection intensity (K) is equal to 2.06 (Allard, 1960);

H is the heritability in the broad sense; and

$\bar{x}$ is the mean value for that character over all the genotypes.

3.10 Downy mildew screening

3.10.1 Inoculum

All experiments were carried out using an asexually-maintained pathogen population derived from plants infected with oospores from the ICRISAT field downy mildew nursery at Patancheru, India. The population was collected and maintained as described by Jones (1994) and Jones *et al.* (1995).

Infected leaves from mature plants of universally susceptible genotype 7042(S) were detached, wiped clean of any sporangiophores already present and incubated in darkness in plastic boxes for 8 h at 20°C and 100% RH. The resulting sporangia were harvested by spraying leaves by de-ionised water and collecting the run-off. The sporangia produced from the leaves were harvested into chilled de-ionised water at approximately 1°C. Suspension was then adjusted to $1x10^5$ sporangia mL^{-1} with water at appropriate temperature. Spraying was carried out using the spray head of a hand-pumped 500 mL sprayer.

3.10.2 Disease incidence determination

40 seeds of all the entries along with parental lines and standard checks including universally susceptible pearl millet genotype 7042(S) (Hash and Witcombe, 1994) were sown in 11.5 cm diameter plastic pots. Each pot was a replicate and there were two pot-replicates for each treatment. Pots were placed on flood-benching in a completely randomised block under glasshouse conditions as described by Jones et al. (1995). Each pot of seedlings was sprayed at the coleoptile-to-one-leaf stage with approximately 4 mL of inoculum. Following inoculation, the glasshouse bench was covered with polythene sheeting for 16-18 h to maintain high humidity. Disease incidence (% of plants showing chlorotic symptoms per pot) was assessed two weeks after inoculation based on number of diseased plants out of total number of plants in a pot.

4. RESULTS

Agronomic phenotyping of BC_4F_2-derived downy mildew resistant (DMR) testcrosses

In the present study data on agronomically important phenotypic characters were recorded and analyzed for 178 BC_4F_2-derived testcrosses and related to those of near-isogenic elite control hybrid HHB 67. Summaries of the agronomic performance of DMR entries, control HHB 67, and the mean of the trial, are presented in Table 3. The coefficient of variability (CV) for most of the characters studied was less than 15% except for effective tillering, stover dry matter percentage, and fresh stover yield where it was 18.2%, 20.6% and 19.5% respectively. These are reasonable levels for CV values for a modestly replicated small-plot field trial.

The analyses of variance indicated statistically significant differences (at $P = 0.01$ and 0.05) for all agronomic characters studied except for grain yield, effective tillers, fresh stover yield and 1000-grain mass, where the differences between treatment means were non-significant. Plant count, effective tillers and harvest index exhibited low broad-sense heritability values of 0.20, 0.30 and 0.37, respectively. The other agronomic characters evaluated showed moderate to high broad-sense heritability values. Entry 51 ranked first with grain yield of 3590 kg ha^{-1} and relatively low downy mildew incidence (DMI). Other good entries included 118 and 97. Compared to the mean of control HHB 67, time to 50% bloom was delayed by one day for entry 51, which also had significantly greater plant height, panicle length, fresh stover yield, biomass, growth index and 1000-grain mass than the control.

Table 3. Agronomic performance of DMR testcross entries compared to the mean of control HHB 67 and the mean of the trial as a whole; Patancheru, rainy season 2000

Entry	DMI	GY	TB	PH	PL	PG	PC	ET	BR	DMP	FSY	BM	HI	GI	TGM
	%	kg ha^{-1}	d	cm	cm	mm	no.	no.	score	%	kg ha^{-1}	kg ha^{-1}	%	kg ha^{-1} d^{-1}	g
113	39.1	2820	36.3	140	18.3	25.7	87	3.7	1.0	0.47	1790	5540	0.51	120	9.58
110	52.4	2970	37.0	135	17.7	26.0	95	3.3	1.0	0.40	2230	6200	0.49	132	9.42
109	54.2	3190	36.0	140	18.0	27.7	94	2.7	1.0	0.38	3110	7060	0.46	153	10.17
97	54.7	3270	38.0	137	18.0	26.3	95	3.3	1.0	0.44	1860	6100	0.54	127	9.62
95	58.8	2990	37.3	139	18.0	26.3	91	3.3	1.0	0.39	2480	6460	0.47	137	9.75
18	61.2	2570	39.3	147	18.7	24.7	99	2.7	2.7	0.34	2260	5670	0.46	117	8.83
106	61.3	2830	36.7	139	17.7	27.0	110	3.0	1.0	0.38	2130	5800	0.50	124	9.63
104	61.8	2680	37.3	140	17.3	25.7	94	3.0	1.0	0.34	1900	5390	0.50	114	9.30
82	66.7	2440	39.0	143	18.0	24.3	94	2.3	1.0	0.34	1320	4510	0.55	92	8.73
96	66.7	2480	36.8	139	17.3	26.3	97	2.3	1.0	0.36	2090	5420	0.46	127	9.67
111	66.7	3130	38.7	137	17.3	25.7	91	3.3	1.0	0.34	2730	6700	0.47	139	9.30
71	67.4	2370	40.7	154	20.7	26.0	83	2.7	3.3	0.36	1840	4940	0.49	98	8.50
155	67.8	2960	38.0	143	20.7	24.3	85	3.3	2.0	0.37	2200	6050	0.50	126	9.50
161	70.4	2040	39.7	149	19.7	23.7	83	2.7	2.3	0.34	1580	4380	0.47	89	8.20
153	71.9	2690	37.0	145	20.3	24.7	88	3.0	2.0	0.36	2250	5820	0.47	124	9.55
46	72.0	2830	39.0	148	20.0	25.7	97	2.7	4.0	0.41	1880	5690	0.50	116	8.80
105	72.2	2770	37.0	141	18.3	27.0	86	3.3	1.0	0.39	1630	5340	0.52	114	9.70
139	72.2	2980	37.0	146	20.3	25.3	105	3.0	2.0	0.32	1990	5990	0.50	128	9.43
61	72.9	2560	37.7	145	19.0	25.0	89	3.0	2.3	0.39	1740	5050	0.52	106	9.43
84	74.1	2810	36.0	141	20.0	26.3	100	3.0	2.7	0.43	1800	5510	0.51	120	9.53
108	74.1	2880	38.0	140	18.0	27.0	96	2.7	1.0	0.33	1750	5380	0.54	112	9.83
48	74.5	2760	37.7	135	18.7	25.0	104	2.7	3.7	0.40	1820	5480	0.51	115	8.57
37	74.6	2750	36.7	139	19.7	25.7	95	3.3	2.3	0.38	2130	5700	0.49	122	9.88
112	74.7	2670	37.0	135	18.0	26.7	106	3.0	1.0	0.44	1750	5280	0.51	113	9.65
51	75.0	3590	38.7	146	19.3	26.0	105	3.0	3.7	0.32	2480	7000	0.52	144	9.93
119	75.0	2940	39.0	140	17.7	25.7	96	3.0	1.0	0.33	1920	5720	0.52	117	9.70
88	76.0	2910	40.3	152	18.3	26.0	90	2.3	1.3	0.35	1840	5530	0.53	110	9.73
107	76.0	3040	36.0	135	17.3	27.0	97	3.3	1.0	0.34	1980	5850	0.53	127	9.42
76	76.8	2480	37.0	143	18.3	24.3	93	3.0	3.7	0.49	1680	4930	0.51	105	8.90
156	76.8	2840	37.7	135	18.7	24.0	104	3.3	1.7	0.34	2050	5830	0.49	123	9.93
Selected DMR entries															
Mean	67.9	2910	37.8	142	18.6	25.7	95.0	3.0	1.8	0.38	2010	5680	0.50	120	9.41
SE (+/-)	9.3	185	0.7	2.2	0.3	0.4	6.8	0.3	0.2	0.03	213	369	0.02	8.2	0.27
HHB 67 (control)															
Mean	97.3	2610	37.5	137	17.5	25.0	88.2	3.3	1.0	0.37	1630	5090	0.52	107	9.05
Maximum	100.0	3010	39.0	143	18.3	26.3	106	4.0	1.0	0.42	1890	5750	0.56	122	9.72
Minimum	92.3	2240	36.0	132	16.3	23.3	70	2.7	1.0	0.32	1250	4410	0.48	91	8.57
SE (+/-)	1.1	182	0.7	1.9	0.3	0.3	7.8	0.4	0.0	0.03	171	342	0.02	7.8	0.26
Trial as a whole															
Mean	86.1	2720	37.7	141	18.7	25.4	92.0	3.1	2.0	0.37	1910	5490	0.50	116	9.30
SE (+/-)	6.2	191	0.7	2.1	0.4	0.4	7.8	0.3	0.2	0.03	215	360	0.02	8.1	2.26
CV (%)	10.2	12.2	3.2	2.6	3.6	2.6	14.8	18.2	20.6	14.3	19.5	11.4	7.6	12.1	4.8
Heritability	0.64	0.43	0.61	0.83	0.87	0.88	0.20	0.30	0.95	0.54	0.65	0.62	0.37	0.61	0.72
F-ratio	2.78	1.77	2.59	5.93	7.52	8.18	1.25	1.42	19.1	2.17	2.87	2.62	1.59	2.53	3.58
lsd															
P = 0.01	22.5**	ns	2.8**	8.6**	1.6**	1.6**	32.3**	ns	0.95**	0.12**	ns	1490**	0.08**	33.2**	ns
P = 0.05	17.1*	ns	2.3*	6.9*	1.3*	1.3*	25.9	ns	0.76*	0.10*	ns	1190*	0.07*	26.7*	ns

DMI = downy mildew incidence; GY = grain yield; TB = time to 50% bloom; PH = plant height; PL = panicle length; PG = panicle girth; PC = plant count; ET = effective tillers; BR = panicle bristling; DMP = dry matter percent; FSY = fresh stover yield; BM = biomass; HI = harvest index; GI = growth index; TGM = 1000-grain mass

*, ** = significant at the *P* = 0.05 and 0.01 levels, respectively; ns = non-significant

Downy mildew incidence (%)

Differences for DMI among DMR testcross entries were significant at $P = 0.01$ and 0.05. The original HHB 67 showed a mean DMI of 97.3% where as the DMR testcross entries had mean value of 67.9% for this trait. Among DMR testcross entries, a range of 39.1% (entry 113) to 76.8% (entry 156) for DMI was observed indicating the segregation of either one or both of the targeted regions from the resistant donor among the BC_4F_2 testcrosses. An overall reduction for DMI of 20-30% was observed among DMR testcross entries. This trait exhibited a broad-sense heritability of 0.64, which is reasonably high.

Plant height (cm)

Significant differences were also revealed for plant height (PH). For DMR testcross entries PH ranged from 135 cm (entries 48, 107, 110, 112 and 156) to 154 cm (entry 71) while the mean PH for the trial was recorded as 141 cm and of controls (HHB 67) as 137 cm. There was an increase of 5 cm in mean PH of the DMR testcross entries as compared to the mean of control HHB 67. A high broad-sense heritability of 0.83 was observed for PH in this trial.

Panicle length (cm)

DMR testcross entries exhibited significant differences for panicle length (PL). Ranging from 17.3 cm (entries 96, 104, 107 and 111) to 20.7 cm (entries 71, 155), panicle length had a mean of 18.6 cm in the DMR testcross entries. Trial mean and mean for control plots (HHB 67) were observed as 18.7 and 17.5 cm, respectively, indicating a modest positive increase in mean of the DMR testcross entries for this trait. PL showed a high broad-sense heritability of 0.87.

Fresh stover yield (kg ha^{-1})

Significant differences were observed in treatment means for fresh stover yield (FSY) at both levels of significance. Ranging from 1320 kg ha^{-1} (entry 82) to 3110 kg ha^{-1} (entry 109), FSY had a mean of 2010 as compared to the trial mean and the mean of control

plots (HHB 67) of 1910 and 1630 kg ha^{-1}, respectively. An increase of 375 kg ha^{-1} in FSY was recorded for the DMR testcross entries over that of the control, with a moderate broad-sense heritability level of 0.65 in this trial.

Biomass (kg ha^{-1})

Biomass (BM) exhibited significant differences in treatment means for the DMR testcross entries. With a mean of 5677 kg ha^{-1}, BM ranged from 4380 kg ha^{-1} (entry 161) to 7060 kg ha^{-1} (entry 109). Trial mean of 5492 kg ha^{-1} was recorded along with the mean of control plots (HHB 67) as 5092 kg ha^{-1}. As compared to the HHB 67 control, an increase of 485 kg ha^{-1} for the DMR testcross entries was obtained. In this trial, BM showed a moderate broad-sense heritability level of 0.62.

Growth Index (kg ha^{-1} d^{-1})

Significant differences between treatment means for growth index (GI) were observed. The DMR testcross entries ranging from 89 kg ha^{-1} d^{-1} (entry 161) to 153 kg ha^{-1} d^{-1} (entry 109) exhibited a mean GI value of 120 kg ha^{-1} d^{-1}. The trial mean and mean of control plots (HHB 67) had values 116 and 107 kg ha^{-1} d^{-1}, respectively. Thus a mean increase of 13 kg ha^{-1} d^{-1} in GI for the DMR testcross entries was observed. Growth index showed a moderate broad-sense heritability of 0.61.

Agronomic phenotyping of BC_4F_3-derived bristled testcrosses

Table 4 presents agronomic performance of 15 BC_4F_3-derived bristled testcrosses compared to the mean of control plots (HHB 67) and the mean of trial. The analysis of data recorded for different phenotypic characters revealed that the coefficient of variability was less than 15% for many characters except for downy mildew incidence (%), plant count, and effective tillers. At the $P = 0.01$ and 0.05 levels of significance, significant differences were detected by analysis of variance for all the characters studied other than grain yield, plant count and growth index. Variation in DMI (H = -0.08), grain yield (H = 0.02), plant count (H = -0.25), effective tiller number (H = -0.86), was essentially not heritable in this trial while that of biomass (H = 0.40) and growth index

(H = 0.29) exhibited low broad-sense heritability values. This suggests that little, if any, genetic variability was present for these six traits in this set of closely related testcrosses.

Maximum grain yield was recorded as 2910 kg ha^{-1} for entry 3 followed by entries 6 and 8, but these were not significantly different from grain yields of other test entries and controls in this trial. Entries 13 and 15 showed lower DMI values of 64.4 and 67.6%, respectively. Entry 3 was delayed by 2 days in terms of time to 50% bloom where as it exceeded the control (HHB 67) in case of plant height, panicle length, fresh stover yield, biomass, growth index and 1000-grain mass, along with long panicle bristles trait that was used as a morphological marker in this backcrossing program.

Time to 50% bloom (d)

Treatment mean differences were observed to be statistically significant for time to 50% bloom (TB). TB of bristled entries was delayed by approximately one day relative to that of the mean of control plots (HHB 67). Some entries were early in TB compared to the controls (HHB 67). TB ranged from 36.3 d (entries 13 and 14) to 38.3 d(entry 3) in the bristled entries whereas the trial mean was observed to be 37.1 d and the controls (HHB 67) had a mean of 36.4 d. A moderate broad-sense heritability of 0.61 was recorded for TB in this trial.

Plant height (cm)

Significant differences were also revealed for plant height (PH). For bristled entries, PH ranged from 143 cm (entry 7, 8) to 154 cm (entry 11, 12) while the mean PH for trial was recorded as 146 cm and of checks as 137 cm. There was an increase of 11cm in mean PH of bristled entries as compared to the mean of control plots (HHB 67). A very high broad-sense heritability of 0.93 was observed for PH in this trial.

Table 4. Agronomic performance of bristled testcross entries compared to the mean of control HHB 67 and the mean of the trial as a whole; Patancheru, rainy season 2000

Entry	DMI	GY	TB	PH	PL	PG	PC	ET	BR	DMP	FSY	BM	HI	GI	TGM
	%	kg ha^{-1}	d	cm	cm	mm	no.	no.	score	%	kg ha^{-1}	kg ha^{-1}	%	kg ha^{-1} d^{-1}	g
15	64.4	2630	37.0	144	19.0	24.7	98	3.0	4.0	0.38	2410	5830	0.45	124	9.10
13	67.6	2590	36.3	152	20.3	24.7	90	3.3	4.0	0.41	2690	6130	0.42	132	8.92
7	81.5	2470	37.0	143	20.3	23.7	98	3.0	2.7	0.37	2470	5750	0.43	122	8.60
6	85.0	2850	37.3	147	19.0	25.3	101	2.7	4.0	0.37	2470	6090	0.47	129	9.72
2	86.4	2470	38.0	148	20.0	25.7	94	2.7	3.3	0.34	2120	5320	0.46	111	9.42
1	87.9	2500	37.0	146	20.0	26.7	87	3.3	4.0	0.44	2360	5610	0.44	120	9.65
3	88.6	2910	38.3	152	19.7	26.0	90	3.3	4.0	0.36	2570	6250	0.46	129	10.03
12	88.9	2530	37.3	154	20.0	24.7	94	2.7	4.0	0.40	2830	6240	0.41	132	8.80
14	89.9	2610	36.3	152	19.7	25.7	106	3.0	4.0	0.49	2430	5910	0.44	128	9.08
10	92.3	2720	36.7	153	20.7	25.3	102	3.0	4.0	0.42	2700	6150	0.44	132	8.77
4	96.5	2600	37.3	146	20.0	25.0	106	2.7	3.3	0.38	2090	5480	0.48	116	8.92
5	98.4	2490	37.0	145	19.7	25.7	96	3.0	3.3	0.38	2080	5340	0.46	114	9.47
9	98.4	2630	37.0	144	19.3	24.7	100	3.0	3.7	0.42	2180	5640	0.47	120	8.03
8	100.0	2740	37.7	143	19.0	26.7	98	3.0	3.3	0.39	2450	5850	0.47	123	9.10
11	ND	2650	37.7	154	20.3	24.3	103	2.7	3.7	0.44	2600	5970	0.44	126	8.68
Bristled entries															
Mean	87.6	2620	37.2	148	19.8	25.2	98	3.0	3.7	0.40	2430	5840	0.45	124	9.09
SE (+/-)	5.4	157	0.4	1.6	0.5	0.3	10.0	0.4	0.2	0.02	122	275	0.01	6.3	0.24
HHB 67 (control)															
Mean	97.3	2480	36.4	137	17.1	24.6	105	2.7	1.0	0.44	2210	5490	0.45	118	8.82
Maximum	100.0	2720	36.7	138	17.3	25.0	116	3.0	1.0	0.47	2390	5970	0.46	129	8.88
Minimum	92.3	2260	36.3	135	17.0	24.0	96	2.3	1.0	0.40	2030	5130	0.44	111	8.78
SE (+/-)	1.1	150	0.5	1.3	0.3	0.3	15.3	0.5	0.0	0.02	141	258	0.02	6.4	0.18
Trial as a whole															
Mean	87.7	2600	37.1	146	19.4	25.1	99	2.9	3.2	0.41	2390	5780	0.45	123	9.04
SE(+/-)	9.8	152	0.4	1.6	0.5	0.3	10.6	0.4	0.2	0.03	120.6	266	0.01	6.1	0.23
CV (%)	15.9	10.2	1.7	1.9	4.3	2.0	18.6	22.4	11.1	11.3	8.7	8.0	4.5	8.6	4.5
Heritability	-0.08	0.02	0.61	0.93	0.83	0.88	-0.25	-0.86	0.96	0.59	0.74	0.40	0.56	0.29	0.76
F-ratio	0.92	1.02	2.58	13.43	5.75	8.06	0.45	0.54	27.8	2.45	3.85	1.67	2.29	1.40	4.11
hsd															
$P = 0.01$	ns	ns	1.6**	7.1**	2.1	1.3**	ns	ns	0.93**	0.13**	536**	1185**	0.04**	ns	1.02**
$P = 0.05$	29.2*	ns	1.3*	5.6*	1.7*	1.0*	ns	ns	0.73*	0.10*	421*	929*	0.03*	ns	0.80*

DMI = downy mildew incidence; GY = grain yield; TB = time to 50% bloom; PH = plant height; PL = panicle length; PG = panicle girth; PC = plant count; ET = effective tillers; BR = panicle bristling; DMP = dry matter percent; FSY = fresh stover yield; BM = biomass; HI = harvest index; GI = growth index; TGM = 1000-grain mass

ns = non-significant

*, ** = significant at the $P = 0.05$ and 0.01level, respectively

Panicle length (cm)

Bristled entries exhibited non-significant differences for panicle length (PL). Ranging from 19.0 cm (entries 6, 8, 15) to 20.7 cm (entry 10) panicle length had a mean of 19.8cm in bristled entries. Trial mean and mean for control plots (HHB 67) was observed as 19.4 and 17.1 cm, respectively, indicating a positive increase in the mean of bristled entries for this trait. PL showed a high broad-sense heritability of 0.87 in this trial.

Panicle bristling (visual rating 1-4)

Significant differences were observed for panicle bristling (BR). Panicle bristling segregated from medium-bristled (score = 2.7) to long-bristled (score = 4.0) panicles with a mean of long bristles in the bristled testcross entries. Trial mean for BR was medium-bristled while it was non-bristled (score = 1.0) for the control plots (HHB 67). A very high broad-sense heritability value of 0.96 was recorded for panicle bristle score in this trial.

Biomass (kg ha^{-1})

Biomass (BM) exhibited significant differences in treatment means for bristled testcross entries; with a mean of 5837 kg ha^{-1}, BM ranged from 5320 kg ha^{-1} (entry 2) to 6250 kg ha^{-1} (entry 3). Trial mean of 5780 kg ha^{-1} was recorded along with the mean of control plots (HHB 67) as 5494 kg ha^{-1}. As compared to the controls, BM showed an increase of 340 kg ha^{-1} for bristled testcross entries was obtained. In this trial, BM showed a moderately low broad-sense heritability of 0.40.

Harvest index (%)

Treatment means manifested significant differences for harvest index (HI) with a mean of 50% for bristled testcross entries. A range of 46% (entry 18) to 55% (entry 82) was expressed for HI. This trait exhibited a trial mean of 50% and mean of control plots (HHB 67) 52%, implying a reduction of 2% in the mean of bristled testcross entries. A moderately low broad-sense heritability of 0.37 was observed for HI in this trial.

Growth index (kg ha^{-1} d^{-1})

Non-significant differences between treatment means for growth index (GI) were observed. Bristled testcross entries ranging from 111 kg ha^{-1} d^{-1} (entry 2) to 132 kg ha^{-1} d^{-1} (entries 10 and 13) exhibited a mean GI value of 124 kg ha^{-1} d^{-1}. The trial mean and mean of control plots (HHB 67) had values 123 and 118 kg ha^{-1} d^{-1}, respectively. A non-significant increase of 6 kg ha^{-1} d^{-1} in GI for bristled testcross entries was also observed. Growth index showed a low broad-sense heritability of only 0.29.

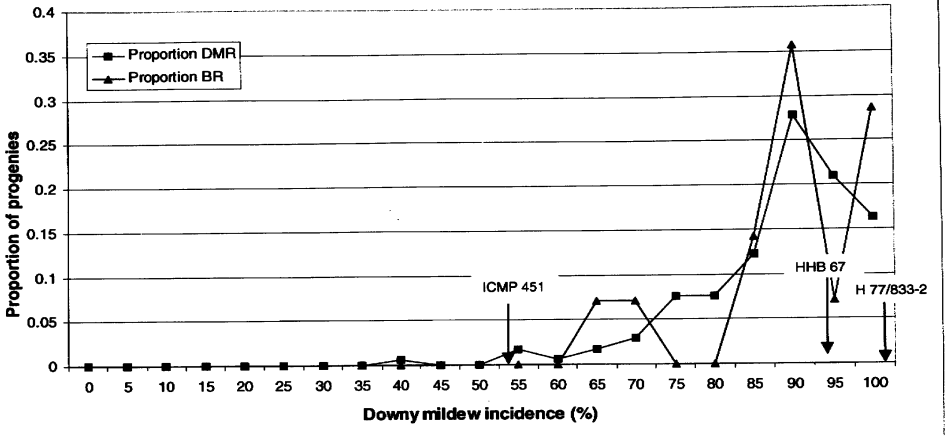
1000-grain mass (g)

Bristled testcross entries treatment means revealed significant differences for thousand grain mass (TGM). With a mean of 9.09 g, bristled testcross entries ranged from 8.03 g (entry 9) to 10.03 g (entry 3). TGM showed mean values for the trial as a whole and control plots (HHB 67) of 9.04 and 8.82 g, respectively. The mean of bristled testcross entries showed a non-significant gain of 0.27 g over that of the trial mean for this trait. A fairly high broad-sense heritability value of 0.76 was also manifested for TGM in this trial.

Downy mildew screening

All the 193 testcrosses, 178 BC_4F_2-derived DMR testcrosses and 15 BC_4F_3-derived bristled testcrosses, were subjected to greenhouse downy mildew (DM) screening against a DM pathogen population from Patancheru, India. The results on downy mildew incidence (DMI, %) are presented graphically in Fig 11. Among the parental lines, donor parent ICMP 451 was fairly resistant to the Patancheru DM isolate with 52% DMI as compared to 100% DMI in case of recurrent parent H 77/833-2. The original HHB 67 had DMI of 97% whereas standard checks 7042(S) and 7042(R) exhibited 98% and 75% DMI, respectively. The proportion of DMR-testcross as well as bristled-testcross progenies were skewed towards susceptibility (Fig. 11), as expected since their pollinatos were still segregating for the targeted DM resistance genes. Based on previous generation pedigrees, segregation for disease resistance against Patancheru isolate among related families of the 193 testcrosses gave best fits to ratios of 1 resistant : 15 susceptible, 3 resistant : 13 susceptible, and 7 resistant : 9 susceptible (Table 5), depending upon the family of testcrosses concerned.

Fig. 11. Distribution of 193 testcross progenies (15 BC_4F_3 testcrosses from bristled backcrossing and 178 BC_4F_2 testcrosses from downy mildew resistance backcrossing) for disease incidence (%) when screened under greenhouse conditions at ICRISAT against a downy mildew pathogen population from Patancheru, India



BR = bristled testcrosses; DMR = downy mildew resistant testcrosses

Table 5. Chi-square estimates for goodness of fit to a range of classical Mendelian segregation ratios for greenhouse screen DM reaction against Patancheru isolate among 193 testcrosses of the pearl millet cross ICMP 451 x H 77/833-2.

BC_4F_1 plant no.	BC_4F_2 plant no.	Linkage group	Mendelian ratios					
			1:63	1:15	9:55	3:13	1:3	7:9
# 1	1-19	LG 1	8.903	**0.166**	6.407	11.939		
# 2	20-41	LG 1	41.208	**1.829**	1.704	5.587		
# 3	42-57	LG 1	192.063	26.666	1.818	**0.000**		
# 4	58-76	LG 4	168.204	22.168	1.063	**0.079**		
# 5	77-92	LG 4	192.063	26.666	1.818	**0.000**		
# 6	93-132	LG 1 & 4	1089.587	224.266	66.917	37.025	16.333	**0.063**
# 7	133-178	LG 1 & 4	243.880	36.780	3.902	**0.312**		
Bristled	179-193	--	105.247	11.020	**0.004**	1.308		

Genotyping of selected DMR entries

Based on DM screening results, pollinators of 38 BC_4F_2 entries, exhibiting lower testcross DMI values, were selected for priority marker genotyping to save time. The genotyping of all other BC_4F_2 entries is also underway. Marker data for those selected 38 entries is presented in Table 6. Out of these 38 entries, 9 entries homozygous for donor genome either in LG 1 or LG 4 or both were identified.

Association between DM screening and marker genotyping results

The marker genotypes for the 9 selected BC_4F_2 progenies in both the target regions, LG 1 and LG 4, along with their pedigree and DMI values have been presented in Table 7. Plants homozygous for donor parent ICMP 451 allele(s) in the targeted regions had lower values of DMI. The marker genotyping results, therefore, are in agreement with the phenotyping results for DMI screening of BC_4F_2 progenies. Nearly all of the BC_4F_2 progenies were segregating for panicle bristling, the secondary target trait.

Table 6. Marker genotype data for 38 BC_4F_2 DMR entries that had lower disease incidence during their testcross DM screening

Plant No			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Trial entry no.			16	37	39	46	48	51	61	71	76	82	84	88	95	96	97	104	105	106	107
Marker	Enzyme	LG																			
Xpsm513	*Hind* III	1	A	H	A	B	B	A	B	B	B	B	B	B	H	H	B	H	H	H	H
Xpsm858	*Dra* I	1	H	H	H	B	B	A	H	A	A	B	B	H	H	H	B	H	H	H	H
Xpsm565	*Hind* III	1	H	H	H	H	H	A	H	A	H	B	B	B	A	H	B	H	H	B	H
Xpsmi757	*Eco R* I	1	H	H	H	H	H	A	H	A	H	B	B	B	-	H	B	H	H	-	H
Xpsm464	*Dra* I	4	B	B	B	B	B	B	H	A	H	A	H	H	B	B	B	B	B	B	B
Xpsmi716	*Dra* I	4	B	B	B	B	H	A	B	A	B	A	H	B	B	B	B	B	B	B	B
Xpsm265	*Hind* III	4	B	B	B	B	H	A	H	H	H	A	H	B	B	B	B	B	B	B	B
Xpsm416	*Dra* I	4	B	B	B	B	H	A	H	A	H	-	-	B	B	B	B	B	B	-	B
DMI (%)			61	75	78	72	75	75	73	67	77	67	74	76	59	67	55	62	72	61	76

Plant No			20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Trial entry no.			108	109	110	111	112	113	114	119	138	139	140	141	153	155	156	161	167	191	193
Marker	Enzyme	LG																			
Xpsm513	*Hind* III	1	H	A	H	A	B	B	A?	H	H	H	H	B	A	H	H	B	A	B	B
Xpsm858	*Dra* I	1	A	A	H	A	B	B	B	B	H	H	H	B	A	H	H	H	A	B	B
Xpsm565	*Hind* III	1	A	A	H	A	B	B	B	B	H	H	H	H	A	H	B	A	A	A	H
Xpsmi757	*Eco R* I	1	-	A	H	A	B	B	-	B	H	H	H	H	A	H	B	-	A	-	-
Xpsm464	*Dra* I	4	B	B	B	B	B	B	B	B	H	A	H	H	B	A	H	H	H	B	B
Xpsmi716	*Dra* I	4	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Xpsm265	*Hind* III	4	B	B	B	B	B	B	B	B	B	A	B	B	B	A	-	B	B	B	B
Xpsm416	*Dra* I	4	B	B	B	B	B	B	B	B	B	A	-	B	B	A	-	B	B	B	B
DMI (%)			74	54	52	67	75	39	79	75	78	72	78	78	72	68	77	70	77	68	64

A = homozygous for donor parent allele; B = homozygous for recurrent parent allele; H = heterozygous; - = not scored

Table 7. Marker genotypes of selected BC_4F_2 plants and selected plants in their progenitor generations along with DMI (%)

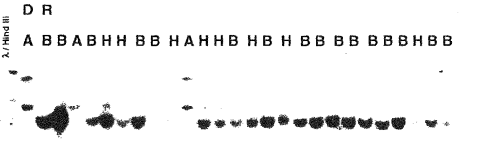
				BC_1F_1			BC_2F_1					BC_3F_1						BC_4F_1							BC_4F_2								
LG	Markers	ICMP 451	H 77/833-2	1	2	3	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	6	7	6	8	21	23	32	36	10	29	33
1	*Xpsm513*	A	B		B		H	H	B	H	H	H	H	B	B	H	H	H	H	H	B	B	H	H	A	B	A	A	A	A	B	H	H
1	*Xpsm858*	A	B	H	B		H	H	B	H	H	H	H	B	B	H	H	H	H	H	H	B	H	H	A	A	A	A	A	A	B	H	H
1	*Xpsm295*	A	B	H	B	H																											
1	*Xpsm565*	A	B				H	H	B	H	H	H	H	B	B	H	H	H	H	H	H	B	H	H	A	A	A	A	A	A	B	H	H
1	*Xpsm757*	A	B	H	B	H	H	B	B	H	H	H	H	B	B	H	H								A	A	A	A	A	A	B	H	H
1	*Xpsm425*			H	B	H						H	H	B	B	H	H															
1	Bristling	Present	Absent	P	P	P	P	A	P	A	A	P	P	A	A	P	A	P	A	P	P	A	A	P	A	P	A	A	P	A	A	A	P
4	*Xpsm409*			H	H	H																											
4	*Xpsm464*	A	B	H	H	H	B	B	H	H	H	B	B	H	H	H	H	B	B	B	H	H	H	H	B	A	B	B	B	H	A	A	A
4	*Xpsm716*	A	B	H	H	H	B	B	H	H	H	B	B	H	H	H	H	B	B	B	H	H	H	H	A	A	B	B	B	B	A	B	B
4	*Xpsm265*	A	B				B	B	H	H	H	B	B	H	H	H	H	B	B	B	H	H	H	H	A	H	B	B	B	B	A	A	A
4	*Xpsm416*	A	B	H	H	H								H	H	H	H								A		B	B	B	B	-	A	A
Expected % of RP genome		0	100	75			87.5					93.75						96.87							96.87								
DMI (%) of parents		52.0	97.3																														
DMI (%) of testcrosses		-		-			-					-						-							75	67	54	67	71	76	67	72	67

Table 8. Summary of QTLs identified using Mapmaker/QTL for the response to downy mildew in pearl millet cross ICMP 451 x H 77/833-2

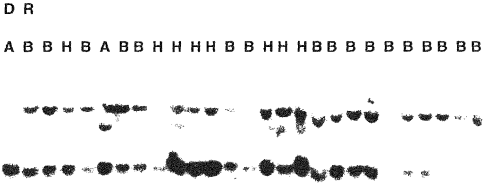
Origin of isolate (location and type of screen)	Linkage Group	QTL name	Flanking markers	Position (cM)	LOD	%Variance accounted for	Weight	Dominance
ICRISAT-Patancheru (IP field, 1994)	1	*QRsg1*	*Xpsm280-Xpsm858*	2.7	5.02	22.0	1.36	-0.41
				3.4			1.53	-0.70
	4	*QRsg4*	*Xpsm716-Xpsm305*	13.5	2.75	16.9	0.91	-0.18
				13.9			1.17	-1.09
	3	*QRsg3a*	*Xpsm325-Xpsm473*	8.1			-0.62	0.82
					10.73	62.8		
ICRISAT-Patancheru (IP field, 1996)	*3*	*QRsg3a*	*Xpsm473-Xpsm617*	*2*	*2.91*	*17.3*	*-0.82*	*-1.26*
	4	*QRsg4*	*Xpsm716-Xpsm305*	13.5	5.72	39.8	0.76	-0.72
	7	*QRsg7*	*Xpsm269-Xpsm618*	*28*	*3.45*	*28.2*	*-0.69*	*-1.04*
			N.B. Significance of	QTLs on	LGs 3	& 7 lost in	combined	model
ICRISAT-Patancheru (IP field, mean)	1	*QRsg1*	*Xpsm280-Xpsm858*	4.0	3.48	20.4	0.98	-0.42
				6.3			0.99	-0.65
	3	*QRsg3a*	*Xpsm473-Xpsm617*	2.7	3.07	17.2	-0.90	-0.77
				2.3			0.77	0.27
	4	*QRsg4*	*Xpsm716-Xpsm305*	17.4	5.51	39.5	1.32	-1.00
				17.4			0.973	0.83
					12.52	59.1		
ICRISAT-Patancheru (UWBGH)	1	*QRsg1*	*Xpsm280-Xpsm858*	3.1	16.60	67.1	23.29	-8.21
				1.8			22.07	8.08
	3	*QRsg3b*	*Xpsm473-Xpsm617*	6.0	2.40	15.7	-8.22	-11.44
				6.8			-7.58	4.82
	2	*QRsg2*	*Xpsm394-Xpsm214*	0.3			5.41	1.40
	4	*QRsg4*	*Xpsm416-Xpsm612*	2.1			4.79	1.35
					25.64	80.2		
ICRISAT-Patancheru (IPGH)	1	*QRsg1*	*Xpsm280-Xpsm858*	1.5	22.46	73.5	31.26	-13.06
				0.9			30.66	13.20
	2	*QRsg2*	*Xpsm394-Xpsm214*	12.4			4.26	4.07
	4	*QRsg4*	*Xpsm416-Xpsm612*	2.0			6.97	-0.09
					35.01	85.4		

Figures in red are for a combined model and includes additional QTLs

Fig. 15. Autoradiographs obtained from screening of BC_2F_1 progenies based on donor parent ICMP 451 and recurrent parent H 77/833-2; heterozygous individuals were selected for further backcrossing to the recurrent parent



Probe: psm575 LG: 1 Enzyme: *Eco*RV Population: BC_2F_1



Probe: psm416 LG: 1 Enzyme: *Dra*I Population: BC_2F_1

D = donor parent = ICMP 451
R = recurrent parent = H 77/833-2
H = heterozygote
A = homozygous for donor parent allele
B = homozygous for recurrent parent allele

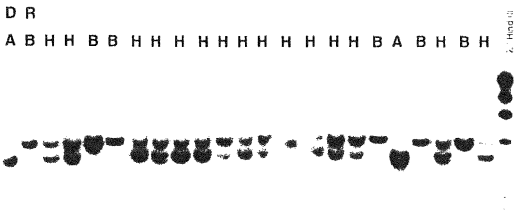
Fig. 16. Autoradiographs obtained from screening of BC_3F_1 progenies based on donor parent ICMP 451 and recurrent parent H 77/833-2; heterozygous individuals were selected for further backcrossing to the recurrent parent

Probe: psm565 LG: 1 Enzyme: *Hind*III Population: BC_3F_1

Probe: psm52 LG: 1 Enzyme: *Dra*I Population: BC_3F_1

D = donor parent = ICMP 451
R = recurrent parent = H 77/833-2
H = heterozygote
A = homozygous for donor parent allele
B = homozygous for recurrent parent allele

Fig. 17. Autoradiographs obtained from screening of BC_4F_1 progenies based on donor parent ICMP 451 and recurrent parent H 77/833-2; heterozygous individuals were selected for further backcrossing to the recurrent parent

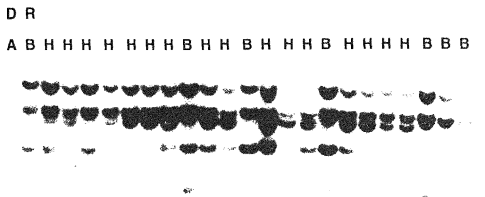


Probe: psm280 LG: 1 Enzyme: *Dra*I Population: BC_4F_1

Probe: psm716 LG: 1 Enzyme: *Dra*I Population: BC_4F_1

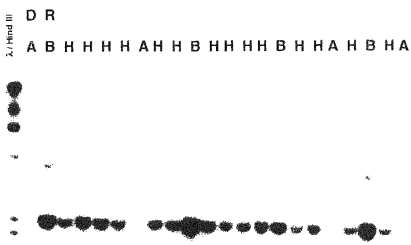
D = donor parent = ICMP 451
R = recurrent parent = H 77/833-2
H = heterozygote
A = homozygous for donor parent allele
B = homozygous for recurrent parent allele

Fig. 18. Autoradiograph obtained from screening of BC_4F_1 progenies based on donor parent ICMP 451 and recurrent parent H 77/833-2; heterozygous individuals were selected for further backcrossing to the recurrent parent



Probe: psm858 LG: 1 Enzyme: *Dra*I Population: BC_4F_1

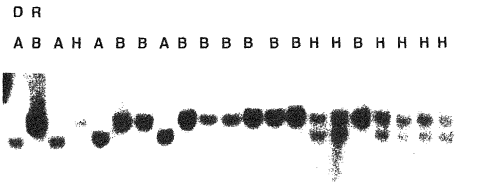
Fig. 19. Autoradiograph obtained from screening of BC_4F_2 progenies based on donor parent ICMP 451 and recurrent parent H 77/833-2; homozygous individuals were selected for further evaluation



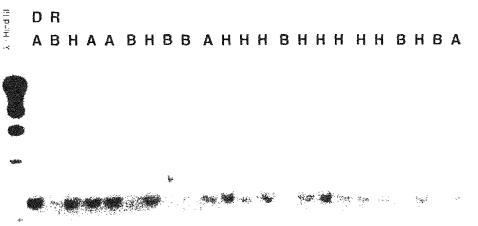
Probe: psm858 LG: 1 Enzyme: *Hind*III Population: BC_4F_2

D = donor parent = ICMP 451
R = recurrent parent = H 77/833-2
H = heterozygote
A = homozygous for donor parent allele
B = homozygous for recurrent parent allele

Fig. 20. Autoradiographs obtained from screening of BC_4F_2 based on donor parent ICMP 451 and recurrent parent H 77/833-2; homozygous individuals were selected for further evaluation



Probe: psm513 LG: 1 Enzyme: *Hind*III Population: BC_4F_2

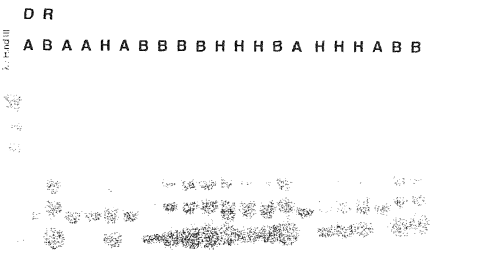


Probe: psm464 LG: 4 Enzyme: *Dra*I Population: BC_4F_2

D = donor parent = ICMP 451
R = recurrent parent = H 77/833-2
H = heterozygote
A = homozygous for donor parent allele
B = homozygous for recurrent parent allele

Fig. 21. Autoradiographs obtained from screening of BC_4F_2 progenies based on donor parent ICMP 451 and recurrent parent H 77/833-2; homozygous individuals were selected for further evaluation

Probe: psm265 LG: 1 Enzyme: *Hind*III Population: BC_4F_2



Probe: psm858 LG: 1 Enzyme: *Dra* I Population: BC_4F_2

D = donor parent = ICMP 451
R = recurrent parent =H 77/833-2
H = heterozygote
A = homozygous for donor parent allele
B = homozygous for recurrent parent allele

5. DISCUSSION

In the present study we have shown that it was possible to transfer QTLs for downy mildew resistance (DMR) in pearl millet (*Pennisetum glaucum* (L.) R. Br.) using marker-assisted selection (MAS) without phenotypic selection, and recorded significant improvement in downy mildew resistance of testcross hybrids of several of the newly developed lines. This is clearly a step that would have been very difficult to achieve before the advent of DNA markers. Also panicle bristling was transferred through conventional backcrossing.

Bristling

Inspite of several well known devices used for scaring off grain-feeding birds, grain yield losses due to birds can be as high as 25-100% in pearl millet. Presence of panicle bristles is said to reduce the vulnerability of grains filling on the panicles to bird damage. There have been several reports indicating panicle bristling to be controlled by a single dominant gene (Krishnaswamy, 1962; Ahluwalia and Shankar, 1964; Athawal and Gill, 1966; Lal and Singh, 1967; and Gill and Athwal, 1970), while others have suggested somewhat more complicated inheritance (Minocha and Sidhu, 1981). Therefore, simple phenotypic selection was followed in the present study using panicle bristling itself as morphological marker to incorporate this trait through conventional backcross breeding into H 77/833-2, the pollinator of a widely grown early-maturing grain hybrid HHB 67. The donor parent used for this trait was ICMP 451. High broad-sense heritability value was observed for panicle bristling confirmed that simple phenotypic selection is sufficient for incorporation of this trait. Continuous variation for bristle length was observed among the BC_4F_2 plants which is in agreement with previous findings of Appa Rao *et al.* (1988). This suggests that although a single major gene largely controls presence/absence of panicle bristles, bristle length and density of bristling on the spike are governed by additional genes.

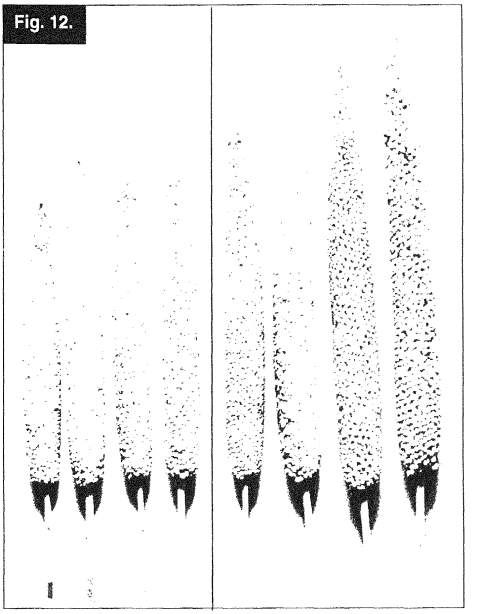
Agronomic phenotyping of BC_4F_3-derived bristled testcrosses

The lower and negative broad-sense heritability values obtained for downy mildew incidence, grain yield, plant count, effective tiller number and growth index suggested that little, if any, genetic variability was present for these traits among the closely related testcrosses and the original HHB 67 control entries in the bristled testcross trial. Significant and positive differences were observed for panicle bristling, plant height, panicle length, fresh stover yield, biomass, growth index and 1000-grain mass without any adverse effect on grain yield. Increases in plant height and panicle length could be a result of linkage of genes controlling these two traits with that for panicle bristling, that maps on to the middle portion of linkage group 1 in this cross (Fig. 3) (Howarth *et al.*, 1994). Increase in fresh stover yield and biomass of the bristled testcrosses compared to control HHB 67 suggests that the linkage between these traits and the panicle bristling morphological marker can be directly used as selection criteria to improve these two traits. Increase in 1000-grain mass when compared to the original HHB 67 was due to the increase in size of grain contributed by ICMP 451.

The slight, but statistically significant, delay in time to 50% bloom of the bristled testcrosses as compared to the control (HHB 67) can be explained by the position of QTLs for these traits on linkage group 1. Time to bloom was mapped to the upper part of LG 1, with alleles from ICMP 451 associated with lateness, while panicle bristling was mapped to the middle of LG1 in this cross.

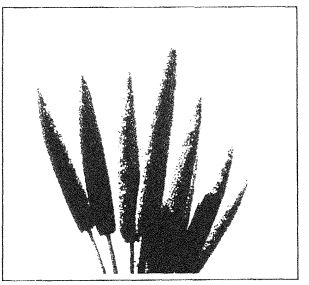
The bristled versions of HHB 67 were considerably identical phenotypically to the original HHB 67 for almost all the traits studied except for the presence of panicle bristles, and significantly increased plant height and panicle length (Fig. 11, Fig. 12 and Fig. 13). Among entries in this trial, entry 13 had long panicle bristles combined with lower downy mildew incidence (67%) and was superior to the original HHB 67 for almost all the traits studied while taking the same time to reach 50% bloom. Based on the better performance of this entry than the original HHB 67 necessitates its further evaluation in large scale multilocation trials.

Fig. 12.



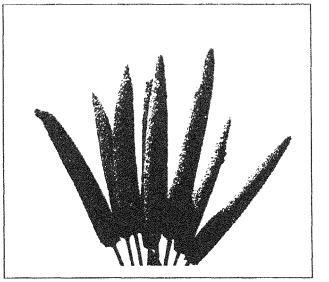
Panicles of the original non-bristled HHB 67, moderately resistant to downy mildew

Bristled, downy mildew resistant version of HHB 67, pollinator improved by MAS



Original HHB 67

Fig. 13.



Bristled version of HHB 67

Fig. 14.

Agronomic phenotyping of BC_4F_2-derived downy mildew resistant testcrosses

In this trial closely related 178 BC_4F_2-derived versions of HHB 67 were compared with the original HHB 67 for their agronomic performance. The mean performance of selected downy mildew resistant (DMR) testcross entries and the mean of all control HHB 67 plots have been presented in Table 3. The DMR testcross entries segregated for downy mildew incidence (%) ranging from moderately resistant to susceptible based on the presence (homozygous or heterozygous) or absence of either one or both of the targeted QTLs in their pollinators.

The new DMR versions of HHB 67 were considerably phenotypically similar to the original HHB 67 with respect to almost all traits studied except for significant increases in plant height and panicle length. The increase in plant height and panicle length could be due to intentional selection for a larger portion of linkage group 1 for downy mildew resistance where QTLs from ICMP 451 for these agronomic traits also mapped (Fig. 3) that resulted in simultaneous selection of these traits as co-variables. In the cross ICMP 451 x H 77/833-2, the position of a QTL for time to 50% bloom lies in the vicinity of the QTL for DM resistance on linkage group 1. This might be the possible explanation for similar expression of this trait in the new DMR versions of HHB 67. A few entries, such as 51, 71, 82, 109, 111 and 153, were found promising having lower downy mildew incidence with higher grain yield than the original HHB 67 and should be further evaluated in multilocation yield trials prior to their possible release as new DMR versions of HHB 67 carrying different combinations of downy mildew resistance gene(s). Regardless of the genetic basis of the transgressive segregants obtained among BC_4F_2 progenies from this crossing program, they may be integrated into further applied pollinator and hybrid breeding work. Proper combination of agronomic traits with downy resistance reaction might yield breeding materials of even greater interest. On the other hand these BC_4F_2 lines have now largely returned to the genetic background of H 77/833-2, so they can serve as good starting materials for construction of NILs to dissect the underlying genetic basis of both disease resistance and agronomic traits such as plant height and panicle length or even grain yield.

Downy mildew screening

Parental line ICMP 451, used as the donor parent, was moderately resistant when screened against the Patancheru isolate of pearl millet downy mildew. This is in marked contrast to H 77/833-2, the recurrent parent, which showed 100% susceptibility to the same pathogen isolate. HHB 67, used as control, had downy mildew incidence (DMI) of 97% whereas standard checks 7042(S) and 7042(DMR) showed 98% and 75% DMI, respectively, indicating high disease pressure during this greenhouse screening of the BC_4F_2-derived downy mildew resistant testcrosses. Various families of the test entries fit to classical Mendelian ratios of 1:15, 3:13 and 7:9, as indicated by non-significant Chi-square estimates, indicating that the various families showed digenic interactions of independently segregating duplicate dominant genes, one basic and one inhibitory gene and two complementary genes, respectively. The proportion of progenies was mostly skewed towards susceptibility in this screen again confirming the level of high disease pressure used, and the fact that many of the testcrosses had been produced with segregating BC_4F_2 plants that were not homozygous for one or more of the two targeted DM resistance genes. The observed segregation pattern of downy mildew resistance in bristled testcrosses was found to best fit a ratio of 9 resistant : 55 susceptible. This suggests the presence of either a dominant inhibitor of resistance (which could be a dominant resistance gene that is no longer effective against the Patancheru pathogen population used in this screen) conferred by two duplicate dominant genes, or the presence of a recessively inherited resistance that is required for effective expression of resistance conditioned by two additional duplicate dominant genes. Further greenhouse disease screening will be required to confirm these preliminary results.

Marker-assisted selection

Marker-assisted selection (MAS) offers a unique opportunity to circumvent many traditional problems associated with phenotypic selection for traits of interest. MAS has the potential to increase the efficiency and flexibility of a breeding program by permitting selection for marker genotypes linked to the target gene or QTLs in seasons or locations where phenotypic selection would be more costly or ineffective. In the present study

MAS was carried out over four backcross generations and one selfing generation in the progeny of crosses between donor parent ICMP 451 and recurrent parent H 77/833-2. The RFLP marker system was used for MAS. Despite the labor-intensive nature of this approach and the resultant limitations on population size that could be used in a given generation, good progress was made. Several quantitative traits have been very effectively manipulated using MAS in maize (Stuber and Edwards, 1986) even though the selections were based on results from a single environment. MAS have been advocated as a highly efficient breeding method because it can offer rapid and precise selection of the target gene(s) (Tanksley *et al.*, 1989). In rice, for example, there have been studies demonstrating the possibility of using MAS to pyramid genes for bacterial blight resistance (Yoshimura *et al.*, 1995; Huang *et al.*, 1997).

In future pearl millet MAS work it may be useful to conduct at least one generation of whole genome genotyping in an earlier stage of backcrossing. This will help to remove donor parent alleles in non-targeted regions more efficiently than selection based only on target regions. Development of PCR-based markers for the region targeted for transfer would allow genotyping a substantially larger number of progenies in each of backcross generations while still reducing marker data generation costs so that intensive phenotypic selection for recurrent parent plant type could be simultaneously practiced. In this way it should be possible to develop NILs of different target regions in only three backcross generations even though the recurrent parent and the donor parent are distantly related. The new lines obtained would be phenotypically identical to their recurrent parents, except for having different combinations of downy mildew resistance genes. In this respect, MAS certainly has a greater advantage over conventional backcross breeding as it is simple to ensure that genetically different resistance genes are being incorporated or even pyramided. As more genes of agronomic importance are being mapped with diagnostic DNA markers in pearl millet, MAS will be increasingly used for genetic improvement of additional traits of this crop.

Pyramiding QTLs by crossing selected BC_4F_2 progenies

The effects of individual QTLs are known to be small. To obtain differences for the resistance system in the progenies that translate to significant differences in terms of DMR in the field, the pyramiding of different resistance QTLs in a common line might be necessary. Pyramids of QTLs can be obtained by crossing plants carrying ICMP 451 resistance alleles at different target regions. In the BC_4F_2 progenies evaluated, plants carrying ICMP 451 alleles at both targeted regions in linkage groups 1 and 4 were found. There can be two methods of QTL pyramiding. One approach is to use plants already carrying favorable alleles at different target regions as recurrent parents. An alternate approach is to first develop NILs carrying different single QTLs, then to cross them to pyramid the QTLs into single line. The first approach can accelerate the process compared with the alternative one by saving one generation of crossing. However there could be practical problems in this pyramiding approach. This type of cross involves two individual plants and not, as in the rest of program, one individual plant and a recurrent parent for which multiple sowings are easy to realize. Therefore the synchronization of the flowering time might be difficult to obtain notably because of segregation for growth duration. However, the problem of non-synchronization can be tackled by the tillering capacity of pearl millet.

One advantage is that the resulting line will have a relatively smaller genetic drag in the non-target regions, because it will come from only one line, compared with the alternative strategy where the resulting lines will accumulate the genetic drag from different lines.

Fine mapping of QTLs on different chromosomes

The other important work for the future could be fine mapping the QTLs for downy mildew resistance that have been transferred from ICMP 451. Several useful recombinations in the BC_4F_2 progenies can be identified for this purpose. For linkage group 1, recombinants carrying ICMP 451 alleles at different and overlapping regions in the vicinity of *Xpsm*280 – *Xpsm*858 – *Xpsm*565 – *Xpsm*757 are readily identified. By comparing their downy mildew reaction phenotypes, they can be used to locate the LG 1 resistance QTL to one of these intervals.

More precise mapping of QTLs will likely require the use of additional recombination in large populations to break up blocks of linked loci (Stuber, 1989). For example, technique such as substitution mapping (Paterson *et al.*, 1990), which utilize selected overlapping recombinant chromosomes generated from backcross lines, should be very useful for fine mapping of QTLs. Fine mapping studies should lead to the determination of whether multiple trait associations with specific genomic regions are due to pleiotropy of a single major gene or due to a group of closely linked genes.

One consideration in using these BC_4F_2 recombinants for QTL fine mapping is that some of them are heterozygous instead of homozygous at some of the targeted marker loci. This may complicate the analysis of QTL effect. But this problem can be overcome by combining PCR techniques with DNA mini-preparation methods so that the genotypes of their progenies at these loci can be determined at seedling stage and only homozygous plants can be used for QTL fine mapping work. Alternatively, one could simply start the fine mapping work from BC_4F_1 plant(s) heterozygous for a small region in the vicinity of the targeted QTL. Greenhouse downy mildew screening of BC_4F_1 plants followed by extensive marker genotyping of resistant segregants in the target region could identify one or more heterozygous plants suitable for backcrossing and/or selfing to develop the fine mapping population. Additional field experiments and greenhouse work using similar populations may allow mapping of several other QTLs for downy mildew resistance on different chromosomes.

Future phenotyping work

In the BC_4F_2 generation plants with the genotypes sought were identified but there is always risk that the individuals displaying the desired genotypes may not carry the QTL as the selection is based on linkage, sometimes quite loose, that can be broken during the generations of backcrossing. The second possible problem could be that the confidence interval for QTL location is often very broad for traits with low heritability (Hyne *et al.*, 1995; and Visscher *et al.*, 1996b) and/or based on mapping populations of

modest size there is often a risk of QTLs being assigned to the wrong interval. For this reason marker-assisted transfer of a large donor segment, followed by phenotyping of a moderate-sized fine mapping population may offer a cost-effective alternative procedure.

The BC_4F_3 seeds coming from selected BC_4F_2 plants could be used for phenotyping. The first step will be to evaluate their downy mildew reaction under greenhouse conditions in comparison to H 77/833-2 and see if they express the expected differences. A small field experiment, with large plots and greater replication, would allow better characterization of differences (if any) in growth duration, plant height and grain and stover yield components. The second step will be to evaluate the best lines (and their hybrids) under field conditions to see how these NILs (and their testcrosses) perform in comparison with H 77/833-2 (and its hybrids HHB 60, HHB67, and HHB 68) under different environments.

Efficiency of MAS

Several important issues regarding the success and efficiency of MAS for QTLs merit further discussion. The number of lines with significantly improved phenotype was not very high. There could be several possible sets of reasons for this. The first set relates to the quality of the initial QTL analyses. It has been observed that a shift in analyses methods from regression on flanking markers to composite interval mapping on the same data set can sometimes lead to different conclusions on the number of QTLs and the direction of their effects. The composite interval mapping method was actually designed to improve the quality of QTL analyses in situations where several QTLs were present on the same chromosome region (Zeng, 1993, 1994). The presence of non-allelic interactions between donor alleles that may be disrupted by recurrent parent alleles during the backcross process is another likely possibility. A recent software taking epistasis into account in the framework of composite interval mapping (Wang *et al.*, 1999) would allow further improvement of the precision in QTL analyses. Another problem could be uncertainty of the QTL position, notably for those with a small effect. Some studies have shown that the confidence interval for QTL location, when it can be determined, is huge by current QTL analyses techniques, sometimes up to 30 cM for a

small mapping population (Hyne *et al.*, 1995; Visscher *et al.*, 1996b). Han *et al.* (1997) described such situations where the target region transferred might not have contained the desired QTL. To limit this risk, we took into account long segments of ICMP 451 but appropriate mapping methodologies are certainly crucial for the success and efficiency of MAS for QTLs.

The second possibility is that the target QTL can be lost during the successive backcrosses through double crossovers between the markers. Some of the intervals between markers followed in this population were long enough to consider this a possibility (e.g. the 37 cM interval between *Xpsm*464 and *Xpsm*716 in linkage group 4). A more saturated map will limit this risk, but besides the cost of adding more markers, some areas seem to be difficult to saturate. The addition of microsatellites to skeleton maps/to genetic linkage maps of this population might allow the selection for better markers. If double crossovers occur, they are impossible to detect in cases where we rely strictly on the marker genotype data to choose plants for several successive rounds of backcrossing because of unreliable phenotyping methods. The situation where a simple phenotyping technique can be applied on a plant-by-plant basis and combined with marker-aided selection either in a two-stage selection scheme or in an index selection scheme should result in better efficiency as shown by Han *et al.* (1997) in barley. One more possible reason could be that the targeted QTLs were actually of intermediate effect. Weak effects are more difficult to assess and additional replications of phenotyping might give clearer conclusions.

Resistance gene deployment strategies in the hybrids using MAS

It is clear that MAS can be used to pyramid several resistance genes in to a single inbred genotype. But, this may not provide durable genetic resistance as the pathogen is exposed to a full homozygous pyramid during hybrid seed production and to a full heterozygous pyramid in the resultant hybrid that is commercially sown. When the near-isogenic A- and B-lines along with R-line used to sow the Foundation Seed production plot for a modified three-way hybrid have been bred by marker-assisted backcrossing to

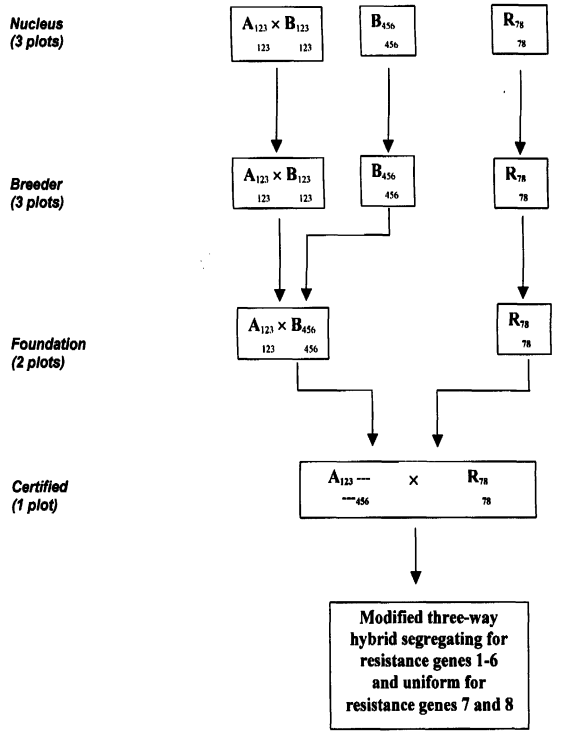
carry small complementary pyramids of resistance genes, the commercially marketed certified seed will produce a population of plants that is agronomically similar to conventional single-cross hybrid, but that is remarkably different from the point of view of a pathogen such as downy mildew that can most easily produce an epidemic on host populations with uniform gene complements (Witcombe and Hash, 2000). The net effect should be delayed development of epidemics (at least those caused by pathogens for which host plant resistance gene complements are inter allelic), and extended useful lifespans of popular hybrid cultivars (and the resistance genes in their parental resistance gene pyramids). The resistance gene complements of A-, B- and R-lines can be changed by breeders from season to season or location to location, further delaying widespread evolution of pathogen races capable of knocking an economically important background genotype out of the market. A modified three-way hybrid seed production scheme can be followed to generate hybrids segregating for pyramids of different resistance gene complements from A-, B- and R-lines that have been bred by marker-assisted selection (Fig. 22).

Future for MAS

The promise of MAS in plant breeding remains, though achieving the practical benefits is clearly taking longer than many had expected. Everything that made MAS an attractive strategy for crop improvement is still true, but the obstacles have turned out to be much bigger than originally thought (Young, 1999). The fact that MAS technology is so challenging should not be a reason for discouragement, but instead, a wake-up call for more ingenuity and better planning and execution of marker-assisted breeding programs.

MAS for polygenic trait improvement is in an important transition phase, and the field is on the verge of producing convincing results. Given the plethora of ongoing experiments and explosion of new molecular technology and applications, new or improved selection schemes should be developed and applied very soon. Technology development, including automation, allele-specific diagnostics and DNA chips, will make marker-assisted selection approaches based on large-scale screening much more powerful and effective (Young, 1999).

Fig. 22. Modified three-way hybrid seed production scheme
(adapted from Witcombe and Hash, 2000)



A = cytoplasmic male sterile seed parents
B = maintainer lines
R = pollinator parent

To take advantage of these powerful technologies, research teams, governmental funding agencies, commodity groups, and even the commercial sector will need to work together to insure that the public breeders are using the best tools possible. These tools will need to go beyond markers themselves to include genome-based knowledge derived from model systems like *Arabidopsis* and rice, as well as data-warehousing and data-mining strategies (Goff, 1999; Meinke *et al.,* 1998). Indeed integrating genomics and bioinformatics in to the field of molecular breeding may prove to be even more significant than DNA markers themselves, and eventually lead to even more profound revolutions in plant breeding.

As Indicated in the recent review by Young (1999), the optimism of a decade ago has today been tempered somewhat by constraints encountered by some current MAS approaches. However, considering the potential for the development of new strategies, the future for polygenic trait improvement through DNA markers, and the contribution of this to plant breeding efforts worldwide appears bright.

Using marker-assisted selection several new versions of HHB 67, with either one or both of downy mildew resistance QTLs introgressed, have been developed. All these new versions of HHB 67 look considerably phenotypically identical to original version of HHB 67 with increased resistances to downy mildew and bird damage. It is clear from the study that traditional plant breeding approaches coupled with molecular techniques can give the desired results in much shorter time than before. As the selection using molecular tools is based on genotype rather than phenotype of the plant and is therefore more precise, so there is no need of large population size for selection. It takes considerably less time to develop/improve a cultivar using molecular techniques as compared to traditional breeding approaches. With the development and use of PCR-based markers cost of breeding has also come down further.

SUMMARY

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the world's sixth most important cereal crop. It provides food for millions of rural people in semi-arid regions of South Asia and Sub-Saharan Africa. Downy mildew (*Sclerospora graminicola* (Sacc.) J. Shroet.) is an obligate, oosporic biotroph with the potential to cause massive yield losses in pearl millet. It is specially problematic in India on genetically uniform single-cross hybrids. The study of host plant resistance to this disease is complicated by both the host and the pathogen being outbreeding and highly variable. Therefore disease reactions of new breeding materials have to be tested in expensive and often unreliable multilocational trials. The development of molecular markers has enabled genes contributing towards complex traits to be mapped using quantitative trait locus (QTL) analyses. Being able to breed for downy mildew resistance at the molecular level has allowed strategies on gene deployment to be implemented and reduces or eliminates linkage drag and the confounding effects of environmental variation associated with conventional disease resistance breeding. Using Mapmaker/QTL, two downy mildew resistance QTLs had been identified using RFLP-based skeleton map for a population derived from a cross between H 77/833-2 and ICMP 451.

At ICRISAT, Patancheru, India, marker-assisted backcross improvement of downy mildew resistance was then conducted in this study using ICMP 451 as the donor parent and H 77/833-2 as the recurrent parent. Based on QTL mapping results, two segments were chosen for introgression of downy mildew resistance from ICMP 451. The targeted regions for downy mildew resistance were on linkage groups 1 and 4. MAS was used to select plants carrying ICMP 451 allele(s) at markers flanking the target regions in the BC_1F_1 through BC_4F_2 generations that were heterozygous or homozygous for donor marker alleles in genomic regions immediately flanking the target QTLs. All the BC_4F_2 plants were testcrossed onto 843A and the resulting hybrids were compared with the original HHB 67 (843A × H 77/833-2) during *kharif* 2000. Data for agronomically

important phenotypic traits of these hybrids were recorded and analyzed, including downy mildew (DM) reaction to a greenhouse screen against Patancheru isolate. Among the parental lines, donor parent ICMP 451 was fairly resistant with 52% downy mildew incidence (DMI) as compared to 100% DMI in case of recurrent parent H 77/833-2. The original HHB 67 had DMI of 97% whereas among the downy mildew resistant (DMR) test entries, DMI ranged from 39% to 76%. An overall reduction of 20-30% was observed for DMI among the segregating DMR entries, most of which were not uniform for presence of the resistance QTLs targeted. A few entries like 6, 8, 10, 21, 23, 29, 32, 33 and 36 were identified that show marker genotypes homozygous for donor parent ICMP 451, allele(s) in the targeted regions along with lower DMI. Agronomic traits of the new DMR versions of HHB 67 were phenotypically similar to those of original HHB 67, with a slight but statistically significant increase in plant height, panicle length, biomass, harvest index and growth index. All of the BC_4F_2 progenies were segregating for panicle bristling. These entries should be evaluated further before selecting one or more for possible release as new DMR versions of HHB 67 carrying different combinations of downy mildew resistance gene(s). Regardless of the genetic basis of the transgressive segregants obtained from among the BC_4F_2 progenies produced by this crossing program, they may be integrated into further applied pollinator and hybrid breeding work.

In another experiment, in view of vulnerability of early-maturing cultivars to grain losses caused by bird damage, panicle bristling gene(s) were transferred from ICMP 451 to H 77/833-2 through conventional backcrossing. Plants from the cross between ICMP 451 × H 77/833-2 were advanced to BC_4F_3 generation using panicle bristling as partially dominant morphological marker trait. Hybrids produced on 843A with uniformly bristled BC_4F_3 progenies were compared with the original HHB 67 in a field trial conducted during *kharif* 2000. Observations were again recorded on different phenotypically important traits. Very little variability was observed for DMI, grain yield, plant count, effective tiller number and growth index among the closely related testcrosses and the original HHB 67 control entry. Significant differences were observed

for panicle bristling, plant height, panicle length, fresh stover yield, biomass, growth index and 1000-grain mass without any adverse effect on grain yield. A slight, but statistically significant delay in time to 50% bloom for the bristled testcrosses was observed as compared to the control HHB 67. Apart from other entries in this trial entry 13 had long panicle bristles combined with low DMI (67%) and was superior to the original HHB 67 for almost all traits studied while taking the same time to reach 50% bloom. Therefore, based on the present findings, this entry can be recommended for further evaluation.

From the present findings, it can be concluded that MAS can be used to pyramid major genes including resistance genes, with the ultimate goal of producing varieties with more desirable characters. With MAS it is now possible for a breeder to conduct many rounds of selection in a year. However, one of the major drawbacks is when the linked marker used for MAS is a distance away from gene of interest, cross-over between the marker and the target gene can occur. This produces a high percentage of false-positives/negatives in the screening process.

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APPENDIX (i)

Southern Transfer based on Reed and Mann, 1985 (NAR 13 [2] 7207-7221)

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.

Purification of DNA inserts from plasmid DNA (Maniatis et al., 1982):

According to this procedure the gene inserts of the clones were cleaved from their vectors using the appropriate restriction endonuclease(s) and fractionated by electrophoresis on a minigel of 1.4% agarose in TBE buffer containing ethidium bromide (0.5 μg/mL). The electrophoresis was carried out with TBE buffer for 3 h at 6 v/cm. The gels were observed on a UV-transilluminator and the desired fragment was transferred on to NA 45 membrane (Schleicher and Schull, Inc., Keene, NH) by placing the membrane in a slit just behind the band of interest and allowing the electrophoresis to resume for further 30 min. The DNA was eluted from the membrane by addition of sufficient high salt buffer (1 M NaCl, 0.1 mM EDTA, 20 mM Tris-HCl, pH 8.0) to cover the membrane followed by incubation at 65°C for 45 min. Ethidium bromide was removed by extraction with TE saturated n-butanol and DNA was precipitated with 0.5 vol of isopropanol at -80°C for 30 min and pelleted in a Sorvall microfuge at 10,000 rpm for 10 min. The pellet was washed in 70% ethanol, dried under vacuum and dissolved in $T_{10}E_1$ buffer.

APPENDIX (iii)

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

5x HSB

dissolve in 800 mL of dH_2O:

175.3 g of NaCl,

30.3 g of PIPES,

7.45 g of Na_2.EDTA.2H2O

adjust to pH 6.8 with 4 M NaOH

make the volume 1 liter

autoclave

Denhardt's III

dissolve in 100 mL of dH_2O at 65°C,

2 g of gelatin,

2 g of Ficoll-400,

2 g of PVP-360,

10 g of SDS,

5 g of sodium pyrophosphate

store on a hotplate

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

Stop bath (3% of HAC)

add 30 mL of HAC (acetic acid) to 970 mL of dH_2O

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

APPENDIX (iv)

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

Entry	GY	Rank	Trial mean	HY	TB	PH	PL	PG	PC	HC	ET	BR	BM	DMP	DMY	FST	HI	GI	TGM
	kg ha^{-1}		%	kg ha^{-1}	d	cm	cm	mm	no.	no.	no.	score	kg ha^{-1}	%	kg ha^{-1}	t ha^{-1}	%	kg ha^{-1}-d^{-1}	g
51	3589	1	132	4520	39	146	19	26	105	297	3.00	4	6999	0.32	2479	7.80	0.52	144	9.93
118	3287	2	121	4118	37	139	18	26	93	296	3.33	1	6663	0.30	2545	8.31	0.51	141	10.07
97	3272	3	120	4235	38	137	18	26	95	294	3.33	1	6096	0.44	1860	4.56	0.54	127	9.62
56	3259	4	120	4105	38	140	19	25	97	295	3.00	4	6834	0.34	2729	8.29	0.49	142	10.32
7	3233	5	119	4164	37	145	20	24	94	319	3.67	2	6308	0.34	2144	6.36	0.52	134	8.92
6	3230	6	119	4101	37	143	19	25	95	313	3.33	2	7070	0.35	2968	8.61	0.46	152	8.97
2	3220	7	118	4119	38	148	18	24	87	305	3.33	2	6591	0.34	2472	7.30	0.49	137	9.15
28	3215	8	118	3992	36	141	21	27	106	288	2.67	3	6332	0.40	2339	5.93	0.52	138	9.97
101	3213	9	118	4123	36	142	17	26	91	298	3.33	1	6757	0.33	2634	8.11	0.48	146	9.65
147	3192	10	117	4360	39	145	20	26	98	301	3.00	2	7079	0.38	2718	7.34	0.46	146	9.40
109	3187	11	117	3949	36	140	18	28	94	281	2.67	1	7057	0.38	3107	8.19	0.46	153	10.17
16	3147	12	116	4024	39	150	19	24	114	291	2.67	2	6879	0.34	2855	8.32	0.47	139	9.33
57	3140	13	116	3888	41	164	19	26	83	231	3.00	4	6744	0.34	2857	8.50	0.47	132	9.88
21	3135	14	115	3802	36	139	17	27	87	276	3.33	1	6032	0.35	2231	6.35	0.52	130	10.28
111	3130	15	115	3976	39	137	17	26	91	294	3.33	1	6703	0.34	2726	8.14	0.47	139	9.30
25	3128	16	115	4044	37	148	21	26	90	283	3.00	2	6416	0.34	2373	7.09	0.49	136	9.48
22	3117	17	115	3983	37	143	21	27	95	288	3.33	3	6674	0.35	2690	7.86	0.47	141	9.92
158	3109	18	114	4011	37	138	19	24	109	327	3.00	2	6589	0.33	2579	7.88	0.49	140	9.48
39	3074	19	113	3905	37	142	20	26	122	301	2.33	2	6362	0.33	2458	7.47	0.49	136	9.95
27	3062	20	113	3940	36	133	18	29	88	285	3.33	1	6260	0.34	2320	6.94	0.49	137	11.28
45	3055	21	112	4044	38	143	18	27	92	279	3.33	1	6109	0.40	2065	5.08	0.51	127	9.43
176	3044	22	112	3904	38	144	19	24	100	287	3.00	2	6100	0.34	2197	6.41	0.51	126	8.80
107	3039	23	112	3868	36	135	17	27	97	303	3.33	1	5852	0.34	1984	6.05	0.53	127	9.42
52	3030	24	112	3780	36	138	19	27	92	267	3.00	4	5755	0.40	1975	5.03	0.53	124	9.88
73	3026	25	111	3960	38	146	18	25	88	299	3.33	4	5900	0.40	1940	4.81	0.52	123	8.78
178	3022	26	111	3918	38	149	21	25	92	291	3.33	2	6458	0.34	2540	7.62	0.47	134	9.15
120	3005	28	111	3834	38	141	18	27	93	259	2.67	1	6202	0.36	2367	6.58	0.48	131	9.97
17	2991	29	110	3534	39	146	19	24	105	273	2.67	2	5879	0.35	2345	6.88	0.51	121	8.35
95	2987	30	110	3981	37	139	18	26	91	301	3.33	1	6458	0.39	2477	6.23	0.47	137	9.75
135	2986	31	110	3887	39	143	20	24	96	293	3.33	2	5443	0.34	1556	4.62	0.55	111	9.42
5	2985	32	110	3770	38	144	20	25	91	282	3.00	2	5934	0.35	2164	6.04	0.52	125	8.90

APPENDIX (iv) contd....

APPENDIX (iv) contd....

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

50	2982	33	110	3821	37	142	19	27	84	264	3.33	4	5938	0.38	2117	5.74	0.51	126	10.43
100	2981	34	110	3809	38	143	18	27	93	258	3.00	1	6040	0.39	2231	5.80	0.50	127	10.05
139	2975	35	109	4001	37	146	20	25	105	305	3.00	2	5994	0.32	1993	6.27	0.50	128	9.43
102	2970	36	109	3825	36	134	18	26	90	308	3.67	2	5766	0.35	1941	5.60	0.52	126	9.92
110	2966	37	109	3969	37	135	18	26	95	316	3.33	1	6203	0.40	2234	5.82	0.49	132	9.42
155	2960	38	109	3846	38	143	21	24	85	291	3.33	2	6046	0.37	2200	6.04	0.50	126	9.50
32	2956	39	109	3847	36	139	20	29	92	261	2.67	2	5923	0.37	2076	5.61	0.51	129	10.08
33	2949	40	109	3861	37	146	21	28	87	264	3.33	3	5540	0.37	1679	4.54	0.53	117	9.92
119	2942	41	108	3801	39	140	18	26	96	273	3.00	1	5717	0.33	1916	6.24	0.52	117	9.70
149	2939	42	108	3728	38	143	20	27	91	273	3.00	3	5584	0.34	1856	5.41	0.53	117	9.23
35	2916	43	107	3864	36	131	20	28	96	285	3.33	2	6481	0.33	2617	7.97	0.46	140	10.28
88	2914	44	107	3687	40	152	18	26	90	225	2.33	1	5530	0.35	1843	5.36	0.53	110	9.73
29	2913	45	107	3802	37	142	20	26	98	279	2.67	2	6339	0.35	2537	7.19	0.46	134	10.13
68	2911	46	107	4050	36	135	19	26	86	342	4.00	4	5715	0.50	1666	3.34	0.52	124	9.33
172	2904	47	107	3811	38	142	20	25	93	291	3.00	2	5788	0.35	1977	5.82	0.50	120	8.50
128	2904	48	107	3700	39	139	18	26	88	247	2.67	1	5951	0.35	2251	6.53	0.50	123	9.72
44	2891	49	106	3688	38	146	19	25	84	273	3.33	4	6023	0.34	2336	6.86	0.49	126	9.55
145	2890	50	106	3813	38	148	19	24	82	284	3.33	2	6134	0.34	2321	6.80	0.48	128	9.43
121	2885	51	106	3720	39	145	18	25	99	289	2.67	1	5894	0.35	2174	6.31	0.50	121	8.77
108	2882	52	106	3625	38	140	18	27	96	244	2.67	1	5379	0.33	1754	5.51	0.54	112	9.83
8	2880	53	106	3911	37	141	19	24	101	315	3.00	2	6162	0.35	2250	6.63	0.47	131	9.22
122	2877	54	106	3736	37	140	18	28	92	276	3.00	2	5631	0.36	1894	5.42	0.52	120	9.60
137	2862	55	105	3718	37	144	21	25	82	285	3.67	2	5422	0.35	1704	4.95	0.53	116	8.72
83	2862	56	105	3660	40	148	20	26	84	228	2.67	4	5133	0.31	1473	4.93	0.56	103	10.08
3	2852	58	105	3738	36	143	19	25	85	271	3.33	2	6118	0.33	2381	7.20	0.48	149	9.22
81	2849	59	105	3670	38	146	19	27	79	252	3.00	3	5588	0.39	1919	5.13	0.52	117	9.08
86	2842	60	105	3759	39	146	19	25	85	247	3.33	3	5605	0.34	1846	5.70	0.51	116	8.95
1	2840	61	105	3800	37	145	20	24	99	306	3.33	2	5737	0.40	1937	5.01	0.50	121	8.72
41	2838	62	104	3659	38	140	20	26	98	268	2.67	2	5802	0.34	2144	6.22	0.49	121	9.88
156	2836	64	104	3782	38	135	19	24	104	303	3.33	2	5832	0.34	2051	6.04	0.49	123	9.93
11	2835	65	104	3632	39	150	20	24	96	258	2.67	2	5723	0.34	2092	6.41	0.50	117	9.18
174	2831	66	104	3799	38	138	20	25	79	287	3.67	2	5650	0.34	1852	5.37	0.51	118	9.27
106	2830	67	104	3669	37	139	18	27	110	289	3.00	1	5796	0.38	2128	5.98	0.50	124	9.63
127	2829	68	104	3708	37	138	18	27	96	285	3.00	1	5806	0.36	2098	5.94	0.49	124	9.38
46	2827	69	104	3803	39	148	20	26	97	282	2.67	4	5687	0.41	1884	4.64	0.50	116	8.80
177	2827	70	104	3730	38	140	19	24	81	294	3.67	2	5814	0.38	2085	5.62	0.49	121	8.92

APPENDIX (iv) contd....

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

113	2820	71	104	3747	36	140	18	26	87	296	3.67	1	5541	0.47	1794	3.94	0.51	120	9.58
91	2820	72	104	3812	37	143	19	26	95	323	3.67	3	5678	0.52	1867	3.51	0.50	122	9.22
84	2810	74	103	3712	36	141	20	26	100	275	3.00	3	5512	0.43	1800	4.48	0.51	120	9.53
75	2808	75	103	3398	38	146	19	26	88	258	3.00	3	5128	0.41	1731	4.12	0.55	108	9.58
55	2804	76	103	3698	37	145	19	25	90	268	3.33	4	5857	0.35	2159	6.25	0.49	124	9.30
138	2801	77	103	3725	36	136	19	25	76	285	4.00	2	5918	0.40	2192	5.54	0.48	128	9.95
129	2797	78	103	3504	39	141	18	26	90	263	3.00	1	5692	0.34	2189	6.48	0.50	116	9.82
14	2790	79	103	3530	40	143	19	24	92	278	3.00	2	5050	0.32	1520	4.66	0.55	101	9.42
167	2785	81	102	3910	37	136	19	25	107	309	3.00	2	6061	0.33	2150	6.59	0.46	129	8.93
132	2783	82	102	3771	37	136	18	27	85	268	3.00	3	5778	0.46	2007	4.25	0.49	123	9.08
94	2780	83	102	3624	38	141	17	26	98	262	3.00	1	6221	0.34	2597	7.72	0.45	129	9.67
105	2773	84	102	3713	37	141	18	27	86	272	3.33	1	5339	0.39	1627	4.34	0.52	114	9.70
140	2770	85	102	3724	37	144	20	24	92	279	3.33	2	5813	0.37	2089	5.87	0.48	125	8.75
34	2764	86	102	3669	36	136	18	26	86	293	3.33	1	5148	0.34	1479	4.40	0.54	111	9.08
24	2762	87	102	3568	36	138	20	28	96	261	3.00	2	4962	0.37	1394	3.98	0.56	107	10.07
48	2761	88	102	3665	38	135	19	25	104	276	2.67	4	5482	0.40	1816	4.78	0.51	115	8.57
54	2749	89	101	3603	38	138	18	27	88	248	2.67	4	5518	0.38	1915	5.00	0.50	115	10.85
37	2749	90	101	3573	37	139	20	26	95	293	3.33	2	5704	0.38	2131	5.80	0.49	122	9.88
9	2745	91	101	3617	38	142	18	24	120	293	2.33	2	5436	0.35	1820	5.39	0.51	114	8.88
114	2739	93	101	3507	36	136	17	26	82	250	3.33	1	5587	0.33	2080	6.44	0.50	121	9.98
13	2739	93	101	3429	38	146	19	25	93	274	2.67	2	5428	0.34	1999	5.93	0.51	113	9.15
53	2728	97	100	3546	38	144	19	26	99	255	2.33	2	5352	0.36	1806	5.21	0.52	112	9.35
12	2726	98	100	3550	40	144	18	24	96	271	3.00	2	5454	0.36	1905	5.46	0.50	110	8.62
30	2724	99	100	3549	36	137	20	27	112	274	2.67	2	5562	0.37	2012	5.53	0.49	121	9.70
80	2719	100	100	3593	37	149	20	27	92	266	2.67	4	5566	0.47	1974	4.32	0.49	120	9.18
23	2718	101	100	3608	38	141	19	27	95	281	3.00	3	5869	0.42	2261	5.17	0.48	125	10.07
163	2717	102	100	3535	39	146	21	24	102	261	2.33	2	6080	0.36	2545	6.96	0.45	125	9.12
15	2716	103	100	3587	40	144	18	23	107	291	2.67	2	5651	0.32	2064	6.48	0.49	112	9.02
141	2712	105	100	3493	37	142	20	25	89	279	3.33	2	4928	0.33	1435	4.67	0.56	104	8.20
136	2710	106	100	3929	38	137	18	25	88	262	3.00	1	6222	0.36	2293	6.42	0.43	131	9.78
90	2707	107	100	3552	39	142	18	25	77	275	3.67	1	5234	0.33	1683	5.21	0.52	107	8.80
134	2695	108	99	3515	38	132	18	25	81	292	3.67	1	5066	0.34	1552	4.60	0.54	106	9.75
72	2689	109	99	3500	39	153	18	25	90	285	3.33	2	5230	0.39	1731	4.54	0.52	108	8.75
153	2689	109	99	3564	37	145	20	25	88	269	3.00	2	5816	0.36	2251	6.41	0.47	124	9.55
49	2686	112	99	3497	36	131	17	26	109	279	2.67	1	5165	0.43	1668	3.89	0.53	112	9.62
89	2686	112	99	3567	37	136	18	28	102	260	2.33	3	4741	0.50	1174	2.36	0.57	102	10.12

APPENDIX (iv) contd....

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

26	2684	114	99	3627	36	143	19	27	87	265	3.00	2	5551	0.35	1925	5.62	0.49	120	10.97
4	2683	115	99	3337	36	143	20	24	97	297	3.33	2	5369	0.36	2032	5.73	0.50	116	8.77
104	2678	116	99	3486	37	140	17	26	94	297	3.00	1	5390	0.34	1903	5.69	0.50	114	9.30
117	2676	117	98	3554	40	145	17	26	90	265	3.00	1	5630	0.33	2076	6.32	0.48	113	9.33
112	2671	118	98	3530	37	135	18	27	106	295	3.00	1	5277	0.44	1748	3.98	0.51	113	9.65
116	2667	119	98	3817	37	139	18	27	88	291	3.67	1	6110	0.33	2293	7.07	0.44	131	9.48
42	2661	123	98	3663	38	149	19	26	93	235	2.67	4	5537	0.35	1873	5.55	0.49	114	9.75
74	2660	124	98	3523	36	140	17	26	95	269	3.00	1	5246	0.51	1723	3.49	0.51	114	8.80
170	2656	125	98	3388	39	138	20	24	98	273	3.00	2	5150	0.33	1762	5.48	0.52	106	8.82
92	2653	126	98	3502	38	147	19	27	95	252	3.00	4	5207	0.48	1705	3.85	0.51	109	9.90
20	2650	128	98	3567	38	146	20	26	105	275	2.67	2	5386	0.35	1820	5.21	0.50	113	9.23
79	2650	128	98	3522	36	133	18	27	91	254	2.67	2	5037	0.51	1515	3.30	0.53	109	9.70
166	2644	132	97	3545	38	141	20	25	77	272	3.33	2	5573	0.34	2028	6.41	0.48	116	9.48
169	2641	133	97	3599	37	140	20	25	93	284	3.00	2	5390	0.37	1791	5.02	0.49	115	8.95
65	2636	134	97	3320	39	149	18	25	104	261	2.67	3	5160	0.35	1839	5.28	0.52	105	8.80
31	2636	134	97	3471	36	138	19	26	94	309	3.33	3	5174	0.41	1702	4.20	0.51	112	9.00
115	2630	137	97	3359	37	136	18	25	97	279	2.67	2	5118	0.42	1760	4.73	0.51	110	9.18
103	2630	137	97	3512	38	137	17	26	89	289	3.33	1	5639	0.36	2127	5.93	0.48	118	9.62
70	2627	139	97	3393	37	149	20	24	99	268	2.67	4	5147	0.42	1754	4.34	0.52	110	8.38
133	2624	140	97	3557	38	139	18	25	97	298	3.33	1	5095	0.41	1538	4.10	0.51	106	8.87
150	2622	142	96	3524	37	137	20	25	93	301	3.33	2	5564	0.31	2040	6.36	0.49	118	9.08
131	2620	144	96	3505	37	141	18	27	95	262	3.00	1	4775	0.38	1271	3.57	0.55	102	9.47
78	2612	148	96	3442	36	133	18	27	90	276	3.33	4	5264	0.47	1822	3.93	0.51	114	10.05
146	2612	148	96	3495	37	142	20	23	89	273	3.00	2	5659	0.36	2164	6.08	0.47	119	8.80
66	2608	152	96	3384	37	146	21	25	83	290	3.67	4	4948	0.36	1564	4.34	0.53	106	8.90
93	2608	152	96	3456	37	135	17	26	95	313	3.67	1	5060	0.47	1605	3.62	0.52	108	8.97
130	2607	154	96	3405	37	140	18	27	90	245	3.00	1	5826	0.40	2421	6.21	0.45	123	9.83
151	2602	156	96	3633	38	144	20	24	97	269	3.00	2	5804	0.36	2171	6.11	0.46	121	8.50
162	2590	158	95	3515	39	143	20	25	82	278	3.33	2	4915	0.35	1401	4.21	0.53	100	9.23
171	2590	159	95	3608	37	144	20	24	110	276	2.33	3	5795	0.36	2187	6.18	0.45	124	8.98
62	2588	160	95	3318	38	143	18	26	101	269	2.67	4	5126	0.45	1808	4.08	0.51	109	9.05
67	2585	161	95	3354	38	150	20	26	93	229	2.33	4	5063	0.38	1708	4.59	0.51	106	9.12
69	2583	162	95	3414	39	142	19	24	107	279	2.67	3	5008	0.38	1594	4.17	0.52	104	9.15
157	2572	163	95	3395	37	140	20	24	101	270	3.00	2	5297	0.35	1902	5.56	0.49	113	8.95
18	2569	164	95	3407	39	147	19	25	99	267	2.67	3	5667	0.34	2259	6.65	0.46	117	8.83
143	2562	165	94	3438	38	141	20	25	89	284	3.33	2	4822	0.29	1384	4.84	0.54	100	9.23

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

38	2559	166	94	3384	37	135	19	27	75	241	3.33	1	5052	0.30	1667	5.84	0.52	107	9.67
61	2557	167	94	3313	38	145	19	25	89	268	3.00	2	5053	0.39	1740	4.48	0.52	106	9.43
152	2555	168	94	3435	37	140	20	25	90	259	3.00	2	5413	0.45	1979	4.37	0.48	115	9.20
142	2552	170	94	3873	38	144	20	25	93	288	3.00	2	6299	0.34	2426	7.26	0.41	131	8.90
19	2550	171	94	3410	40	149	18	26	74	250	3.00	2	5674	0.36	2265	6.37	0.46	113	9.52
59	2542	173	94	3306	38	149	17	25	89	264	3.00	4	4888	0.43	1582	3.73	0.53	103	9.10
36	2538	174	93	3556	38	148	19	26	99	280	2.67	2	5641	0.40	2085	5.10	0.46	119	9.35
85	2525	175	93	3410	37	142	19	26	96	266	3.00	4	4964	0.50	1554	3.47	0.51	105	9.73
77	2499	178	92	3390	38	142	20	26	93	218	2.00	3	5085	0.39	1696	4.54	0.50	107	9.17
60	2489	181	92	3291	39	147	19	25	97	267	2.67	4	5044	0.44	1753	4.45	0.50	103	8.62
96	2479	182	91	3325	37	139	17	26	97	243	2.33	1	5417	0.36	2092	5.84	0.46	127	9.67
76	2479	182	91	3241	37	143	18	24	93	250	3.00	4	4925	0.49	1683	3.44	0.51	105	8.90
98	2478	184	91	3327	38	141	17	25	92	262	3.00	1	5086	0.35	1759	5.21	0.49	106	9.13
123	2478	185	91	3258	40	146	18	27	66	210	3.67	1	4691	0.34	1434	4.32	0.53	95	9.27
64	2457	186	90	3406	36	147	20	25	90	285	3.33	3	4900	0.46	1494	3.48	0.50	106	9.12
58	2456	187	90	3314	36	137	19	25	87	287	3.33	3	4883	0.52	1569	3.25	0.51	106	9.32
160	2442	188	90	3407	37	134	18	24	98	299	3.00	1	4906	0.39	1500	3.94	0.50	104	8.63
82	2435	190	90	3181	39	143	18	24	94	240	2.33	1	4505	0.34	1323	4.03	0.55	92	8.73
63	2428	191	89	3269	37	139	19	25	75	243	3.00	4	5033	0.51	1764	3.39	0.49	107	9.18
10	2425	192	89	3644	39	149	20	25	86	270	3.33	2	5686	0.33	2042	6.06	0.44	117	8.98
175	2412	194	89	3186	38	139	20	25	93	264	3.00	2	4588	0.34	1402	4.18	0.53	95	9.42
40	2402	195	88	3307	38	138	20	27	107	285	3.00	2	4828	0.35	1520	4.45	0.50	101	9.45
148	2393	196	88	3297	38	142	20	24	80	230	3.00	2	5198	0.33	1902	5.70	0.47	108	8.93
144	2388	197	88	3189	38	143	20	25	92	251	2.67	2	5105	0.35	1916	5.45	0.48	107	9.05
71	2372	198	87	3103	41	154	21	26	83	224	2.67	3	4938	0.36	1835	4.99	0.49	98	8.50
125	2336	199	86	3101	40	141	18	25	87	244	3.00	1	5291	0.34	2190	6.70	0.45	107	9.15
47	2335	200	86	3264	40	155	19	26	105	214	2.00	4	5069	0.34	1805	5.44	0.47	101	9.32
126	2325	201	86	3166	37	136	17	26	68	282	4.00	1	5044	0.37	1878	5.24	0.48	107	9.23
154	2311	203	85	3133	38	138	19	24	90	268	3.00	2	4633	0.39	1500	3.79	0.50	97	8.87
87	2306	204	85	3120	40	144	19	26	76	227	3.00	4	4862	0.41	1743	4.35	0.48	98	9.65
43	2303	205	85	3129	38	144	18	25	95	242	2.67	4	4661	0.37	1532	4.36	0.49	97	8.98
99	2288	206	84	3015	36	135	18	27	92	247	2.67	1	4324	0.43	1308	3.22	0.53	94	9.40
173	2253	208	83	3134	37	135	20	25	89	277	3.00	2	4559	0.34	1425	4.15	0.49	97	8.97
168	2218	211	82	3119	39	137	17	24	80	262	3.67	1	4777	0.43	1658	3.86	0.48	99	8.73
124	2177	212	80	3066	39	144	17	25	92	223	2.67	1	5400	0.36	2334	6.34	0.41	112	9.40
159	2155	213	79	2924	36	130	18	25	95	251	2.67	1	4324	0.38	1400	3.86	0.50	94	8.97

APPENDIX (iv) contd....

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

165	2132	214	78	2871	39	136	20	24	92	273	3.33	2	4395	0.36	1524	4.22	0.49	90	9.18
164	2087	215	77	3041	40	146	20	24	93	235	2.33	2	4751	0.32	1710	5.43	0.45	96	8.93
161	2038	216	75	2801	40	149	20	24	83	226	2.67	2	4380	0.34	1580	4.61	0.47	89	8.20
180	3014	27	111	3869	37	133	18	25	95	317	3.67	1	5749	0.42	1880	4.60	0.53	122	8.57
199	2861	57	105	3669	37	132	18	26	79	281	3.33	1	5489	0.35	1821	5.21	0.53	117	9.30
194	2838	63	104	3699	38	137	18	25	78	294	4.00	1	5472	0.36	1773	4.92	0.52	115	9.18
213	2813	73	104	3616	37	140	18	25	92	310	3.33	1	5261	0.36	1645	4.62	0.54	111	9.02
201	2787	80	103	3788	37	137	18	25	106	306	3.00	1	5466	0.40	1678	4.44	0.51	117	8.97
202	2745	91	101	3802	36	137	18	25	94	317	3.33	1	5620	0.38	1817	5.28	0.49	122	8.92
179	2738	95	101	3621	38	140	18	26	96	315	3.33	1	5413	0.42	1793	4.32	0.52	114	8.97
198	2733	96	101	3520	37	136	17	25	95	296	3.33	1	4940	0.39	1420	3.54	0.56	105	9.48
191	2715	104	100	3585	37	135	17	25	95	302	3.33	1	4854	0.35	1269	3.76	0.56	104	8.72
212	2688	111	99	3680	38	136	18	25	96	303	3.00	1	4976	0.36	1296	3.79	0.54	104	9.20
184	2663	120	98	3495	37	140	17	25	77	288	3.67	1	5179	0.36	1683	4.79	0.52	110	9.25
188	2663	121	98	3577	37	134	17	26	82	285	3.67	1	5324	0.37	1747	4.74	0.51	114	9.30
216	2662	122	98	3476	38	135	18	25	88	290	3.33	1	5230	0.35	1754	5.27	0.52	109	9.42
181	2653	127	98	3573	37	135	18	25	84	286	3.33	1	4821	0.34	1248	3.78	0.55	103	9.48
207	2650	130	98	3590	37	135	18	25	92	282	3.00	1	5017	0.41	1427	3.45	0.53	107	8.82
190	2650	130	98	3295	37	139	18	25	83	283	3.33	1	4934	0.42	1639	4.19	0.54	106	9.03
193	2635	136	97	3388	38	135	18	25	89	264	3.00	1	5102	0.37	1714	4.67	0.52	106	9.25
183	2623	141	97	3354	38	135	18	25	96	299	3.00	1	4977	0.37	1622	4.58	0.53	104	8.97
182	2622	143	96	3479	37	134	17	25	88	284	3.33	1	5079	0.35	1601	4.73	0.52	107	8.85
208	2616	145	96	3415	38	135	18	26	73	265	3.67	1	5231	0.38	1816	4.78	0.51	110	8.98
205	2615	146	96	3630	38	137	17	24	96	290	3.00	1	5369	0.38	1739	4.60	0.48	111	8.82
214	2612	148	96	3511	37	137	18	26	81	284	3.67	1	5224	0.39	1714	4.99	0.50	111	9.25
187	2609	150	96	3524	37	135	17	25	101	313	3.33	1	5313	0.37	1788	4.81	0.49	113	8.82
200	2608	151	96	3442	38	138	17	25	84	268	3.00	1	5084	0.33	1643	5.10	0.52	107	8.87
185	2605	155	96	3539	37	140	17	25	92	293	3.00	1	5428	0.34	1888	5.54	0.49	115	8.85
192	2592	157	95	3734	38	135	18	25	87	248	2.67	1	5225	0.33	1491	4.88	0.50	108	9.12
206	2553	169	94	3322	38	135	17	25	87	285	3.33	1	4979	0.41	1658	4.12	0.52	105	8.98
204	2544	172	94	3658	38	143	18	25	81	312	4.00	1	5128	0.32	1470	4.76	0.49	107	9.18
189	2517	176	93	3321	39	139	17	25	82	291	3.67	1	4936	0.41	1615	4.50	0.51	101	9.00
211	2501	177	92	3269	39	139	17	25	70	276	4.00	1	4893	0.38	1624	4.28	0.52	102	9.28
196	2496	179	92	3401	37	134	18	25	90	280	3.00	1	5024	0.37	1623	4.51	0.50	107	9.07
215	2490	180	92	3147	37	138	18	25	82	264	3.33	1	4757	0.36	1610	4.52	0.52	101	9.28

APPENDIX (iv) contd....

Summary of the mean agronomic performance of 178 BC_4F_2-derived DMR testcrosses along with mean of checks (HHB 67) and mean of trial

195	2441	189	90	3262	37	135	18	25	89	263	3.33	1	5104	0.38	1841	4.92	0.49	109	9.07
203	2423	193	89	3219	37	135	17	25	92	293	3.33	1	4804	0.36	1584	4.68	0.51	103	8.62
210	2324	202	86	3145	39	135	17	25	95	281	3.00	1	4727	0.35	1582	4.59	0.50	98	8.72
197	2268	207	83	2930	39	137	17	25	78	244	3.33	1	4415	0.32	1484	4.68	0.52	91	9.72
186	2252	209	83	3029	39	138	16	24	95	264	3.00	1	4553	0.33	1524	4.63	0.50	93	8.68
209	2244	210	83	2913	39	138	17	25	93	263	3.00	1	4412	0.35	1499	4.43	0.51	92	8.72
SE (+/-)	191			234	0.69	2.09	0.39	0.38	7.83	20.44	0.32	0.23	360.38	0.03	214.98	0.57	0.02	8.06	0.26
mean	2718			3582	37.68	141	19	25	92	276	3.08	1.95	5492	0.37	1910	5.32	0.50	116	9.30
CV (%)	12.18			11.31	3.16	2.57	3.64	2.59	14.75	12.82	18.15	20.56	11.36	14.33	19.49	18.63	7.57	12.07	4.84
F-ratio	1.77			1.51	2.59	5.93	7.52	8.18	1.25	1.28	1.42	19.13	2.62	2.17	2.87	4.69	1.59	2.53	3.58
h^2	0.20			0.15	0.35	0.62	0.68	0.71	0.08	0.08	0.12	0.86	0.35	0.28	0.38	0.55	0.16	0.34	0.46
H	0.43			0.34	0.61	0.83	0.87	0.88	0.20	0.22	0.30	0.95	0.62	0.54	0.65	0.79	0.37	0.61	0.72

GY = grain yield; HC = head yield; TB = time to 50% bloom; PH = plant height; PL = panicle length; PG = panicle girth; PC = plant count;
HC = head count; ET = effective tillers; BR = panicle bristles; BM = biomass; DMP = dry matter percent; DMY = dry matter yield;
FSY = fresh stover yield; HI = harvest index; GI = growth index; TGM = 1000-grain mass
SE = standard error of means; CV = coeficient of variability; h^2 = heritability on plot basis; H = heritability on mean basis

APPENDIX (v)

Summary of the mean agronomic performance of 15 BC_4F_3 derived bristled testcrosses along with mean of checks (HHB 67) and mean of trial

Entry	GY	Rank	Trial mean	HY	TB	PH	PL	PG	PC	HC	ET	BR	BM	DMP	DMY	FST	HI	GI	TGM
	kg ha^{-1}		%	kg ha^{-1}	d	cm	cm	mm	no.	no.	no.	score	kg ha^{-1}	%	kg ha^{-1}	t ha^{-1}	%	kg ha^{-1}d^{-1}	g
15	2628	8	101	3425	37.0	144	19	25	98	289	3.0	4.0	5831	0.38	2407	6.40	0.45	124	9.10
13	2589	11	100	3435	36.3	152	20	25	90	296	3.3	4.0	6127	0.41	2693	6.46	0.42	132	8.92
7	2467	16	95	3279	37.0	143	20	24	98	283	3.0	2.7	5749	0.37	2470	6.73	0.43	122	8.60
6	2851	2	110	3616	37.3	147	19	25	101	282	2.7	4.0	6088	0.37	2471	6.66	0.47	129	9.72
2	2473	15	95	3204	38.0	148	20	26	94	246	2.7	3.3	5320	0.34	2116	6.20	0.46	111	9.42
1	2499	13	96	3254	37.0	146	20	27	87	267	3.3	4.0	5609	0.44	2355	5.34	0.44	120	9.65
3	2908	1	112	3682	38.3	152	20	26	90	279	3.3	4.0	6250	0.36	2569	7.16	0.46	129	10.03
12	2533	12	97	3405	37.3	154	20	25	94	258	2.7	4.0	6236	0.40	2831	7.20	0.41	132	8.80
14	2605	9	100	3483	36.3	152	20	26	106	286	3.0	4.0	5914	0.49	2431	5.06	0.44	128	9.08
10	2716	5	104	3455	36.7	153	21	25	102	282	3.0	4.0	6154	0.42	2699	6.49	0.44	132	8.77
4	2595	10	100	3387	37.3	146	20	25	106	265	2.7	3.3	5475	0.38	2088	5.55	0.48	116	8.92
5	2488	14	96	3265	37.0	145	20	26	96	278	3.0	3.3	5341	0.38	2076	5.47	0.46	114	9.47
9	2630	7	101	3462	37.0	144	19	25	100	299	3.0	3.7	5635	0.42	2175	5.20	0.47	120	8.03
8	2737	3	105	3398	37.7	143	19	27	98	269	3.0	3.3	5851	0.39	2453	6.31	0.47	123	9.10
11	2645	6	102	3370	37.7	154	20	24	103	270	2.7	3.7	5973	0.44	2604	5.89	0.44	126	8.68
17	2721	4	105	3584	36.3	138	17	25	104	289	2.7	1.0	5974	0.46	2390	5.13	0.46	129	8.88
18	2458	17	95	3156	36.7	135	17	25	116	261	2.3	1.0	5378	0.47	2222	4.75	0.46	116	8.78
16	2263	18	87	3104	36.3	138	17	24	96	292	3.0	1.0	5131	0.40	2028	5.02	0.44	111	8.80
SE (+/-)	152.4			188	0.4	1.6	0.5	0.3	10.6	15.0	0.4	0.2	266	0.03	120.6	0.36	0.01	6.12	0.23
mean	2600			3387	37.1	146.3	19.4	25.1	99	277	2.9	3.2	5780	0.41	2393	5.95	0.45	122.89	9.04
CV (%)	10.15			9.61	1.71	1.88	4.26	2.00	18.59	9.34	22.39	11.11	7.98	11.31	8.73	10.45	4.53	8.62	4.46
F-ratio	1.02			0.69	2.58	13.43	5.75	8.06	0.45	0.90	0.54	27.77	1.67	2.45	3.85	4.65	2.29	1.40	4.11
h^2	0.01			-0.12	0.34	0.81	0.61	0.70	-0.23	-0.03	-0.18	0.90	0.18	0.33	0.49	0.55	0.30	0.12	0.51
H	0.02			-0.45	0.61	0.93	0.83	0.88	-1.25	-0.11	-0.86	0.96	0.40	0.59	0.74	0.79	0.56	0.29	0.76

GY = grain yield; HC = head yield; TB = time to 50% bloom; PH = plant height; PL = panicle length; PG = panicle girth; PC = plant count; HC = head count; ET = effective tillers; BR = panicle bristles; BM = biomass; DMP = dry matter percent; DMY = dry matter yield; FSY = fresh stover yield; HI = harvest index; GI = growth index; TGM = 1000-grain mass

SE = standard error of means; CV = coeficient of variability; h^2 = heritability on plot basis; H = heritability on mean basis

Appendix (vi) (Continued)

UPPER PERCENTAGE POINTS OF THE STUDENTIZED RANGE, $q_\alpha = \frac{\bar{x}_{max} - \bar{x}_{min}}{s_x}$

treatment means									α	Error df
12	13	14	15	16	17	18	19	20		
7.32	7.47	7.60	7.72	7.83	7.93	8.03	8.12	8.21	.05	5
10.70	10.89	11.08	11.24	11.40	11.55	11.68	11.81	11.93	.01	
6.79	6.92	7.03	7.14	7.24	7.34	7.43	7.51	7.59	.05	6
9.49	9.65	9.81	9.95	10.08	10.21	10.32	10.43	10.54	.01	
6.43	6.55	6.66	6.76	6.85	6.94	7.02	7.09	7.17	.05	7
8.71	8.86	9.00	9.12	9.24	9.35	9.46	9.55	9.65	.01	
6.18	6.29	6.39	6.48	6.57	6.65	6.73	6.80	6.87	.05	8
8.18	8.31	8.44	8.55	8.66	8.76	8.85	8.94	9.03	.01	
5.98	6.09	6.19	6.28	6.36	6.44	6.51	6.58	6.64	.05	9
7.78	7.91	8.03	8.13	8.23	8.32	8.41	8.49	8.57	.01	
5.83	5.93	6.03	6.11	6.20	6.27	6.34	6.40	6.47	.05	10
7.48	7.60	7.71	7.81	7.91	7.99	8.07	8.15	8.22	.01	
5.71	5.81	5.90	5.99	6.06	6.14	6.20	6.26	6.33	.05	11
7.25	7.36	7.46	7.56	7.65	7.73	7.81	7.88	7.95	.01	
5.62	5.71	5.80	5.88	5.95	6.03	6.09	6.15	6.21	.05	12
7.06	7.17	7.26	7.36	7.44	7.52	7.59	7.66	7.73	.01	
5.53	5.63	5.71	5.79	5.86	5.93	6.00	6.05	6.11	.05	13
6.90	7.01	7.10	7.19	7.27	7.34	7.42	7.48	7.55	.01	
5.46	5.55	5.64	5.72	5.79	5.85	5.92	5.97	6.03	.05	14
6.77	6.87	6.96	7.05	7.12	7.20	7.27	7.33	7.39	.01	
5.40	5.49	5.58	5.65	5.72	5.79	5.85	5.90	5.96	.05	15
6.66	6.76	6.84	6.93	7.00	7.07	7.14	7.20	7.26	.01	
5.35	5.44	5.52	5.59	5.66	5.72	5.79	5.84	5.90	05	16
6.56	6.66	6.74	6.82	6.90	6.97	7.03	7.09	7.15	.01	
5.31	5.39	5.47	5.55	5.61	5.68	5.74	5.79	5.84	.05	17
6.48	6.57	6.66	6.73	6.80	6.87	6.94	7.00	7.05	.01	
5.27	5.35	5.43	5.50	5.57	5.63	5.69	5.74	5.79	.05	18
6.41	6.50	6.58	6.65	6.72	6.79	6.85	6.91	6.96	.01	
5.23	5.32	5.39	5.46	5.53	5.59	5.65	5.70	5.75	.05	19
6.34	6.43	6.51	6.58	6.65	6.72	6.78	6.84	6.89	.01	
5.20	5.28	5.36	5.43	5.49	5.55	5.61	5.66	5.71	.05	20
6.29	6.37	6.45	6.52	6.59	6.65	6.71	6.76	6.82	.01	
5.10	5.18	5.25	5.32	5.38	5.44	5.50	5.54	5.59	.05	24
6.11	6.19	6.26	6.33	6.39	6.45	6.51	6.56	6.61	.01	
5.00	5.08	5.15	5.21	5.27	5.33	5.38	5.43	5.48	.05	30
5.93	6.01	6.08	6.14	6.20	6.26	6.31	6.36	6.41	.01	
4.91	4.98	5.05	5.11	5.16	5.22	5.27	5.31	5.36	.05	40
5.77	5.84	5.90	5.96	6.02	6.07	6.12	6.17	6.21	.01	
4.81	4.88	4.94	5.00	5.06	5.11	5.16	5.20	5.24	.05	60
5.60	5.67	5.73	5.79	5.84	5.89	5.93	5.98	6.02	.01	
4.72	4.78	4.84	4.90	4.95	5.00	5.05	5.09	5.13	.05	120
5.44	5.51	5.56	5.61	5.66	5.71	5.75	5.79	5.83	.01	
4.62	4.68	4.74	4.80	4.85	4.89	4.93	4.97	5.01	.05	∞
5.29	5.35	5.40	5.45	5.49	5.54	5.57	5.61	5.65	.01	

Appendix (vi)

UPPER PERCENTAGE POINTS OF THE STUDENTIZED RANGE, $q_\alpha = \frac{\bar{x}_{max} - \bar{x}_{min}}{s}$

Error *df*	α	p = number of									
		2	3	4	5	6	7	8	9	10	11
5	.05	3.64	4.60	5.22	5.67	6.03	6.33	6.58	6.80	6.99	7.17
	.01	5.70	6.97	7.80	8.42	8.91	9.32	9.67	9.97	10.24	10.48
6	.05	3.46	4.34	4.90	5.31	5.63	5.89	6.12	6.32	6.49	6.65
	.01	5.24	6.33	7.03	7.56	7.97	8.32	8.61	8.87	9.10	9.30
7	.05	3.34	4.16	4.68	5.06	5.36	5.61	5.82	6.00	6.16	6.30
	.01	4.95	5.92	6.54	7.01	7.37	7.68	7.94	8.17	8.37	8.55
8	.05	3.26	4.04	4.53	4.89	5.17	5.40	5.60	5.77	5.92	6.05
	.01	4.74	5.63	6.20	6.63	6.96	7.24	7.47	7.68	7.87	8.03
9	.05	3.20	3.95	4.42	4.76	5.02	5.24	5.43	5.60	5.74	5.87
	.01	4.60	5.43	5.96	6.35	6.66	6.91	7.13	7.32	7.49	7.65
10	.05	3.15	3.88	4.33	4.65	4.91	5.12	5.30	5.46	5.60	5.72
	.01	4.48	5.27	5.77	6.14	6.43	6.67	6.87	7.05	7.21	7.36
11	.05	3.11	3.82	4.26	4.57	4.82	5.03	5.20	5.35	5.49	5.61
	.01	4.39	5.14	5.62	5.97	6.25	6.48	6.67	6.84	6.99	7.13
12	.05	3.08	3.77	4.20	4.51	4.75	4.95	5.12	5.27	5.40	5.51
	.01	4.32	5.04	5.50	5.84	6.10	6.32	6.51	6.67	6.81	6.94
13	.05	3.06	3.73	4.15	4.45	4.69	4.88	5.05	5.19	5.32	5.43
	.01	4.26	4.96	5.40	5.73	5.98	6.19	6.37	6.53	6.67	6.79
14	.05	3.03	3.70	4.11	4.41	4.64	4.83	4.99	5.13	5.25	5.36
	.01	4.21	4.89	5.32	5.63	5.88	6.08	6.26	6.41	6.54	6.66
15	.05	3.01	3.67	4.08	4.37	4.60	4.78	4.94	5.08	5.20	5.31
	.01	4.17	4.83	5.25	5.56	5.80	5.99	6.16	6.31	6.44	6.55
16	.05	3.00	3.65	4.05	4.33	4.56	4.74	4.90	5.03	5.15	5.26
	.01	4.13	4.78	5.19	5.49	5.72	5.92	6.08	6.22	6.35	6.46
17	.05	2.98	3.63	4.02	4.30	4.52	4.71	4.86	4.99	5.11	5.21
	.01	4.10	4.74	5.14	5.43	5.66	5.85	6.01	6.15	6.27	6.38
18	.05	2.97	3.61	4.00	4.28	4.49	4.67	4.82	4.96	5.07	5.17
	.01	4.07	4.70	5.09	5.38	5.60	5.79	5.94	6.08	6.20	6.31
19	.05	2.96	3.59	3.98	4.25	4.47	4.65	4.79	4.92	5.04	5.14
	.01	4.05	4.67	5.05	5.33	5.55	5.73	5.89	6.02	6.14	6.25
20	.05	2.95	3.58	3.96	4.23	4.45	4.62	4.77	4.90	5.01	5.11
	.01	4.02	4.64	5.02	5.29	5.51	5.69	5.84	5.97	6.09	6.19
24	.05	2.92	3.53	3.90	4.17	4.37	4.54	4.68	4.81	4.92	5.01
	.01	3.96	4.54	4.91	5.17	5.37	5.54	5.69	5.81	5.92	6.02
30	.05	2.89	3.49	3.84	4.10	4.30	4.46	4.60	4.72	4.83	4.92
	.01	3.89	4.45	4.80	5.05	5.24	5.40	5.54	5.65	5.76	5.85
40	.05	2.86	3.44	3.79	4.04	4.23	4.39	4.52	4.63	4.74	4.82
	.01	3.82	4.37	4.70	4.93	5.11	5.27	5.39	5.50	5.60	5.69
60	.05	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65	4.73
	.01	3.76	4.28	4.60	4.82	4.99	5.13	5.25	5.36	5.45	5.53
120	.05	2.80	3.36	3.69	3.92	4.10	4.24	4.36	4.48	4.56	4.64
	.01	3.70	4.20	4.50	4.71	4.87	5.01	5.12	5.21	5.30	5.38
∞	.05	2.77	3.31	3.63	3.86	4.03	4.17	4.29	4.39	4.47	4.55
	.01	3.64	4.12	4.40	4.60	4.76	4.88	4.99	5.08	5.16	5.23

SOURCE: This table is abridged from Table 29, *Biometrika Tables for Statisticians*, vol. 1, Cambridge University Press, 1954. It is reproduced with permission of the *Biometrika* trustees and the editors, E. S. Pearson and H. O. Hartley. The original work appeared in a paper by J. M. May, "Extended and corrected tables of the upper percentage points of the 'Studentized' range," *Biometrika*, **39**: 192–193 (1952).

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Title of thesis	:	Marker-assisted improvement of pearl millet *(Pennisetum glaucum)* downy mildew resistance in elite hybrid parental line H 77/833-2.
Full name of the degree holder	:	**Arun Sharma**
Admission No.	:	96A58D
Title of degree	:	Doctor of Philosophy in Plant Breeding
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Degree awarding University/Institute	:	CCS Haryana Agricultural University Hisar - 125004, India.
Major subject	:	Plant Breeding
Total number of pages in thesis	:	103 + xvii
Number of words in the abstract	:	Approx. 300

Abstract

Present investigation was carried out at International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 1998 to 2000. Polygenic systems of host plant disease resistance are sensitive to environmental variation and vertifolia effects of oligogenes. Therefore the expression of resistance is often inconsistent. Use of molecular markers like restriction fragment length polymorphism (RFLP) markers, linked to resistance gene(s) provide a rapid and effective method of breeding improved hybrid parental lines through marker-assisted selection (MAS). Available information on quantitative trait loci (QTLs) for downy mildew resistance (DMR) in the cross ICMP 451 × H 77/833-2, was used to conduct marker-assisted backcrossing to introgress two previously identified DMR QTLs into elite hybrid parental line H 77/833-2 using ICMP 451 as the donor parent.

Bird damage being another major limiting factor in pearl millet cultivation, panicle bristling gene(s) was/were transferred from donor parent ICMP 451 to recurrent parent H 77/833-2 through conventional backcrossing.

We then evaluated the agronomic performance of the downy mildew resistant and bristled testcrosses based on improved versions of H 77/833-2 with that of the original hybrid (HHB 67). Phenotypic data was recorded on 14 agronomic traits including plant height, panicle length, days to 50% bloom, effective tillers, 1000-grain mass and grain yield. A greenhouse downy mildew screening of all the testcrosses was also conducted to observe downy mildew incidence (%). The field and greenhouse evaluations revealed that new versions of HHB 67 were considerably identical phenotypically to the original HHB 67 except for slight but statistically significant positive genetic drag in terms of plant height and panicle length along with increased downy mildew resistance. Further evaluation, in multilocation trials, of these new versions of HHB 67is necessary before there possible release.

Major Advisor 4/6/2001

Signature of Degree Holder

Head of Department 5/6/01