ABSTRACT

IDENTIFICATION OF QTLs FOR YIELD AND ITS COMPONENT TRAITS, AND DOWNY MILDEW [*Sclerospora graminicola* (Sacc.) J. Schröt.] RESISTANCE IN PEARL MILLET [*Pennisetum glaucum* (L.) R. Br.]

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This study was formulated to improve the yield potential of hybrids of PT 732A/B, which is one of the elite and important male-sterile lines used in hybrid breeding programs in Tamil Nadu. Identification of downy mildew resistance genomic regions was also set as an additional objective. One hundred and thirty-six F_2 derived F_4 self-bulks of a pearl millet mapping population (skeleton-mapped F_2 individuals) derived from PT 732B × P 1449-2 were used as the basic source population for this study. PT 4450, an elite pollinator inbred was used for producing testcross hybrids for each of the 136 F_4 self-bulks. To identify the QTLs for yield and its component traits, the testcross hybrids were raised at two locations in Tamil Nadu namely, at Tamil Nadu Agricultural University, Coimbatore and at Regional Research Station, Bhavanisagar during October 2001. Disease resistance screening was also conducted at these two

locations using selfed seeds of F_4 self-bulks during October 2001. Eighteen QTLs were identified from the two locations for nine agronomic traits using plant height, time to 50% stigma emergence and plant height together with time to 50% stigma emergence as predictors of the remaining yield-related traits. Among these nine traits, time to 50% stigma emergence, panicle circumference, plant height, panicle length and grain yield per season registered one QTL, thousand-grain mass registered two QTLs, grain yield per day registered three QTLs and single-panicle grain mass registered four QTLs. The across-locations data set produced six QTLs for five traits. Genomic regions on LG 4 and LG 7 controlled these traits. For downy mildew resistance, five different QTLs were detected on four linkage groups using disease incidence percentage and arc-sin radians values. Of these two QTLs were identified from the Coimbatore data set on LG 2, two from the Bhavanisagar data set on LG 1 and LG 4 and one from the across-locations data set on LG 7. Grain yield performance of hybrids for Tamil Nadu conditions can be improved by marker-assisted back crossing of these QTLs regions into seed parent pair PT 732A/B. Marker-assisted transfer of resistance QTLs and pyramiding of resistance genes may improve resistance to downy mildew disease.

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Introduction

1. INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a principal food cereal grown on about 27 million ha of drought-prone soils in the semi-arid regions of the Indian subcontinent and Africa (FAO and ICRISAT, 1996) with a grain yield averaging 500-600 kg/ha. It is also used as forage in Australia, South Africa and the USA and ranks as the fifth cereal in order of global economic importance. Pearl millet has the capacity to tolerate drought and low soil fertility, but responds well to water and favourable soil conditions (Kumar and Andrews, 1989). So this crop provides scope for increased production in regions too arid for sorghum (Burton, 1983).

Yield is the ultimate target of any heterosis-breeding program. A major problem of economic concern with the use of inbred CMS lines in hybrid breeding is their low yield in seed production plots. Good yielding ability and seed set, particularly in A-line, is needed to practically and economically maintain and use such inbreds. Increased and stabilized pearl millet grain production is essential for the well being of millions of people who live in these arid and semi-arid tropical regions.

Inheritance of the majority of economically important plant traits such as grain yield and its components can be classified as polygenic or quantitative. Even traits considered to be simply inherited, such as disease resistance, may be oligogenic or "semi-quantitative" for which trait expression is governed by several genes (e.g., a major gene plus several modifiers). The challenge to strategically use new tools (such as DNA-based markers) to increase the contribution of "science" to the "art plus science" equation for plant improvement therefore applies to most, if not all, traits of importance in plant breeding programs.

Sclerospora graminicola (Sacc.) J. Schröt. is an obligate biotrophic pseudo fungus that causes downy mildew disease on pearl millet, often resulting in devastating yield losses. The study of host plant resistance to this pathogen has been hindered by the fact that resistance in the host shows continuous variation (Shinde *et al.*, 1984) and resistance is regionally variable (ICRISAT, 1989). So breeding material has to be tested in expensive, time consuming and often unreliable multilocational traits. This regional variability has been found to be principally due to genetic variability of pathogen populations rather than environmental difference between locations (Ball and Pike, 1984). Molecular markers linked to host plant resistance genes would allow resistance to different pathogen population to be selected for at a single location in the absence of the pathogen variants. Linkage drag and the confounding effects of environmental variation associated with conventional breeding methods would also be reduced or eliminated.

The establishment of saturated molecular maps using restriction fragment length polymorphism (RFLP) and other DNA marker techniques make it possible to dissect Mendelian factors underlying complex traits such as grain yield. Systematic studies on mapping quantitative trait loci (QTL) have been conducted in a number of crop species (Paterson *et al.*, 1991; Tanksley and Hewitt, 1988; Stuber *et al.*, 1992) for various traits.

In this study, characterization was done for QTL for yield and its component traits and resistance to downy mildew disease. The objectives of this study were:

- Estimate the mean performance of mapping population testcross hybrids for yield and its component traits
- Determine correlations between grain yield and its component traits

- Estimate the number and location of QTL significantly affecting the variation of grain yield and its component traits across two locations in Tamil Nadu
- Determine the magnitude of the genetic effects of QTL for an elite and economically important tester and
- Identify QTLs for downy mildew disease resistance under field condition.

2. REVIEW OF LITERATURE

2.1. Pearl millet

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a cereal belonging to the genus *Pennisetum*, which contains about 140 grassy tropical species. Pearl millet is grown almost exclusively as human food, and indeed is the staple cereal of 90 million people who live in agroclimatic zones where there are severe abiotic stress limitations to crop production mainly due to heat, low and erratic rainfall, and soil type (low inherent fertility and moisture holding capacity, and in some cases low pH or high levels of aluminium saturation). Since fertilizers are seldom used and cultivation is largely by hand or animal traction actual grain yields are low in these regions (500 to 600 kg/ha), yet in the agroecologies where this crop is grown, its yield is higher and more reliably obtained than those from other possible tropical dry land cereal crops such as sorghum or maize. Grain is always the principal object of cultivation, but the stover is often secondarily important as animal fodder, and stems can also be used as fuel, for fencing, and roofing.

2.2. Molecular marker importance

There is such an enormous amount of diversity in the DNA of higher plants that no two organisms are likely to be identical in DNA base sequence. Thus, there is a tremendous amount of DNA variation present in natural populations of plants. These variations have been detected in restricted (*i.e.*, enzymatically digested) genomic DNA of plants and have paved way for the development of molecular markers (Winter and Kahl, 1995). Genetic engineering and biotechnology hold great potential for application in plant breeding as they promise to reduce the time taken to produce crop varieties with desirable characters.

With the use of molecular techniques, it would now be possible to hasten the transfer of desirable genes among varieties and to introduce novel genes from related species (Mohan *et al.*, 1997). Molecular markers detect unambiguous, single-site genetic differences that can easily be scored and mapped in most segregating populations. It is not difficult in populations of most crop species to identify and map 10-50 segregating molecular markers per chromosome pair (Kearsey, 1998). DNA markers can increase efficiency in breeding programs in a number of ways.

i. The ability to screen in the seedling stage for traits that are expressed late in the life of the plant.

ii. The ability to screen for traits that are extremely difficult, expensive, or time consuming to score phenotypically.

iii. The ability to distinguish between the homozygous and heterozygous conditions of many loci in a single generation without progeny testing.

iv. The ability to perform simultaneous, marker-aided selection to screen for a character or complex of characters that could not previously be included in the program because of cost or difficulty of conventional methods based on phenotypic screens.

Molecular markers can accelerate the generation of new varieties and allow connection of phenotypic characters with the genomic loci responsible for them. However, the real advantage of using molecular markers is to permit efficient backcross transfer of desirable alleles in a directed manner that would not be practical with conventional phenotypic selection procedures.

Polygenic characters that were previously very difficult to analyze using traditional plant breeding methods can now be readily studied and it is now relatively easy to establish genetic relationships between even sexually incompatible crop species (Mohan *et al.*, 1997). The ability to map genes contributing towards variation in complex traits with enough accuracy to be useful for plant breeding applications has been made possible through the development of comprehensive molecular marker maps (Jones *et al.*, 1997).

The following is a list of DNA marker techniques that have been developed over the years (Mohan *et al.*, 1997; Gupta and Varshney, 2000):

Acronym	Technique	Reference
AFLP	Amplified Fragment Length Polymorphism	Vos *et al.*, 1995
ALP	Amplicon Length Polymorphism	Ghareyazie *et al.*, 1995
AP-PCR	Arbitrarily Primed PCR	Welsh and McClelland, 1990
AS-PCR	Allele Specific PCR	Sarkar *et al.*, 1990
CAPS	Cleaved Amplified Polymorphic Sequence	Lyamichev *et al.*, 1993
DAF	DNA Amplification Fingerprinting	Caetano-Anolles *et al.*, 1991
IMP	Inter-MITE (Miniature Inverted-repeat Transposable Elements) Polymorphism	Chang *et al.*, 2001
ISA=ISSR	Inter-SSR Amplification = Inter Simple Sequence Repeat	Zietkiewiez *et al.*, 1994
MP-PCR	Microsatellite-Primed PCR	Meyer *et al.*, 1993
MFLP	Microsatellite-anchored fragment length polymorphism	Yang *et al.*, 2002
RAMS	Randomly Amplified Microsatellite	Ender *et al.*, 1996
RAPD	Random-Amplified Polymorphic DNA	Williams *et al.*, 1990
REMAP	Retrotransposon-Microsatellite Amplified Polymorphism	Kalendar *et al.*, 1999
RFLP	Restriction Fragment Length Polymorphism	Botstein *et al.*, 1980
SAP	Specific Amplicon Polymorphism	Williams *et al.*, 1991
SCAR	Sequence Characterized Amplified Region	Williams *et al.*, 1991
SNP	Single Nucleotide Polymorphism	Nikiforov *et al.*, 1994
SSCP	Single Strand Conformation	Orita *et al.*, 1989

	Polymorphism	
SSLP	Microsatellite Simple Sequence Length Polymorphism	Rongwen *et al.*, 1995
SSLP	Minisatellite Simple Sequence Length Polymorphism	Jarman and Wells, 1989
SSR	Simple Sequence Repeat	Hearne *et al.*, 1992
STMS	Sequence Tagged Micro-satellite Sites	Beckmann and Soller, 1990
STS	Sequence Tagged Site	Fukuoka *et al.*, 1994

2.3. Importance of RFLP marker and its application

Among the various DNA-based molecular markers, RFLPs were the first to be used in human genome mapping (Botstein *et al.*, 1980) and later they were adopted for plant genome mapping (Helentjaris *et al.*, 1986a; Helentjaris, 1987; Paterson *et al.*, 1988; Weber and Helentjaris, 1989). RFLP is the most reliable DNA polymorphism that can be used for accurate scoring of genotypes. It has provided a relatively rapid means of producing genetic maps of densely spaced marker loci in numerous crop species (Ellis, 1986; Helentjaris *et al.*, 1986a; Landry *et al.*, 1987; Burr *et al.*, 1988; Mohan *et al.*, 1997). The four primary advantages of RFLP markers over morphological markers are co-dominance, frequent polymorphism, absence or limited influence of the environment, and absence of pleiotropic effects (Botstein *et al.*, 1980; Beckmann and Soller, 1983). Since RFLP markers have no known effect on the phenotype of the plant, they are ideal for studying quantitative traits (Stuber, 1992).

RFLP analysis employs cloned DNA sequences to probe specific regions of the genome for variations that are seen as changes in the length of DNA fragments produced by digestion with restriction endonucleases (Landry *et al.*, 1987). In plants, RFLPs were first been used in tomato, maize and rice to saturate their already extensive genetic maps

based on morphological markers and isozyme markers (Bernatzky and Tanksley, 1986; Helentjaris *et al.*, 1986a; McCouch *et al.*, 1988).

Prior to the availability of SSR markers, two types of DNA markers have been most commonly used for most crop plant molecular marker-based linkage map development and subsequent quantitative trait locus (QTL) mapping: RFLP markers (Botstein *et al.*, 1980) and RAPD markers (Williams *et al.*, 1990). Both detect DNA polymorphism and monitor the segregation of a DNA sequence among progeny of a genetic cross permitting construction of a genetic linkage map. However co-dominant RFLP markers are more robust and repeatable than RAPD markers, which are inherited in a dominant manner.

RFLP and RAPD marker allelic differences between plants are inherited in the same fashion as conventional Mendelian genes, thus genetic linkage maps of these molecular markers can be constructed using conventional methods. Such RFLP linkage maps indicate the locations of specific restriction site and/or insertion/deletion polymorphisms in 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.

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.*, 1987; Stuber *et al.*, 1987; Weller *et al.*, 1988; Mohan *et al.*, 1997) and to monitor response to recurrent selection (Stuber *et al.*, 1980, 1982). RFLP markers facilitate the selection of progenies with desirable genotypes in a relatively short span of time.

However, compared to more recently developed molecular marker techniques, RFLP analysis is labour intensive and time consuming (Mohan *et al.*, 1997).

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. Hash (1991), Gale and Witcombe (1992), Hash *et al.* (1997; 1999) and Hash and Bramel-Cox (2000) emphasized the opportunities for potential use of RFLP in plant breeding with particular reference to downy mildew resistance in pearl millet. A number of recent papers suggest that the use of RFLPs as markers offers a clear advantage in breeding for important qualitative and quantitative traits (Edwards *et al.*, 1987; Melchinger, 1990; Paterson *et al.*, 1991; Arunachalam and Chandrashekaran, 1993; Mohan *et al.*, 1997; Young, 1999), and for improving our understanding of the physiological mechanisms of complex traits (Jones *et al.*, 1997; Prioul *et al.*, 1997).

2.4. Developing a mapping population

The most critical decisions in constructing linkage maps with DNA markers are those made in developing the mapping population. In making these decisions, several factors must be kept in mind, the most important of which is the goal of the mapping project. Young (1994) reviewed the important factors for a mapping project, the success or failure of which is mainly dependent on which parents are chosen for crossing, the size of the population, how the cross is advanced, and which generations are used for DNA and phenotypic analysis. Hash and Witcombe (1994) described the procedures being used at ICRISAT for development and multiplication of pearl millet mapping populations, the

parentage of mapping populations then available, and the traits for which they might be used for QTL mapping.

2.5. Mapping QTL using testers

Most of the early QTL studies published on grain yield and yield components involved either individual plants or replicated progenies of segregating population derived from biparental crosses by selfing or backcrossing (Lubberstedt *et al.*, 1997). However in hybrid pearl millet breeding, the main selection criterion is testcross performance and line performance *per se* is only of secondary importance because these measures are poorly correlated, especially for yield characters. Use of testcross progenies in QTL mapping studies provides information about the influence of the tester and hence, is important for both basic research and application of marker-aided selection (MAS).

Lubberstedt *et al.* (1997) crossed 380 F_3 lines obtained by selfing F_2 individuals from a maize mapping population, and the two parental lines, with two diverse dent inbred testers to map QTLs affecting testcross performance for important forage maize traits and to investigate their consistency across-environments and testers. They detected seven (starch yield) to 16 (plant height) QTLs in each testcross series, explaining between 52 to 71% of σ_g^2 in a simulation test.

For forage maize, Lubberstedt *et al.* (1997) found good agreement across testers for dry matter concentration and plant height, but not for other traits including dry matter yield and *in vitro* digestibility of the whole plant. Hence at least for most of the relevant forage maize traits, it appears the separate QTL mapping is necessary for each tester.

Lubberstedt *et al.* (1998) evaluated four independently-derived mapping populations crossed with same tester, in maize. They observed that consistency for QTL

position across all four populations, which were greater for dry matter concentration, starch concentration, and plant height than for dry matter yield, *in vitro* digestible organic matter and protein concentration. Results from their study indicated QTLs were poorly consistent among crosses within the flint heterotic pool, suggesting prior to MAS, QTL mapping must be performed separately for each population.

The consistency of QTL mapping results across testers will be largely reflected by the genotypic correlation among testers and the predominant type of gene action for each trait. Thus, for a given sample selection response from MAS for testcross performance of traits with mainly additive gene action should be comparable for testcross progenies with other related testers. Melchinger *et al.* (1998) found little evidence for digenic epistasis among the detected QTLs, particularly when re-examined in an independent sample. On the contrary, differences in the testcross performance of F_3 lines with each tester were due to the presence or absence of common QTLs. This suggests that non-epistatic gene effects are major determinants of general and specific combining ability in hybrid performance, as was also concluded that numerous classical quantitative genetic experiments.

Austin *et al.* (2000) reported that QTLs detected with only one tester were not necessarily detected for the other testers especially for grain yield. Austin *et al.* (2001) used three different testers in maize. Results indicated that regions containing QTL effects for a single tester appear to be less stable across test environments and less likely to be detected for mean testcross performance across testers than those associated with QTL effects for two to three testers. Mean testcross effects (MTC), however, appear to be

less sensitive to environmental factors with the majority of QTLs with the largest MTC effects being consistently detected across test environments.

Yadav *et al.* (2002a) used pearl millet testcross F_1 hybrids for phenotyping QTLs associated with traits determining grain and stover yield under terminal drought stress conditions, rather than using inbred progenies for several reasons:

1. to restore heterotic vigour to the inbred mapping population that might otherwise be too weak for effective screening under stress conditions (pearl millet is highly cross-pollinated in nature and suffers considerably from inbreeding depression);

2. to use the dominantly inherited early flowering of the tester to reduce variation in flowering time among the test units in order to focus the mapping on specific drought tolerance traits rather than traits or responses associated with drought escape; and finally;

3. to have test units that approximate the genetic structure of the F_1 hybrids grown by farmers rather than F_3 or F_4 inbred lines.

2.6. Linkage mapping

Linkage mapping is putting marker loci (and QTLs) in order, indicating the relative distances among them, and assigning them to linkage groups on the basis of their recombination values from all pair-wise and three-point combinations. The first map of the human genome based on molecular markers (Botstein *et al.*, 1980) fuelled the development of molecular marker-based genome maps in other organisms, and has led to the recent genomic sequencing of humans, mice, *Arabidopsis* and rice.

The theory of linkage mapping is same for DNA markers as in classical genetic mapping based on morphological markers, however, several new considerations must be kept in mind. This is primarily a result of the fact that potentially unlimited numbers of

DNA markers can be analyzed in a single mapping population. DNA-based maps can be related to existing cytogenetic maps through the use of aneuploid or substitution lines (Helentjaris *et al.*, 1986b; Sharp *et al.*, 1989; Young *et al.*, 1987) or *in situ* hybridization (ISH) (Zhang *et al.*, 2000).

Since DNA marker technology was first applied to plants, there has been an explosion in the development and application of genetic linkage maps (Mohan *et al.*, 1997). Using these new DNA-based markers, scientists have constructed maps in species where only poorly populated classical maps existed before (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1991; Liu *et al.*, 1994), located genes governing quantitative characters often in great detail and taken the first steps towards gene cloning based on genetic map position. Detailed genetic linkage maps are also fundamental tools for studies on selection, identification and organization of plant genomes (Tanksley, 1993; Beckmann and Soller, 1986; Landry and Michelmore, 1987).

2.6.1. Achievements in different crops

Using RFLPs as genetic markers, Helentjaris *et al.* (1986a) constructed linkage maps for maize and tomato. 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 (Bernatzky and Tanksley, 1986). Since then, DNA marker-based genetic linkage maps for many plant species have been constructed (Helentjaris, 1987; McCouch *et al.*, 1988; Heun *et al.*, 1991; Tanksley, 1993; Mohan *et al.*, 1997)

A detailed map of lettuce was constructed by Landry *et al.* (1987) using 53 genetic markers. These included 41 RFLP loci, 5 downy mildew resistance genes, 4 isozyme loci and 3 morphological markers covering 404 cM.

McCouch *et al.* (1988) reported the construction of an RFLP-based genetic linkage map of rice. The map comprised of 135 loci corresponding to clones selected from a *Pst*I genomic library covering 1,389 cM of the rice genome. Causse *et al.* (1994) developed a rice genetic map using ca. 800 RFLPs that expanded the length of the rice linkage map to 1491 cM. Chao *et al.* (1989) attempted RFLP mapping in hexaploid 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.

2.6.2. Computer software packages for constructing genetic linkage maps

Advances in computer technology have been essential to progress in DNA marker-based genetic linkage maps. The theory behind linkage mapping with DNA markers is identical to mapping with classical genetic markers, but the complexity of the problem has dramatically increased because of the larger numbers of markers that must be used. This increase in numbers of segregating loci (and the number of progenies in which they are segregating) relative to studies of classical genetic markers has necessitated the development of complex computer algorithms and software packages specifically for this purpose.

Construction of a genetic linkage map from a DNA marker data set requires computer software packages capable of running χ^2 contingency table analysis. The program, LINKAGE-1 (Suiter *et al.*, 1983) carries out this type of analysis automatically

and also compares the observed allelic distributions to expected distributions. In a different strategy for optimizing the use of DNA marker information, the computer program "HyperGene" converts genotypic data into a "graphical genotype" (Young and Tanksley, 1989a,b), in which a complete genome of an individual from the mapping population is displayed.

MAPMAKER/EXP is a linkage analysis software package for constructing primary linkage maps of markers segregating in experimental crosses. It performs full multipoint linkage analysis for dominant, recessive and co-dominant (e.g. RFLP-like) markers in BC_1 backcrosses, F_2 and F_3 (self) intercrosses and recombinant inbred lines (Lander *et al.*, 1987; Lincoln *et al.*, 1992a, b).

The software package Joinmap (Stam 1993; Stam and Van Ooijen, 1995) analyses all types of mapping populations, and can combine maps of different mapping populations provided there are common markers. Another software for linkage mapping is Gmendel from Oregon State University, USA (Holloway and Knapp, 1994). The package Mapmanager, with different versions such as QTX, QTXP and QTX-Classic for Macintosh- and IBM compatible computers (Manly, 1993; Manly and Olsen, 1999), can be used to analyse the results of genetic mapping experiments using backcrosses or recombinant inbred lines.

In addition with these packages QTL Cartographer and PLABQTL are seldom used to carry out the genetic linkage analysis using molecular markers.

2.7. Pearl millet genetic map

The first detailed molecular marker-based genetic linkage map of pearl millet was published in 1994, and was comprised primarily of RFLP markers (Liu *et al.*, 1994).

They placed 181 loci on a linkage map by studying segregation in two F_2 populations. Two crosses (LGD × ICMP 85410 and Tift $23D_2B_1$ × IP 18292) were employed. The total length of this map, which comprised seven linkage groups, (apparently corresponding to the seven pearl millet chromosome pairs) was 303 cM and the average distance between loci was about 2 cM. The individual linkage groups (LG) varied from 90 cM for LG1 to only 30 cM for LG6 (Devos *et al.*, 1995). This pearl millet genetic linkage map was unusual among grass genomes in that it was particularly short, but this difference is expected to reduce with time. Subsequent studies have extended the length of the pearl millet genetic linkage map to circa 700 Haldane cM, but to date no significant linkage has been detected between the marker loci in these seven linkage groups and telomeric sequences that are expected to cap the ends of each (Katrien M. Devos, pers. comm.). This suggests that the pearl millet genetic linkage map will eventually extend to at least 1400 cM (Haldane).

2.8. Quantitative Trait Loci

2.8.1. Origin

A "QTL", the acronym for Quantitative Trait Locus (plural = QTLs = quantitative trait loci), is one of the genes or gene blocks that underlie quantitative traits (Gelderman, 1975). Before the discovery of molecular markers, QTLs were referred to as polygenes (Mather, 1949). QTL analysis is predicated on associations between phenotypic values for the quantitative trait and the marker alleles segregating in the mapping population. It has two essential stages: the mapping of markers and the association of the trait phenotype values with the marker genotypes. The basic theory underlying marker mapping has been available since 1920.

Sax (1923) first reported association of simply inherited genetic markers with a quantitative trait in plants when he observed segregation for seed size associated with segregation for a seed coat colour marker in beans (*Phaseolus vulgaris* L.). Rasmusson (1935) demonstrated linkage of flowering time (a quantitative trait) in peas (*Pisum sativum* L.) with a simply inherited gene for flower colour. Everson and Schallet (1955) found morphological markers that flanked a chromosomal region affecting yield in barley (*Hordeum vulgare* L.).

Extensive work in *Drosophila melanogaster* (Mather and Harrison, 1949) demonstrated the effects of individual chromosomes on quantitative traits. Cavalli (1952) crossed lines of *D. melanogaster* selected for high and low abdominal bristle number, and found evidence of linkage between polygenes. Harrison and Mather (1950) and Gibson and Thoday (1962) by selection experiments in *D. melanogaster*, were able to locate polygenes for bristle number on a particular chromosome. Thoday (1961) developed methods for detecting linkage of polygenes with marker loci. In domesticated animals, associations of quantitative traits with segregation for blood group markers have been reported (Niemann-Sorenson and Robertson, 1961). In hexaploid wheat (*Triticum aestivum* L.) monosomics have been used to identify association of quantitative traits with individual chromosomes (Law, 1967). These earlier studies provided a background of theory and observation for more recent work with molecular markers (Dudley, 1993).

The first use of a reasonably complete crop linkage map based on RFLP markers was reported in tomato by Paterson *et al.* (1988). They resolved quantitative traits to discrete Mendelian factors in an inter-specific backcross of tomato, mapping at least six QTLs controlling fruit mass and four QTLs for soluble solids.

2.8.2. Mapping QTLs for grain yield and its related traits

Grain yield is generally controlled by a number of quantitative trait loci and is affected by environmental factors, making it difficult to manipulate and improve in a breeding program. Grain yield can be dissected into a number of component traits such as individual grain mass, spikelet number, grain number per panicle, effective tiller number per plant, and plant population density that depend upon the crop concerned. These component traits are also under QTL control and the effects of individual QTLs on phenotypic variation are relatively small. Some of them, however, are less environmentally sensitive and have higher heritabilities than grain yield itself (Bezant *et al.*, 1997; Yano and Sasaki, 1997). Therefore, while looking for QTLs controlling grain yield, QTLs for yield and yield components should also be determined to provide useful information.

The advent of molecular markers, and in particular RFLP has greatly facilitated the detection of QTLs controlling yield components and the relationship between grain yield and its components. Using molecular linkage genetic maps, it is possible to estimate the number of loci controlling statistically significant portions of genetic variation in a segregating population and to characterize these loci with regard to map position, gene action, phenotypic effects, pleiotropic effects and epistatic interaction with other QTLs (Xiao *et al.*, 1996). It has been demonstrated that correlated components of yield or other traits often have QTLs mapping at similar locations. This has been observed in maize (Abler *et al.*, 1991; Veldboom *et al.*, 1994; Austin and Lee, 1996), tomato (Paterson, et al., 1991), barley (Tinker *et al.*, 1996; Bezant *et al.*, 1997), rice (Xiao *et al.*, 1996), and pearl millet (Yadav *et al.*, 2002a).

In potato, tuber starch content and tuber yield are quantitative traits that are easy to determine under field conditions Schafer-Pregl *et al* (1998) mapped QTLs for tuber starch content and tuber yield in two F_1 populations derived from crossing non-inbred dihaploid potato breeding lines A total of 18 putative QTLs for tuber starch content were identified on all 12 potato linkage groups and 8 putative QTLs for tuber yield were identified on eight linkage groups Also, twenty-six putative QTLs were reproducibly detected in two environments and/or mapping populations

Orf *et al.* (1999) measured and compared QTLs for agronomic traits of soybean in a large R_1 population derived from crosses between three different sets of population. QTLs were identified for all the primary and derived traits with a significance level ≥ LOD 3, on 17 of the 20 soybean linkage groups and these QTLs tended to be clustered on only three of the linkage groups. QTLs with major effect ($R^2 > 10\%$) were identified for all the observed characters and for many of these characters explained more than half of the observed heritable variation.

Campell *et al.* (1999) conducted a study to determine associations between kernel traits and molecular markers and to identify QTLs affecting kernel traits in a soft × hard wheat cross. They identified QTLs for kernel traits located on chromosomes 1A, 2B, 2D, 3B, 7A and 7B. Particularly the *pinB* marker on chromosome arm 5Ds explained over 60% of the phenotypic variation for kernel texture

Shah *et al.* (1999) were able to locate QTLs for a number of agronomically important traits such as grain yield, kernel number per spike, 1000-grain weight, spike number, grain volume weight, plant height and anthesis date to the long arm of 3A chromosome using a substitution line.

two-row barley cross Harrington/TR 306 on the basis of evaluation of 145 DH line in 30 field experiments (Spaner *et al*., 1999). They compared among groups of lines with contrasting markers genotypes on chromosome 7 (5H) and confirmed that a QTL on the "plus" arm of that chromosome affects grain yield and plant height.

2.9. QTL × environment interactions

One of the major goals for plant breeders is to develop genotypes with a high yield potential and the ability to maintain yield across-environments. The effect of QTL × environment interaction has been addressed in several studies in which QTL have been mapped in the same population in different environments (Paterson *et al*. 1991; Stuber *et al*. 1992; Hayes *et al*. 1993; Yan *et al*., 1999; Yadav *et al*., 2002b).

Paterson *et al*. (1991) investigated the prediction value of QTLs across-environments in tomato by comparing QTL maps of an F_2 population and its derived F_3 families. They showed that only 4 out of 29 QTLs were detected in all testing environments. Stuber *et al*. (1992) studied genotype × environment interaction for QTLs of maize by field evaluation of backcross families in six diverse environments, but limited evidence was found.

Zhuang *et al*. (1997) repeated studies of an F_2 and two equivalent F_3 populations of an *indica-indica* cross of rice grown in three different environments. In all three trials QTLs for yield components were frequently detected in the same intervals. They identified 23 of the 29 QTLs for yield and its component traits and 9 of the 15 QTLs for plant stature in more than one trial. They indicated that detection of chromosomal segments harboring QTL was hardly affected by environmental factors, perhaps because the environmental difference themselves were small.

A doubled haploid rice mapping population of 123 lines from IR 64/Azucena was used to analyse the genotypic × environmental interaction for eight different plant-type traits in rice (Yan *et al.*, 1999). Four to nine QTLs affecting different plant-type traits were detected. They suggested that QTLs with substantial main effects could be used in MAS across-environments. QTL × environment interaction effects were detected more than QTL main effects for plant height, which might indicate that gene expression for this trait could be greatly affected by environments.

In order to identify QTLs controlling agronomic trait variation and their consistency under Mediterranean conditions in barley, a progeny of 167 RILS and their parents Tadmore and Er/APM were grown under six environments (Teulat *et al.*, 2001). A total of 24 QTL consistent across all the testing environments were detected using multiple environment analysis. Out of these QTLs, 11 presented main effects, seven presented QTL × environment interaction, and six presented both effects.

Liao *et al.* (2001) used a rice doubled haploid population and a rice recombinant inbred line population derived from crosses between a tropical *japonica* variety, Azucena, and two *indica* varieties, IR 64 and IR 1552, in both field and pot experiments, for detecting QTLs and epistasis for rice panicle number in different genetic backgrounds and different lowland irrigated rice production environments. Their results indicated that the effect of genetic background on QTLs was greater than that of environments, and epistasis between QTLs is more sensitive to genetic backgrounds and environments than main effect QTLs. Main effect QTLs and epistatic QTLs could be interchangeable depending on the genetic backgrounds and probably on the environments where they are identified.

Cao *et al.* (2001) studied QTLs with epistatic effects and environment interaction effects for plant height of rice using mixed model-based QTL mapping with a doubled haploid mapping population from IR 64/Azucena tested in four different environments. The results demonstrated all QTLs detected were involved in epistatic interactions while only 64% of were found with significant additive effects. QTL × environment were detected more often than QTL main effects for plant height, which indicates that gene expression for this trait could be greatly affected by test environment.

2.10. Pearl millet downy mildew and its importance

The millet downy mildew pathogen was first described as *Protomyces graminicola* on *Setaria verticillata*. Schröter in 1879 renamed it as *Sclerospora graminicola* (Ullstrup, 1973). Downy mildew was first reported on *Setaria viridis* (L.) P. Beauv. by Farlaw (1884), and later reported on pearl millet (Butler, 1907) and other hosts (Bhat, 1973). This disease is of great economic importance in India but also causes yield losses in many countries in Africa, including Burkina Faso, Chad, Eritrea, Ghana, Mali, Mozambique, Niger, Nigeria, Senegal, Sudan, Togo, Tanzania and Zambia. This pathogen has been reported to cause disease on pearl millet in more than 20 countries around the world (Singh *et al.*, 1993).

Pearl millet downy mildew caused by [*Sclerospora graminicola* (Sacc.) J. Schröt.] is a highly destructive and widespread disease in Africa and Asia. Over the past 40 years, pearl millet production area in India has come down for many reasons. One of the major causes of this reduction has been the disease downy mildew, caused by the oomycetic pseudo-fungus (*S. graminicola*). Downy mildew is the most devastating disease of pearl millet in India. A major epidemic there occurred in the early 1970s,

closely following the release and widespread adoption of several closely related, genetically uniform pearl millet single-cross hybrids (Dave, 1987; Singh *et al.*, 1987; Hash, 1997).

2.10.1. Downy mildew – Screening techniques

The life cycle of *Sclerospora graminicola* (Sacc.) J. Schröt. is comprised of both sexual and asexual phases. The sexual stage produces oospores, which are soil or seed borne and provide the primary source of inoculum each season (Shetty, 1987). Sexual oospores are thick-walled, spherical, brownish yellow, and 22 to 35 µm in diameter. Oospores form following sexual recombination in colonized host tissue and can survive from 8 months to 13 years under laboratory conditions (Wilson, 1999). The asexual sporangia are produced at night under conditions of moderate temperatures and high relative humidity. Maximum sporangia production occurs at 20°C a temperature that is in fact well below the optimum for pearl millet growth. No sporulation is recorded at relative humidity levels below 70%. Sporangia germinate via a germ tube and generally do not remain viable for very long after daybreak. Early attempts to screen for sources of resistance to pearl millet downy mildew depended on "sick plots" i.e., plots into which infected, oospore-bearing pearl millet plants had been ploughed for several years (Nene and Singh, 1976). The test materials were sown in these plots and infection was initiated by the oospores in the soil. Large-scale field screening techniques have since been developed, based on pre-sown infector rows that provide sporangial inoculum (Williams *et al.*, 1981). This technique involves the sowing of infector rows (every fifth or ninth row) with a mixture of susceptible cultivars three weeks before sowing test material. It is most

effective when the infector rows are sown in sick plots, although it will also work well if the infector row seedlings are inoculated with sporangial inoculum.

Singh and Gopinath (1985) described a laboratory downy mildew screening technique using a micro-syringe that is more effective than field screening in producing downy mildew infection in susceptible genotypes. The procedure resembles natural infection but provides greater inoculum uniformity, and does not affect normal host activity. A modified greenhouse method for assessing resistance to downy mildew given by Weltzien and King (1995) is more rapid and is suitable for use throughout the year, independent of season. In this method, instead of inoculating plants individually, seedlings at the coleoptile to one-leaf stage were spray-inoculated with an aqueous suspension of freshly prepared sporangia (about 10^5 sporangia mL^{-1}).

Singh *et al.* (1997) explained all laboratory/greenhouse screening techniques available for this disease including dip inoculation, spray inoculation, drop inoculation, injection inoculation, and settling tower inoculation as well as field screening techniques based on infector rows. Jones *et al.* (2001) discussed effective ways to maintain infection potential of inoculum by spraying a chilled suspension of sporangia. Spraying seedlings with a suspension of sporangia that had been chilled before zoospore release gave uniform and adequately high disease pressure over many hours. Thus there has been tremendous improvement over the past 30 years in the screening methods available to detect the genetic differences in host plant resistance to pearl millet downy mildew (Singh *et al.*, 1997; Hash, 1997; Hash and Witcombe, 2000 in press).

2.10.2. QTL for downy mildew resistance in pearl millet

The first fairly detailed molecular marker map for pearl millet was constructed by Liu *et al.* (1994) so that QTL analysis is now possible in this crop. QTLs for host-plant resistance to downy mildew caused by *S. graminicola* pathogen populations from India, Nigeria, Niger, and Senegal were mapped using the cross LGD-1-B-10 (susceptible) × ICMP 85410 (resistant) (Jones *et al.*, 1995). Host-plant resistance QTLs were detected that were effective against each of the four pathogen populations. To locate genes in mapping populations other than those for which RFLP maps exist, a skeleton map needs to be transferred to the new mapping population. In pearl millet less than 40 single-copy probe-enzyme combinations will produce such a map, with an average map distance of less than 15 cM between marker loci (Liu *et al.*, 1994).

Jones *et al.* (2002) demonstrated that field screening and greenhouse pot screening of seedlings detect the same QTLs for host-plant resistant to pearl millet downy mildew using F_2 derived F_4 self bulks of a mapping population derived from a cross of resistant line P 7-3 and susceptible 7042 (S).

Howarth *et al.* (unpublished) identified QTLs for downy mildew resistance and seedling heat tolerance from pearl millet mapping populations produced from crosses ICMP 451 × H 77/833-2 and H 77/833-2 × PRLT 2/89-33. Hash *et al.* (unpublished) worked with mapping populations from crosses PT 732B × P 1449-2, 81B × ICMP 451 and 841B × 863B to locate QTLs for resistance to pearl millet downy mildew. QTLs for host-plant resistance effective against downy mildew African and Indian pathogen populations were identified in new mapping population based on cross W 504 × P 310 (Kolesnikova, 2001), and Tift 238D1 × IP 18293 (Azhaguvel, 2001). To date over 65

QTLs for pathogen-population-specific host plant resistance to pearl millet downy mildew have been detected (C.T. Hash, pers. comm.)

2.10.3. QTL mapping for disease resistance in other crops

With DNA markers and QTL mapping, complex forms of disease resistance and their underlying genes are now far more accessible to applied plant breeders and pathologists. Quantitative genetics is unsuited for dissecting polygenic resistance characters into discrete genetic loci or defining the roles of individual genes in disease resistance. With QTL mapping, the role of specific resistance loci can be described, race-specificity of partial resistance genes can be assessed, and interactions between resistance genes, growth stage of plant development and the environment can be analyzed (Melchinger, 1990; Young, 1996).

The quantitative host-plant resistance system for rice blast caused by *Pyricularia oryzae* has been especially well characterized (Wang *et al.*, 1994). Two dominant qualitative resistance loci were identified on chromosomes 4 and 11 of rice (Yu *et al.*, 1991). Another disease system that has been studied with QTL mapping is late blight of potato caused by *Phytophthora infestans*, an oomycetic pseudo-fungus distantly related to *Sclerospora graminicola*. Leonards-Schippers *et al.* (1994) identified eleven genomic segments on nine chromosomes that were associated with host plant resistance to potato late blight.

Inheritance of disease reaction to leaf spot caused by *Cercospora zeae-maydis* in three maize F_2 populations was examined to study quantitative resistance using RFLP markers (Bubeck *et al.*, 1993). One QTL on maize chromosome 2 was found to be significantly associated with resistance in all three populations.

A study of resistance to bacterial wilt caused *Pseudomonas solanacearum* in tomato was reported by Danesh *et al.* (1994) using DNA marker genotypes and disease resistance reactions for 71 F_2 individuals. Two genomic regions were significantly associated with resistance, one on chromosome 6 and another on chromosome 10. Loci contributing towards quantitative variation in disease resistance have been mapped in tomato for resistance against insects (Nienhuis *et al.*, 1987), in potato for resistance against cyst-nematode (Kreike *et al.*, 1993), in peas for resistance against ascochyta blight (Dirlewanger *et al.*, 1994), and in maize for northern corn leaf blight (Freymark *et al.*, 1993) and stalk and ear rot (Pè *et al.*, 1993).

Manzanares-Dauleux *et al.* (2000) identified QTLs against clubroot disease of *Brassica napus* caused by *Plasmodiophora brassicae*. Inheritance of *Cercospora* leaf spot resistance in sugar beat was studied by Nilsson *et al.* (1999) and they identified QTLs for this trait. In sugar beet, four QTLs associated with *Cercospora* resistance on chromosomes III, IV, VII and IX were revealed using composite interval mapping (Setiawan *et al.*, 2000). Four QTLs were localized for the leaf rust (*Puccinia hordei*) resistance in barley, which explained 96.1% of the segregating genetic variation (Kicherer *et al.*, 2000). Brown stem rot (*Phialophora gregata*) resistance QTLs were identified by Lewers *et al.* (1999) in a RIL mapping population of soybean using 146 RFLPs, 760 AFLPs and 4 probes for resistance gene analogs (RGAs).

2.11. QTL analysis: Statistical methods

Jayakar (1970) suggested mathematical-statistical methods for the detection and estimation of linkage between a qualitative marker gene and a locus influencing a quantitative character. Since then, experimental designs for determination of linkage

between marker loci and QTL have been widely described (Elston and Stewart, 1971; Geldermann, 1975; Hill, 1975; Soller and Beckmann, 1983, 1990; Jensen, 1989; Lander and Botstein, 1989; Knapp *et al.*, 1990).

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.

Two classical approaches used for QTL detection are marker-by-marker ANOVA and multiple marker methods. The principle of the ANOVA is to test whether there are significant differences between the phenotypic means of the genotype classes at a particular marker locus (Prioul *et al.*, 1997). Churchill and Doerge (1994) described an empirical method, based on the concept of permutation tests, for estimating threshold values for declaring significant QTL effects. Van Ooijen (1999) presented methods that provide reasonably accurate approximations to LOD significance thresholds for QTL analysis, which were obtained by large-scale simulations.

Marker-QTL association detection can be conducted 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' (Weller, 1987; Jensen, 1989; Lander and Botstein, 1989; Knapp *et al.*, 1990), although simpler approaches are also possible (Thoday, 1961; Weller, 1987; Haley and Knott, 1992).

Lander and Botstein (1989) described a 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. In this, interval mapping is applied in a "straight forward" fashion to several population types. Each interval between adjacent pairs of markers along a chromosome is scanned and the likelihood profile of a QTL being at any particular point in each interval is determined.

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_1 populations.

Particularly in the case of cross-pollinating crop populations, interval mapping has been enhanced to "all marker mapping". To calculate the likelihood of a segregating QTL, the segregation information of all linked markers is employed. Each segregating marker may follow a different segregation type, with two to four alleles (Maliepaard and Van Ooijen, 1994).

An alternate approach was developed by Knapp *et al.* (1990) and Haley and Knott (1992) for QTL analysis using regression. It produces results very similar to interval mapping both in terms of accuracy and precision, but has the advantage of speed and simplicity of programming. This method uses the coefficient of regression of the phenotype on the genotype of the different markers (Martinez and Curnow, 1992; Wu and Li, 1994). A significant regression coefficient is indicative of an association between the marker locus and gene(s) contributing to phenotypic differences. The significance of the association is affected by the degree of linkage between the marker and the QTL and the type and magnitude of genetic effects of the QTL.

Estimating the location and the size of the effects of QTLs using flanking markers was discussed by Martinez and Curnow (1992) in the framework of a backcross using a regression model as the analytical tool. Conneally *et al.* (1985), in the field of linkage analysis, proposed the use of a confidence interval based on limits of the χ^2 distribution of the likelihood ratio test between two positions. This idea leads to a very simple construction of the confidence interval. Mangin *et al.* (1994) described a method for constructing the confidence interval of the QTL location parameter, developed in the local asymptotic framework, leading to a linear model at each position of the putative QTL.

Kearsay and Hyne (1994) further developed the marker regression approach. It attempts to model to all the marker means on a given chromosome simultaneously, and obtains significance tests by weighted least squares or by simulation. The method involves regressing the additive difference between the marker genotype means at a locus against the function of the recombination frequency between the locus and the putative QTL.

Hackett (1997) described diagnostic tools based on residuals, likelihood profiles and regression coefficients for fitting QTL models. These are used to assess the agreement between linkage data and fitted normal mixture models for interval mapping.

2.11.1. QTL mapping software

Normally all QTL mapping software require input of the data for

1. The quantitative trait value(s) for each progeny
2. The genotype (molecular markers) for each progeny

There are over one hundred genetic analysis software packages available. Here is the brief list of some of the most commonly used software packages for these analyses.

- MapMaker/QTL (*ftp://genome.wi.mit.edu/pub/mapmaker3/*) is the original QTL mapping software for Macintosh and IBM computers (Lincoln *et al.*, 1992b). It is user-friendly, freely distributed, and runs on almost all platforms. It will analyze F_2 or backcross data using standard interval mapping procedures.

- MQTL is an IBM-compatible computer program for composite interval mapping in multiple environments (Van Ooijen and Maliepaard, 1996). It can also perform simple interval mapping. Currently, MQTL is restricted to the analysis of data from homozygous progeny (doubled haploids, or recombinant inbred lines). Progeny types with more than two marker classes (e.g. F_2) are not handled.

- PLABQTL (*http://www.uni-hohenheim.de/~ipspwww/soft.html*) is a freely distributed IBM-compatible computer program for composite interval mapping and simple interval mapping of QTLs (Utz and Melchinger, 1995; Utz *et al.*, 2000). Its main purpose is to localize and characterize QTLs in mapping populations derived from a biparental cross by selfing or production of double haploids. Currently, this program is the easiest software to use for composite interval mapping.

- QTL Cartographer (*http://statgen.ncsu.edu/qtlcart/cartographer.html*) is a QTL-mapping software written for UNIX, Macintosh, DOS or Windows computer operating systems. It performs single-marker regression, interval mapping, and composite interval mapping. It permits analysis of F_2 or backcross populations. It displays map positions of QTLs using the GNUPLOT software.

QTL Cartographer was developed by the group of Zeng at North Carolina State University (Zeng, 1993, 1994; Basten *et al.*, 1994, 1997). It allows markers to be chosen as cofactors to reduce the background genetic noise and increase the resolution of QTL detection. This provides an effective strategy for improving the ability to detect QTLs of small effect provided that the number of progenies in the mapping population is reasonably large.

- MapQTL *(http://www.cpro.dlo.nl/cbw/)*. A composite interval mapping method similar to that implemented in QTL Cartographer has been developed by Jansen and co-workers at Wageningen University (Jansen, 1993; Jansen and Stam, 1994) called multiple QTL modeling (MQM).

- Multimapper (Sillanpaa and Arjas, 1998), based on Bayesian modeling and inference, treats the number of quantitative trait loci as an unobserved random variable using ideas similar to composite interval mapping. This method is introduced for inbred lines and it can be applied also in situations involving frequent missing genotypes.

- Qgene is a QTL mapping and marker-aided breeding package written for Macintosh computer operating systems. It has a user-friendly graphical interface and produces graphical outputs. QTL mapping is conducted by either single-marker regression or interval regression.

- QTLSTAT is based on interval mapping using nonlinear regression for F_2, backcross, RIL and DH populations and outputs results in graphical form (Knapp *et al.*, 1992; Liu and Knapp, 1992).

- PGRI calculates based on the functions of t-test, conditional t-test, linear regression, multiple QTL modeling and permutation tests (Lu and Liu 1995). It is for F_2, backcross, RIL, heterozygous F_1 and open-pollinated populations.
- SAS (SAS, 1999) is a general statistical analysis software package. It can detect QTL by identifying associations between marker genotype and quantitative trait phenotype by single-marker analysis approaches such as ANOVA, t-test, and regression (e.g. PROC ANOVA, PROC GLM or PROC REG).

2.12. Reliability of QTL mapping

Kearsey and Farquhar (1998) reported that the available analytical methods locate QTL with poor precision unless the heritability of phenotypic data used for mapping a particular trait is high. Also the estimates of the QTL effects, particularly dominance effects, tend to be inflated because only large estimates are detected as being statistically significant. This is especially problematic where mapping population size is less than optimal (as it usually is).

Darvasi *et al.* (1993) showed that the 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. 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), but in fact, even loose linkages can be exploited in an applied breeding program (Sharma, 2001).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

F_2 derived F_4 self-bulks of a pearl millet mapping population (skeleton-mapped F_2 individuals) obtained from a cross of two pearl millet inbreds, PT 732B and P 1449-2, were used as the basic material. PT 732B (Appadurai *et al.*, 1982), an elite d_2 dwarf hybrid seed parent maintainer line developed at Tamil Nadu Agricultural University (TNAU) and P 1449-2 (ICRISAT, 1997; Singh, 1990) is a tall, downy mildew resistant parent, which is a selection developed at ICRISAT from a germplasm accession originating from Mali. PT 4450, an elite pollinator inbred was used as a male parent to produce testcross hybrids on each of the F_4 self-bulk mapping population progenies. PT 4450 is an elite restorer line being used to produce the commercial hybrid CoHCU-8 (PT 732A × PT 4450) in Tamil Nadu.

3.1. Test units

One hundred and thirty-six F_2 plants were derived from a single F_1 plant from the cross PT 732 × P 1449-2 were previously selfed at ICRISAT and skeleton mapped at John Innes Centre, UK. The F_2 plants were advanced to the F_4 seed generation at ICRISAT without selection. For this study the F_4 self-bulks of this mapping population were crossed with pollen from elite restorer line PT 4450, and the resulting 136 testcross hybrids, along with control hybrid CoHCU-8, and testcross of the two mapping population parental lines, were evaluated in replicated field trials.

3.1.1. Seed multiplication of testcross hybrids

One hundred and thirty-six F_4 self-bulks along with the two parental inbred lines (PT 732B and P 1449-2) and the pollinator (PT 4450) were sown in April 2001 (summer season at TNAU, Coimbatore).

Seeds were sown in a well-prepared nursery. Emerged seedlings were transplanted to the main field. The mapping population was raised in plots accommodating three rows each having 4m length. The adopted spacing was 30 cm between plants and 60 cm between rows. The pollinator was raised along with the mapping population. Two sowings were taken for the F_4 self-bulks so as to make effective crossing of all the lines. To get the synchronisation of flowering multiple sowing were taken of the pollinator line: one week before the F_4 lines, two weeks accompanying the F_4 lines, and one week after the second sowing of the F_4 lines. This plan provided sufficient time to make crosses as well as allow synchronisation of flowering. During flowering, pollen from the PT 4450 was collected and used to pollinate protected stigmas of multiple panicles of all the 136 F_4 self-bulks. Standard package of agronomic practices were carried out during the entire crop growth period.

3.1.2. Selfing

In addition to crossing, selfing was also carried out in all the F_4 self-bulks so as to get F_5 self-bulk seeds for field screening against pearl millet downy mildew.

3.1.3. Evaluation of test cross hybrids

Testcross hybrids were evaluated for phenotyping grain yield performance and its component traits during October 2001 (Rainy season at Tamil Nadu, 2001). Field trials

were conducted in two environments, one at TNAU, Coimbatore itself and another at the TNAU Regional Research Station (RRS), Bhavanisagar. Testcross seeds from all 136 lines, their parents and the commercial hybrid control COHCU-8 were evaluated in an alpha design with 18 × 18 plots. The testcross hybrids were sown in plots of 2 rows × 4 m with three replications. Inter-row spacing was maintained at 0.6 m and plots initially over sown, were thinned within two weeks of seedling emergence to a uniform plant stand of approximately 12 plants per row (30 cm spacing between plants within the row) in both environments, for an average final plant population density of 50,000 per ha. Recommended cultural practices were followed during the entire crop growth period.

3.1.4. Screening for downy mildew resistance

Selfed seeds from the F_4 self-bulks mapping population progenies were used for screening against downy mildew in both locations (*i.e.* TNAU, Coimbatore and RRS, Bhavanisagar) during October 2001. Screening was done in sick plot conditions *i.e.* fields, having sufficient oospore inoculum. The infector-row technique was followed (Williams *et al.*, 1981, as modified by Singh *et al.*, 1993) to screen against downy mildew.

The disease screening was done in the following way:

The line 7042 (S) was sown as an infector in every 5^{th} row, 3 weeks prior to sowing of the test materials to develop a viable sporangial load for the test materials. At two-leaf stage the infector rows were spray inoculated with a viable sporangial suspension (10^6 sporangia mL^{-1}) during the late evening hours, after irrigation. Frequent furrow irrigation was given during the first 15 days after inoculation to promote high humidity favoring a higher frequency of infected plants at an early growth stage.

The F_5 selfed seed bulks produced by selfing of F_4 self-bulks mapping population progenies were sown three weeks after the infector rows sown in the intercrossing rows after the infection rows have developed 50-60% disease incidence. A well-known susceptible control (HB 3) genotype was also sown along with the test material after every 20 entries to monitor variation in the level of disease incidence across the field.

Test materials and controls were sprayed with viable sporangial inoculum (10^6 sporangia mL^{-1}) when they reached two-leaf stage to increase the likelihood of disease development in genetically susceptible individuals.

All the test lines and controls were sown in rows of 4m length with two replications. Standard package of practices were followed.

3.2. Observations recorded in mapping population testcross hybrid yield trials

The following observations were noted in the F_1 testcross hybrids from both locations.

Time to 50% stigma emergence in days (FT)

Flowering time was recorded as the number of days from sowing until 50% of the plants in each plot produced stigmas on their main stem panicles.

Plant height (PH)

Plant height was measured from the base of the stem to the tip of the panicle at maturity. Data was recorded on five random plants from the middle of each row, and was recorded in cm.

Productive tiller number (PT)

Number of productive tillers per m^2 was taken by counting the panicles from individual plants occupied per m^2 area from the middle portion of the rows.

Panicle length (PL)

Length of panicle on the main stem was measured for the same plants considered for plant height in each plot and recorded in cm.

Panicle circumference (PCR)

Girth of the panicle was measured in cm using vernier caliper on all those plants for which panicle length was measured, and this was converted to circumference by multiplying girth by π.

Grain yield per season (GY)

Panicles were threshed and their grains cleaned. Weight of the grains in grams was recorded from each plot.

Thousand-grain mass (TGM)

One thousand grains were counted and their weight (in grams) was recorded for all the entries.

Grain yield per day (GYD)

This is calculated by dividing plot grain yield per season with total number of days taken to attain physiological maturity (approximated as time to 50% stigma emergence + 25) and expressed in grams per plot per day.

Single-panicle grain mass (SPGM)

This is the ratio between plot grain yield and the number of productive tillers per plot and was expressed in grams.

Single-panicle grain number (SPGN)

This is derived from the ratio of single panicle grain mass and thousand grain mass and expressed in numbers.

Grain number per unit area (GNPS)

Grain number per panicle surface unit area is obtained by the following formula:

$$GNPS = \frac{\text{Panicle grain number}}{\text{Panicle circumference} \times \text{panicle length}}$$

3.3. Scoring of disease incidence for downy mildew screening trials

Diseased plants were identified by the scoring method developed at ICRISAT (Singh *et al.*, 1997).

3.4. Statistical analysis

The statistical analyses were done using the program, GENSTAT 5th edition (1993). Analysis of variance, F-ratio and heritability (mean and plot basis) were calculated for each observed or calculated trait for single-site data sets from Coimbatore and Bhavanisagar, and across-locations, for both yield trials and downy mildew screening trials.

3.4.1. Linkage map construction

A previously constructed RFLP marker-based genetic linkage map for the cross PT 732B × P 1449-2, developed at John Innes Centre by Dr. Katries Devos and co-workers, using the 136 progenies in the current study was used to locate the QTLs. This map consists of seven linkage groups with different lengths, which vary from 27.6 to 177.6 cM (Haldane), and accommodates a total of 60 RFLP markers (Figures 1.1-1.3). The linkage map was constructed using the program MAPMAKER/EXP 3.0 (Lander *et al.*, 1987).

3.4.2. QTL analysis

3.4.2.1. Data processing for yield trials

Plot values for grain yield and yield components data from Coimbatore, Bhavanisagar and across-locations were subjected into square root and log-transformations before regression analysis. Time to 50% stigma emergence, plant height and time to 50% stigma emergence together with plant height were used as predictors of plot yield performance. All the traits were regressed with these predictors individually and the residuals from this analysis were then used to map QTLs for grain yield and its component traits. This procedure was adopted after initial QTL analyses suggested very strong effects of flowering time and plant height QTLs (perhaps linked) on nearly all other agronomic traits studied.

3.4.2.2. Data processing for downy mildew screening trials

Data recorded from Coimbatore, Bhavanisagar and across-locations were converted into percentage disease incidence values and these were subjected to QTL analysis. These

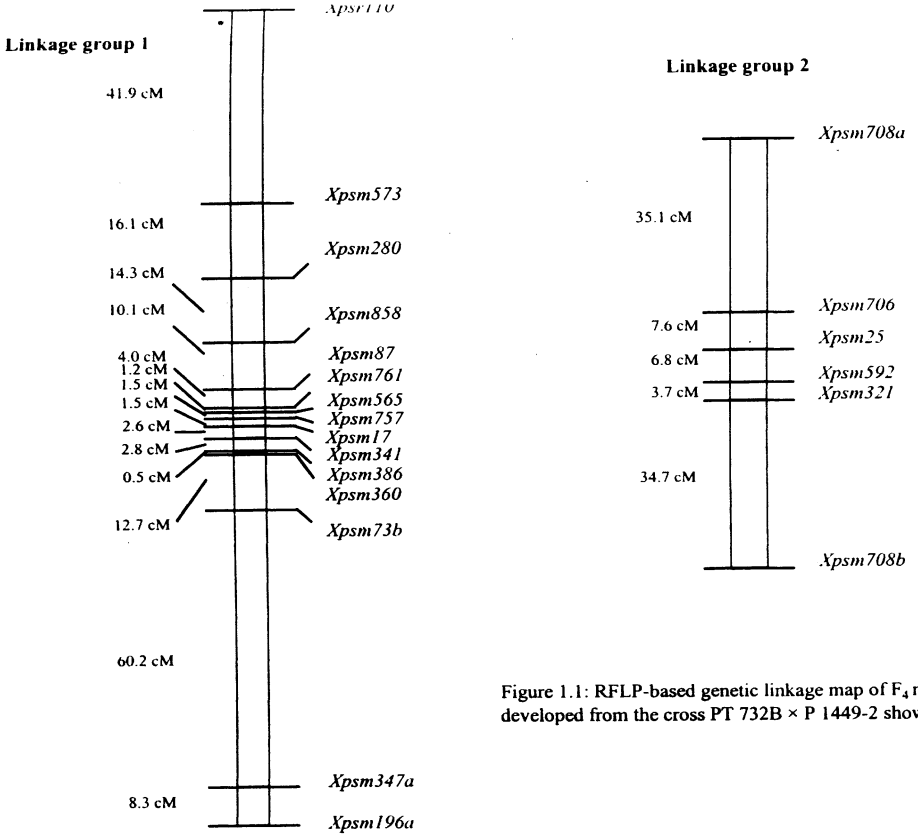


Figure 1.1: RFLP-based genetic linkage map of F_4 mapping population developed from the cross PT 732B × P 1449-2 showing LG 1 and LG 2

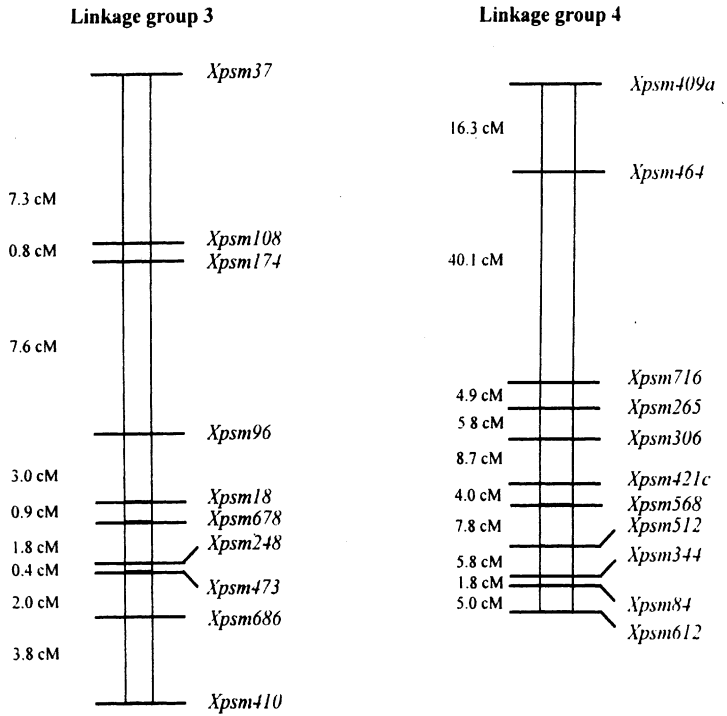


Figure 1.2: RFLP-based genetic linkage map of F_4 mapping population developed from the cross PT 732B × P 1449-2 showing LG 3 and LG 4

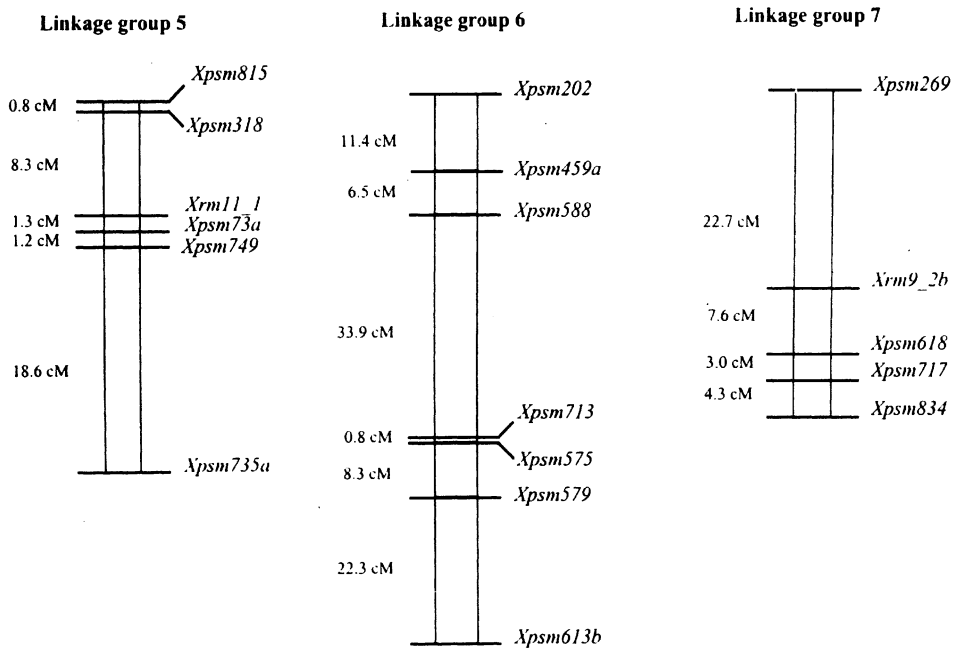


Figure 1.3: RFLP-based genetic linkage map of F_4 mapping population developed from the cross PT 732B × P 1449-2 showing LG 5, LG 6, and LG 7

data were also transformed into arc-sin values (radians) and used for detecting downy mildew resistance QTLs.

3.4.2.3. Mapping QTLs for yield trials

Residual data from Coimbatore, Bhavanisagar and across-locations from 136 mapping population testcross hybrids were sorted into progeny order corresponding to the marker genotype data set. QTL mapping was then carried out using MAPMAKER/QTL version 1.1b (Lander and Botstein, 1989; Lincoln *et al.*, 1992a). An additive genetic model from the program was used because testcross progenies derived from a heterozygous F_2 plant are a sample of the two parental alleles in combination with the tester allele, and the average of the heterozygote is the average of the two homozygotes (Cowen, 1988; Beavis *et al.*, 1994; Yadav *et al.*, 2002), so only additive effects are detected in such testcrosses and dominance effects can not be detected.

3.4.2.4. Mapping QTLs for downy mildew screening trials

Percentage of mean disease incidence and radians from arc-sin transformation were used for detecting downy mildew resistance QTLs from screens using the Coimbatore, Bhavanisagar and across-locations data sets. MAPMAKER/QTL version 1.1b was used to identify these QTLs. A free genetics model was considered as suitable because phenotyping was done in the F_2-derived F_5 self-bulk population.

RESULTS

4. RESULTS

Eleven important agronomic traits, including grain yield and its components, were phenotyped and their mean performance were recorded. Analysis of variance was calculated for all the traits at Coimbatore, Bhavanisagar and across-locations. Individual location data and pooled data showed significant difference for all the characters under study and interactions between genotypes and the locations were not significant for any of the characters, permitting interpretation of these traits using only the across-location means (Tables 1-3). Heritability calculation showed significant higher values for most of the traits (>50%), which is a prerequisite for effective QTL mapping.

4.1. Mean performance for different traits

Time to 50% stigma emergence

Testcross hybrids at both locations took a minimum of 40 days for completing 50% stigma emergence. Similarly 47 days was the maximum for completing 50% stigma emergence at both trial sites. Heritability for this trait was only 51% at Coimbatore and reached its maximum value (79%) when the statistical analysis was performed using pooled data from across the two test sites.

Plant height

The trial at Coimbatore had shorter statured plants than that conducted at Bhavanisagar, but maximum height was almost the same for both locations (177 cm). Heritability for this trait reached maximum at Coimbatore, while Bhavanisagar had lower heritability values.

Panicle length

Highest mean values for panicle length was obtained in Bhavanisagar. This location had also highest maximum values for panicle length (32.4 cm). Bhavanisagar and across-locations data showed maximum heritability values.

Panicle circumferences

The two locations had similar minimum mean values for panicle circumference, but Coimbatore had highest maximum panicle circumference (10.7 cm), where as Bhavanisagar registered the highest mean values for this trait (8.6 cm). Heritability for this trait was more than 90% at both locations and across-locations.

Productive tiller number

Data from both locations revealed that mean performance for minimum number productive tiller number were same. The maximum number of productive tillers was also same for both locations. Across-locations data had the highest broad sense heritability (73%) for this trait.

Thousand-grain mass

It was observed that thousand-grain mass reached minimum value (6.1g) at Coimbatore and had a maximum value of 12.6 g at Bhavanisagar. Individual locations and across-locations had high broad-sense heritability values for the trait (97 to 98%).

Single-panicle grain mass

Bhavanisagar had highest mean value (8.9 g) but Coimbatore registered the maximum observed value (14.4 g) for this trait. Broad-sense heritability calculated across-locations was the higher (83%) than that from individual locations data.

Single-panicle grain number

Values in Bhavanisagar ranged from 685 to 1302 g and the mean values attained the maximum of 953 g. Heritability (plot basis) was very low in both Locations (31 and 21%, for Coimbatore and Bhavanisagar respectively) but broad-sense heritability was more than 50% for the across-locations analysis.

Grain yield per day

Bhavanisagar had maximum values for grain yield per day and it also had the highest mean values. Where as Coimbatore had the minimum value for this trait. Heritability (broad-sense) for grain yield per day was 87% when pooled data were taken for consideration.

Grain number per unit panicle surface area

Both locations registered similar minimum and maximum values for grain number per unit panicle surface area. Also both locations had low plot-basis heritability but broad sense heritability at across-locations had higher values (55%).

Table 1: ANOVA for mapping population testcross hybrids for different traits from the trial conducted at Coimbatore, 2001-2002

Traits	Grain yield (g/m^2)	Flowering (days to 50% stigma emergence)	Plant height (cm)	Panicle length (cm)	Panicle circumference (cm)	Productive tiller number (per m^2)	1000-grain mass (g)	Single-panicle grain mass (g)	Single-panicle grain number	Grain yield per day (g/m^2/day)	Grain number (per cm^2 of panicle surface)
Mean	559	44	133	27	8.5	56	9.3	8.7	941	8.2	5.2
SE (±)	23.9	0.8	3.2	0.9	0.3	4.2	0.3	0.7	76.1	0.4	0.4
CV (%)	7.4	3.2	4.2	6.0	5.0	11	4.8	13.6	14.0	7.9	15.7
Minimum	454	40	90	21.7	7.0	46	6.1	5.5	672	6.5	3.5
Maximum	668	47	178	31.3	10.7	87	11.9	14.4	1219	10.1	7.5
F ratio	6.43***	4.73***	57.36**	6.6**	15.17***	4.95***	39.84***	7.28**	2.31**	7.72**	2.37***
h^2 (plot basis)	60	60	95	65	83	57	93	67	31	69	31
h^2 (mean basis)	90	79	98	85	93	80	97	86	57	87	57

*** Significant at the 0.01 level of probability

Table 2: ANOVA for mapping population testcross hybrids for different traits from the trial conducted at Bhavanisagar, 2001-2002

Traits	Grain yield (g/m^2)	Time to 50% stigma emergence (days)	Plant height (cm)	Panicle length (cm)	Panicle circumference (cm)	Productive tiller number (per m^2)	1000 grain mass (g)	Single-panicle grain mass (g)	Single-panicle grain number	Grain yield per day ($g\ m^2$ day)	Grain number (per cm^2 of panicle surface)
Mean	568	43	135	27	8.6	56	9.5	8.9	953	8.3	5.4
SE (±)	25.4	0.8	9.0	0.9	0.3	5.1	0.3	0.8	90.1	0.4	0.5
CV (%)	7.8	3.3	11.5	5.7	5.7	13.5	4.7	16.1	16.4	8.3	19.2
Minimum	456	40	94	21.7	7.0	51	6.7	5.6	685	6.5	3.9
Maximum	681	47	176	32.4	10.0	88	12.6	12.6	1302	10.4	7.3
F ratio	5.4***	4.1***	5.4***	7.3**	10.1***	3.0***	40.1***	4.6***	1.8***	6.4***	1.7***
h^2 (plot basis)	59	51	60	68	75	40	93	55	21	64	19
h^2 (mean basis)	81	76	82	86	90	67	98	78	45	94	42

*** Significant at the 0.01 level of probability

Table 3: ANOVA for mapping population testcross hybrids for different traits from across- locations pooled data, 2001/2002

Traits	Grain yield (g/m^2)	Time to 50% stigma emergence (days)	Plant height (cm)	Panicle length (cm)	Panicle circumference (cm)	Productive tiller number (per m^2)	1000 grain mass (g)	Single-panicle grain mass (g)	Single-panicle grain number	Grain yield per day ($g \cdot m^2 \cdot day$)	Grain number (per cm^2 of panicle surface)
Mean	564	43	134	27	8.5	66	9.4	8.8	947	8.3	5.3
SE (±)	34.9	1.2	9.5	1.3	0.4	6.6	0.4	1.1	117.9	0.6	0.6
CV (%)	7.6	3.3	8.7	5.8	5.4	12.3	4.8	14.9	15.3	8.1	17.6
Minimum	468	41	94	22.2	7.1	55	6.6	5.8	800	6.6	3.8
Maximum	666	46	174	31.0	9.9	84	11.8	11.7	1160	10.0	7.2
σ_g^2 / SE σe^2	7.7***	7.4***	8.0***	7.9**	7.84***	6.82***	8.26***	5.51***	5.43***	7.47***	5.51***
h^2 (broad sense)	85	79	90	87	91	73	98	83	54	87	55

*** Significant at the 0.01 level of probability

4.2. Correlation studies

Grain yield

Grain yield per season is the ultimate trait that was taken first as an explanatory variable and correlated with other traits to find the relative contribution of each constant trait to the observed yield variation. The results are shown in the Table 4.

Plant height, panicle circumference, thousand-grain mass, single-panicle grain mass, single-panicle grain number, grain yield per day and grain number per unit panicle surface area had positive correlations with grain yield per season. Traits like time to 50% stigma emergence, panicle length and productive tiller number were correlated negatively with grain yield at both locations. Coimbatore showed the highest positive correlations for plant height (0.620), panicle circumference (0.642), grain yield per day (0.983) and single-panicle grain number (0.283). For grain number per unit panicle surface area, Bhavanisagar registered the higher correlation. In both locations the trait grain yield per day was closely correlated with grain yield per season.

Coimbatore had higher values for traits negatively correlated with grain yield per season *i.e.* time to 50% stigma emergence (-0.520) and productive tiller number (-0.421) than did Bhavanisagar where panicle length was highly negatively correlated (-0.527) with grain yield per season.

Time to 50% stigma emergence

Productive tiller number and panicle length were the two traits showing positive relationships with time to 50% stigma emergence at both locations. Other characters including grain yield per season showed a negative correlation with time to 50% stigma emergence.

Coimbatore showed higher values for negatively correlated traits. For all other traits like plant height, panicle circumference, single-panicle grain mass, single-panicle grain number, grain yield per day and grain number per unit area except thousand-grain number, showed lower values at Bhavanisagar.

Plant height

Plant height exhibited similar relationships as that of grain yield per season had with other traits. Panicle circumference, thousand-grain mass, single-panicle grain mass, single-panicle grain number, grain yield per day and grain number per unit area were the positively related traits with plant height and among these traits Coimbatore had higher values for panicle circumference, thousand-grain mass, single-panicle grain mass and grain yield per day. On the other hand Bhavanisagar had higher positive values for single-panicle grain number and grain number per unit area. Productive tiller number and panicle length were the negatively correlated traits with plant height.

4.3. Mean performance of F_5 population in downy mildew screening trials for disease incidence

The original values for total and diseased plant count per plot were converted into disease incidence (%) and arc-sin transformation of this number in radians. These data were used for further statistical calculations. The results from ANOVA for disease reaction performance at Coimbatore, Bhavanisagar and across-locations and their coefficients of variation are shown in Table 5.

Results from ANOVA revealed that Bhavanisagar had a higher disease pressure with half of the population succumbing to downy mildew (52.6%). In Coimbatore the

Table 4: Correlation matrix of mapping population testcross hybrids at Coimbatore, Bhavanisagar and across locations, 2001/2002

	GY	FT	PH	PT	PL	PCR	TGM	SPGM	SPGN	GYPD	GNPS	Environment
GY	1											Across locations
	1											Coimbatore
	1											Bhavanisagar
FT	-0.509**	1										Across locations
	-0.520**	1										Coimbatore
	-0.507**	1										Bhavanisagar
PH	0.603**	-0.540**	1									Across locations
	0.620**	-0.571**	1									Coimbatore
	0.584**	-0.515**	1									Bhavanisagar
PT	-0.392**	0.377**	-0.479**	1								Across locations
	-0.421**	0.429**	-0.534**	1								Coimbatore
	-0.363**	0.330**	-0.426**	1								Bhavanisagar
PL	-0.495**	0.470**	-0.627**	0.425**	1							Across locations
	-0.469**	0.456**	-0.658**	0.450**	1							Coimbatore
	-0.527**	0.484**	-0.599**	0.402**	1							Bhavanisagar
PCR	0.609**	-0.522**	0.661**	-0.467**	-0.598**	1						Across locations
	0.642**	-0.557**	0.733**	-0.536**	-0.607**	1						Coimbatore
	0.573**	-0.490**	0.584**	-0.398**	-0.592**	1						Bhavanisagar
TGM	0.762**	-0.512**	0.609**	-0.451**	-0.555**	0.653**	1					Across locations
	0.790**	-0.503**	0.673**	-0.483**	-0.570**	0.681**	1					Coimbatore
	0.733**	-0.526**	0.541**	-0.418**	-0.542**	0.623**	1					Bhavanisagar
SPGM	0.769**	-0.511**	0.619**	-0.868**	-0.539**	0.612**	0.692**	1				Across locations
	0.791**	-0.543**	0.658**	-0.867**	-0.534**	0.667**	0.718**	1				Coimbatore
	0.746**	-0.487**	0.578**	-0.870**	-0.548**	0.556**	0.664**	1				Bhavanisagar
SPGN	0.276**	-0.181*	0.243**	-0.747**	-0.176*	0.184*	-0.075	0.658**	1			Across locations
	0.283**	-0.243**	0.232**	-0.740**	-0.162*	0.234**	-0.043	0.654**	1			Coimbatore
	0.268**	-0.129	0.251**	-0.753**	-0.190*	0.136	-0.106	0.661**	1			Bhavanisagar
GYPD	0.983**	-0.658**	0.640**	-0.421**	-0.531**	0.641**	0.774**	0.780**	0.279**	1		Across locations
	0.983**	-0.665**	0.659**	-0.454**	-0.503**	0.675**	0.793**	0.803**	0.298**	1		Coimbatore
	0.982**	-0.658**	0.618**	-0.387**	-0.563**	0.605**	0.753**	0.756**	0.261**	1		Bhavanisagar
GNPS	0.131	-0.089	0.148	-0.600**	-0.316**	-0.120	-0.169*	0.489**	0.860**	0.133	1	Across locations
	0.085	-0.099	0.095	-0.569**	-0.301**	-0.103	-0.175*	0.442**	0.839**	0.095	1	Coimbatore
	0.172*	-0.080	0.195	-0.627**	-0.331**	-0.137	-0.164*	0.531**	0.877**	0.167*	1	Bhavanisagar

GY: Grain yield(g/m^2); FT: Time to 50% stigma emergence (days); PH: Plant height (cm); PL: Panicle length (cm); PCR: Panicle circumference (cm)
PT: Productive tiller number (per m^2); TGM: 1000-grain mass (g); SPGM: Single-panicle grain mass (g); SPGN: Single-panicle grain number
GYPD: Grain yield per day (g/m^2/day); GNPS: Grain number (per cm^2 of panicle surface)
* Significant at 0.5 level of probability, ** Significant at the 0.1 level of probability

disease incidence was also severe, reaching nearly 50%. Heritability for the disease incidence values (for both percentage diseased plants and the arcsin-transformed data) were high enough for use to map QTLs. Disease incidence (%) at Bhavanisagar had the highest heritability (plot basis). This location also showed highest heritability for arc-sin transformed disease incidence.

4.4. Information on linkage map

A Previously constructed RFLP linkage map was used for this study. This linkage map was developed from the cross PT 732 B × P 1449- 2 using 58 RFLP probes detecting 60 loci. Seven linkage groups (LGs; singular = LG) were constructed using MAPMAKER/EXP multipoint analysis with the LOD threshold value of 2.0 and a recombination fraction of 0.5. The minimum and maximum length of linkage groups varied from 27.6 cM (LG 3) to 177.6 cM (LG 1). These linkage groups were used for mapping QTL for both in test cross hybrids for yield and its components traits and in F_2 population for mapping downy mildew resistance QTLs.

Linkage group 1

LG 1 has a length of 177.6 cM (Haldane units) and this is the lengthiest LG in pearl millet. It accommodates 15 markers with different marker intervals. LOD score of this group was very high (-365.0), much higher than the other linkage groups.

Table 5: ANOVA for percentage and arcsin-transformed values for downy mildew incidence from trials conducted at Coimbatore, Bhavanisagar and across-locations, 2001/2002

Parameters	Coimbatore		Bhavanisagar		Across Locations	
	percentage	radians	percentage	radians	percentage	radians
SE (±)	12.70	0 20	11 57	0.17	8 57	0.11
Mean	49.2	0 57	52.6	0.61	50 9	0.55
CV (%)	36.52	49 45	31.10	38.01	23 81	27.94
F ratio	3 83**	3 03**	4 73**	4 82**	4 04**	3 86**
h^2 (plot basis)	59	50	65	66	60	59
h^2 (mean basis	74	67	79	79	75	74
σ_x^2 SE σe^2	3 4**	4 2**	3 9**	3.3**	3 8**	4.1**

** Significant at the 0 1 level of probability

Linkage group 2

This group has six markers with the total distance of 87 9 cM The order of the markers on this linkage group is *Xpsm708a*, *Xpsm706*, *Xpsm25*, *Xpsm592*, *Xpsm321* and *Xpsm708b* The LOD score of this linkage group was -179 39

Linkage group 3

The total length of this linkage group is 27 6 cM This is the shortest pearl millet linkage group although it accommodates 10 markers. The LOD score of this group is -162.28.

Linkage group 4

This group has the length of 100 0 cM and has 11 markers with optimum inter-marker intervals to detect QTLs.

Linkage group 5

Six markers occupied LG 5 The maximum likelihood position of the marker intervals is *Xpsm815*, *Xpsm328*, *Xpsm73A*, *Xrm11_1*, *Xpsm749* and *Xpsm735a* The total length of this linkage group is 30 2 cM and its LOD score is -137 84.

Linkage group 6

LG 6 accommodates seven markers in a length of 83 1 cM This group has the LOD score of -205 52

Linkage group 7

This is the smallest linkage group in terms of number of markers. It has only five markers and their correct order is *Xpsm269*, *Xrm9_2b*, *Xpsm618*, *Xpsm717* and *Xpsm834*. The length of this group is 37.6 cM with LOD score of -143 03

4.5. Mapping QTLs

The constructed linkage map from the cross PT 732 B × P 1449-2 using 136 individuals was used for mapping QTLs for yield and its related traits. Software package MAPMAKER/EXP version 3.0b was used for constructing linkage groups and MAPMAKER/QTL version 1.1 b was used for detecting QTLs.

4.5.1. MAPMAKER/QTL

The interval mapping method as implemented in MAPMAKER/QTL was used with a LOD of 2.0 as threshold value for detecting significant QTLs. The additive genetic model from this software package was used as the phenotyping was done in testcross hybrids. For this, the command "sequence [all: additive]" was used to restrict the genetic model only to additive effects.

For mapping downy mildew resistance QTLs, phenotyping was done in the F_4 self bulks. So, all possible genetic models (additive, dominant and recessive) were considered. This is carried out by using the command "sequence [all]". Combined effects of multiple QTLs were calculated by multiple QTL models for two QTLs, three QTLs etc. The qualifying criteria for accepting a multiple QTL model was a LOD score of two units more than the highest LOD score of the best model having one less QTL.

LOD_n = Minimum qualifying LOD score for acceptance of a multiple QTL model with (n) QTLs.

= 2 + LOD (n-1)

$LOD_{(n-1)}$ = Maximum LOD score for observed model with (n-1) QTLs.

4.6. QTLs for agronomic traits

A total of 18 QTLs were identified across seven linkage groups for nine traits, but genomic regions flanked by only seven markers loci controlled all these QTLs. The details of the QTLs detected on different linkage groups are shown in Tables 6-22. Graphical representation of LOD values obtained from different types of transformation for different traits are shown in Figures 2.1-7.3.

Mean values at Coimbatore, Bhavanisagar and across-locations from the mapping population testcross [(PT 732B × P 1449-2) × PT 4450] consisting of 136 hybrids were used for mapping QTLs for the different traits. Square root and log-transformed values from Coimbatore, Bhavanisagar and across-locations were used to map these QTLs in an attempt to reduce distribution abnormalities in the trait data set. Plant height, time to 50% stigma emergence, and plant height together with time to 50% stigma emergence were used as predictors of other traits using liner regression, and the residuals from these regressions were used to locate QTL positions. Most of the detected QTLs are situated on LG 4. LG 2, LG 6 and LG 7 are the other groups having QTLs. No QTLs were detected on LG 1, LG 3 and LG 5.

Time to 50% stigma emergence

A single QTL was identified for time to 50% stigma emergence at Coimbatore, using log-transformed data. This QTL for time to 50% stigma emergence is situated on LG 4 and explained 7.8% of observed phenotypic variation with a LOD value of 2.1. The additive effect of the P 1449-2 parent allele at this QTL decreased flowering by 0.5 day

Plant height

A single QTL was mapped on LG 4 for plant height. This QTL had its minimum LOD score of 2.83 at Bhavanisagar when log transformed values were used. But the maximum LOD value of 6.95 was obtained at Coimbatore when square root transformed values were used. At this maximum LOD a maximum explanation of observed phenotypic variance was (23.9%) also obtained. Additive genetic model gave the maximum value of 0.7984 for this maximum LOD score, which corresponds to an increase of plant height by one cm when the P 1449-2 parent allele is present.

Panicle circumference

For panicle circumference one QTL was identified on the bottom of LG 4. The panicle circumference QTL was observed between the marker loci *Xpsm512* and *Xpsm344* when regressed against plant height and time to 50% stigma emergence at both locations. The significant LOD score for this QTL ranged from 2.46 to 7.46. The phenotypic variance ranged from 10.1 to 26.6%, depending upon the data manipulations used prior to QTL mapping. At the maximum LOD value (7.46) the additive effect of the allele from P 1449-2 increased the panicle circumference by 6.5 cm.

Panicle length

This trait had a single QTL, which is located between the marker loci *Xpsm568* and *Xpsm512* on LG 4 Nearly all types of transformation of data from both locations and all the residuals from different types of functions detected this QTL This QTL at the LOD score of 6 52 explained 22 7% of the observed phenotypic variance at Coimbatore

Thousand-grain mass

Two QTLs were identified for thousand-grain mass These QTLs are both located on LG 4 but at different intervals (*Xpsm306- Xpsm421c* and *Xpsm568- Xpsm512*). A maximum LOD score of 7 4 was obtained for this trait by using square root transformation of data from Bhavanisagar when plant height used as a predictor But the maximum portion of observed phenotypic variance (11 6%) was explained when time to 50% stigma emergence was used as a predictor of this trait

Grain yield per season

One QTL for grain yield per season was mapped at the bottom of LG 4 This QTL was detected when grain yield per season was regressed on time to 50% stigma emergence from both types of transformation. Square root transformations and log transformations gave more or less similar LOD scores (2 6) and R^2 values (10 0) They also exhibited similar additive effects (0 51), which correspond to an increase of grain yield per season by 0 3 g/m^2 when a P 1449-2 allele replaced that of PT 732B

Grain yield per day

Three QTLs were identified for grain yield per day at various intervals on LG 4. These intervals are *Xpsm84* to *Xpsm612*, *Xpsm568* to *Xpsm512* and *Xpsm306* to *Xpsm421c*. The middle QTL between marker loci *Xpsm568* and *Xpsm512*, recorded the maximum LOD (2.71) and explained the largest portion of the observed phenotypic variance (10.7). This was obtained by regressing grain yield per day against time to 50% stigma emergence using log-transformed data from Bhavanisagar.

Productive tiller number

A maximum of four QTLs were obtained for this trait on four different linkage groups (LG 2, LG 4, LG 6 and LG7). The maximum LOD peak of 2.92 was found at Coimbatore using log transformation together with time to 50% stigma emergence as a function. A maximum of 15.4% for R^2 was explained by a single QTL, which was located on LG 2 (between *Xpsm321* and *Xpsm708b*) with the additive effect of 0.6909 corresponding to a decrease of tiller number by 0.3 m^{-2} when the PT 1449-2 allele was replaced for that of PT 732B at this locus.

Single-panicle grain mass

Xpsm84- Xpsm612, *Xpsm579- Xpsm613b* and *Xrm9_2b- Xpsm618* are the three marker loci intervals accommodating QTLs for this trait on LG 4, LG 6 and LG 7 respectively. The QTL on LG 6 explained more of the observed phenotypic variance (15.4%) than other QTLs, and had a LOD value of 2.19. However the QTL on LG 4 had the highest LOD score (3.58) and explained 12.4 % of observed phenotypic variance.

Table 6: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height as a predictor of square root-transformed values of other traits at Coimbatore.

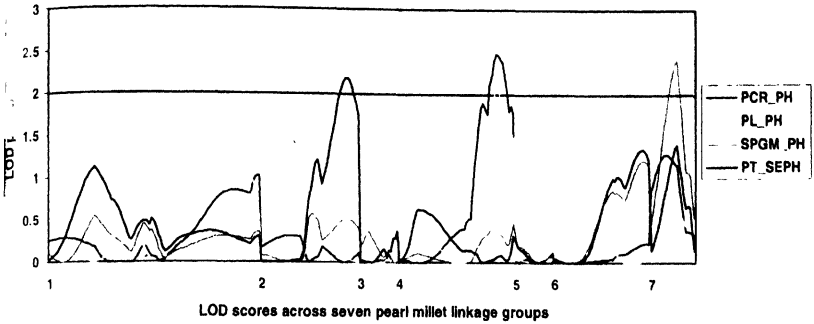
Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*568-*Xpsm*512	4	4.0	2.5	10 5	0 5156	0 3 cm
Panicle length	*Xpsm*568-*Xpsm*512	4	4.0	2.0	8.3	-0 4580	0 2 cm
Single-panicle grain mass	*Xrm*9-2b-*Xpsm*618	7	0	2.4	9 0	0 4114	0 2 g

Table 7: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using time to 50% stigma emergence as a predictor of square root-transformed values of other traits at Coimbatore.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*512-*Xpsm*344	4	2.0	7.4	26.4	0.8126	0.7 cm
Plant height	*Xpsm*512-*Xpsm*344	4	4.0	7.0	23.9	0.7974	0.6 cm
Panicle length	*Xpsm*512-*Xpsm*344	4	2.0	6.5	22.5	-0.7574	0.6 cm
Productive tiller number	*Xpsm*84-*Xpsm*612	4	4.0	2.6	9.0	-0.4597	0.2
	*Xpsm*579-*Xpsm*613b	6	12.0	2.9	13.6	-0.5339	0.3
Single-panicle grain mass	*Xpsm*84-*Xpsm*612	4	4.0	3.58	12.4	0.5391	0.3 g
	*Xpsm*579-*Xpsm*613b	6	10.0	2.73	12.3	0.5119	0.3 g

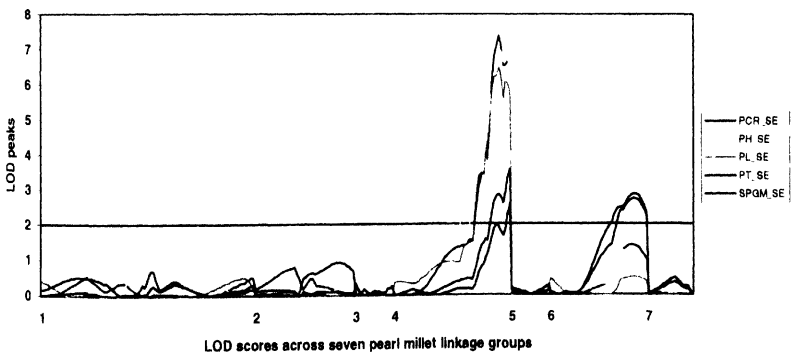
Table 8: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height together with time to 50% stigma emergence as a predictor of square root-transformed values of other traits at Coimbatore.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Productive tiller number	*Xpsm*321-*Xpsm*708b	2	24.0	2.2	14.8	0.6909	0.5



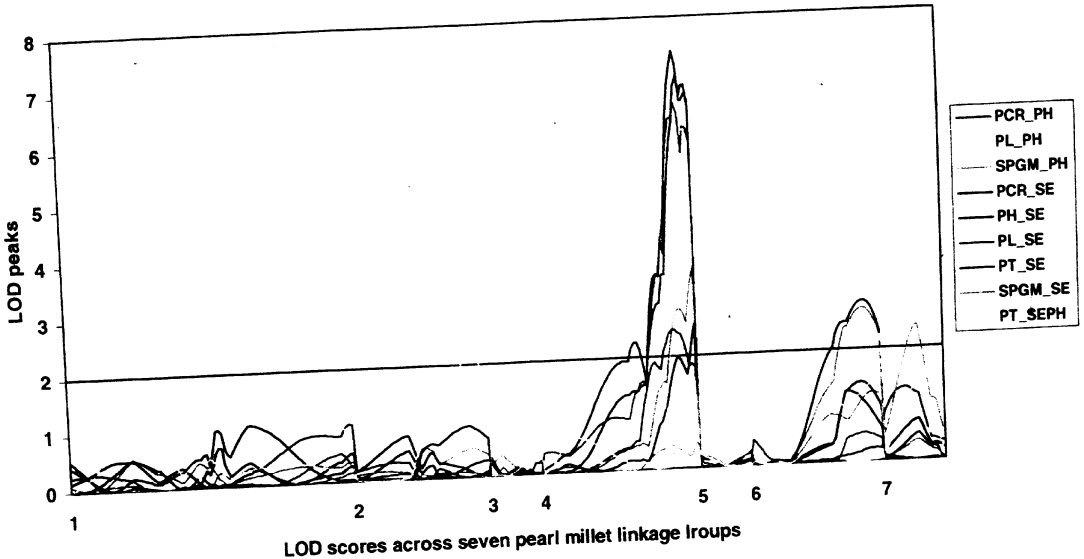
Legend: PCR-panicle circumference; PL-panicle length; SPGM-single-panicle grain mass; PT-productive tiller number. _PH- plant height as predictor; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 2.1: QTL LOD peaks for various traits using plant height and time to 50% stigma emergence together with plant height as predictors of log-transformed values from Coimbatore yield trial.



Legend: PCR-panicle circumference; PH-plant height; PL- panicle length; PT- productive tiller number; SPGM-single-panicle grain mass; _SE- time to 50% stigma emergence as predictor

Figure 2.2: QTL LOD peaks for various traits using time to 50% stigma emergence as a predictor of square root-transformed values from Coimbatore yield trial.



Legend: PCR- panicle circumference: PL- panicle length: SPGM- single-panicle grain mass: PH- plant height: PT- productive tiller number; _PH- plant height as predictor: _SE- time to 50% stigma emergence as predictor: _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 2.3: Comparison of QTL LOD peaks for various traits using different types of predictors of square root-transformed values from Coimbatore yield trial

Table 9: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height as a predictor of log-transformed values of other traits at Coimbatore. QTL indicated in bold-italics are those that were not detected at LOD threshold of 2.0 but have significantly contribution.

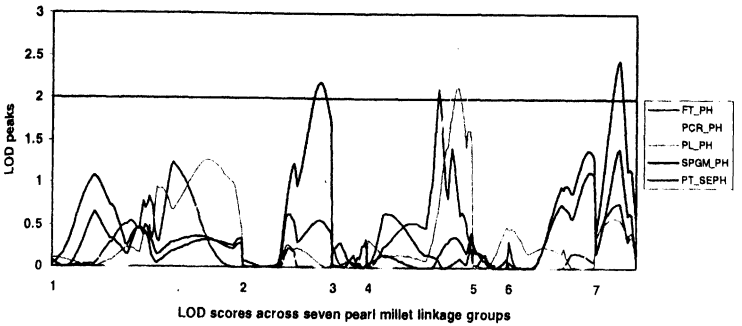
Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Time to 50% stigma emergence	***Xpsm306- Xpsm421c***	***4***	***0.0***	***2.1***	***7.8***	***-0.457***	***0.4 day***
Panicle circumference	*Xpsm*568- *Xpsm*512	4	6.0	2.5	10.1	0.4976	3.5 cm
Panicle length	*Xpsm*568- *Xpsm*512	4	6.0	2.1	8.4	-0.4559	0.4 cm
Single-panicle grain mass	*Xrm*9-2b- *Xpsm*618	7	0.0	2.5	9.1	0.4145	2.6 g

Table 10: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using time to 50% stigma emergence as a predictor of log-transformed values of other traits at Coimbatore. QTL indicated in bold-italics are those that were not detected at LOD threshold of 2.0 but have significantly contribution.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Grain yield per day	*Xpsm84-Xpsm612*	*4*	*2.0*	*2.0*	*7.1*	*0.4210*	*2.6 g*
Panicle circumference	*Xpsm*512- *Xpsm*344	4	2.0	7.5	26.6	0.8158	6.5 cm
Plant height	*Xpsm*512- *Xpsm*344	4	4.0	6.9	23.7	0.7957	6 cm
Panicle length	*Xpsm*512- *Xpsm*344	4	2.0	6.5	22.7	-0.7609	0.2 cm
Productive tiller number	*Xpsm*84- *Xpsm*612	4	4.0	2.6	9.2	-0.4659	0.3
	*Xpsm*579- *Xpsm*613b	6	12.0	2.92	13.8	-0.5376	0.3
Single-panicle grain mass	*Xpsm*512- *Xpsm*344	4	4.0	3.58	12.4	0.5390	3.5 g
	*Xpsm*579- *Xpsm*613b	6	12.0	2.76	12.9	0.5210	3.3 g

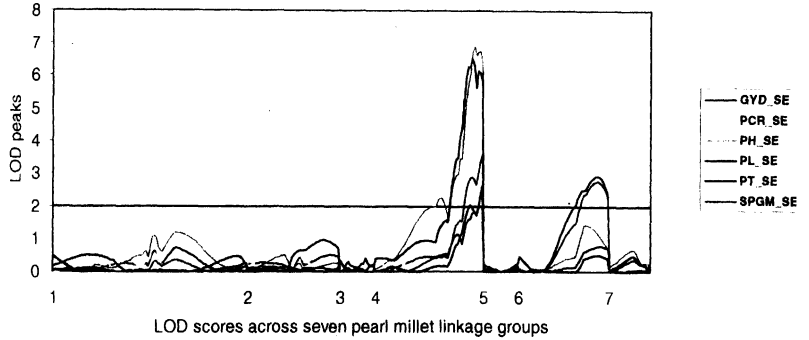
Table 11: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height together with time to 50% stigma emergence as a predictor of log-transformed values of other traits at Coimbatore.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Productive tiller number	*Xpsm*321-*Xpsm*708b	2	22.0	2.2	15.4	0.6924	4.925



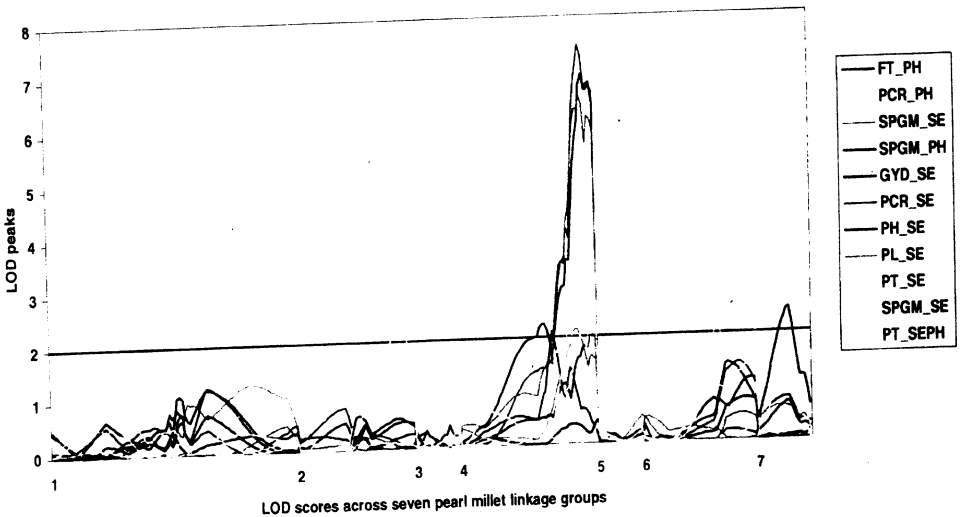
Legend: FT- time to 50% stigma emergence; PCR- panicle circumference; PL-panicle length; SPGM- single-panicle grain mass; PT- productive tiller number; _PH- plant height as predictor; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 3.1: QTL LOD peaks for various traits using plant height and time to 50% stigma emergence together with plant height as predictors of log-transformed values from Coimbatore yield trial.



Legend: GYD- grain yield per day; PCR- panicle circumference; PH- plant height; PL- panicle length; PT- productive tiller number; SPGM- single-panicle grain mass; _SE- time to 50% stigma emergence as predictor

Figure 3.2: QTL LOD peaks for various traits using time to 50% stigma emergence as a predictor of log-transformed values from Coimbatore yield trial.



Legend: FT- time to 50% stigma emergence: PCR- panicle circumference: SPGM- single-panicle grain mass; GYD- grain yield per day; PH- plant height: PL- panicle length: PT- productive tiller number; _PH- plant height as predictor: _SE- time to 50% stigma emergence as predictor; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 3.3: Comparison of QTL LOD peaks for various traits using different types of predictors of log-transformed values from Coimbatore yield trial

Table 12: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height as a predictor of square root-transformed values of other traits at Bhavanisagar.

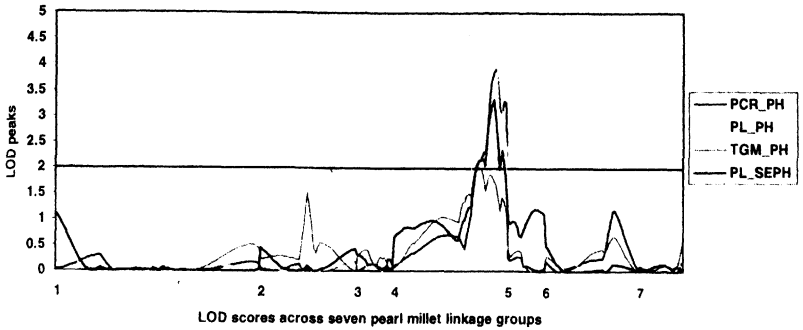
Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*512- *Xpsm*344	4	2.0	3.9	15.2	0.6247	0.4 cm
Panicle length	*Xpsm*512- *Xpsm*344	4	0.0	4.5	15.7	-0.6156	0.4 cm
Thousand-grain mass	*Xpsm*306- *Xpsm*421c	4	8.0	7.4	2.015	0.4390	0.2 g

Table 13: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using time to 50% stigma emergence as a predictor of square root-transformed values of other traits at Bhavanisagar.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Grain yield per day	*Xpsm*568-*Xpsm*512	4	4.0	2.7	10.5	0.5163	0.3 g
Grain yield	*Xpsm*568-*Xpsm*512	4	4.0	2.6	10.3	0.5118	0.3 g
Panicle circumference	*Xpsm*512-*Xpsm*344	4	2.0	5.9	21.6	0.7401	0.5 cm
Plant height	*Xpsm*512-*Xpsm*344	4	4.0	2.8	10.0	0.5162	0.3 cm
Panicle length	*Xpsm*512-*Xpsm*344	4	0.0	6.0	20.3	-0.6970	0.5 cm
Thousand-grain mass	*Xpsm*568-*Xpsm*512	4	6.0	3.0	11.6	0.5364	0.3 g

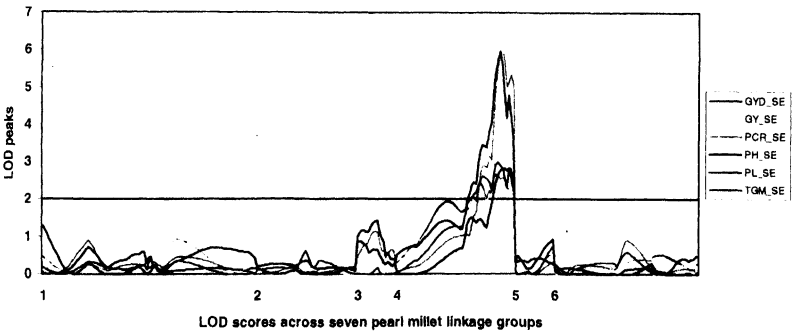
Table 14: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height together with time to 50% stigma emergence as a predictor of square root-transformed values of other traits at Bhavanisagar.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle length	*Xpsm*512-*Xpsm*344	4	0.0	3.3	11.9	-0.5354	0.3 cm



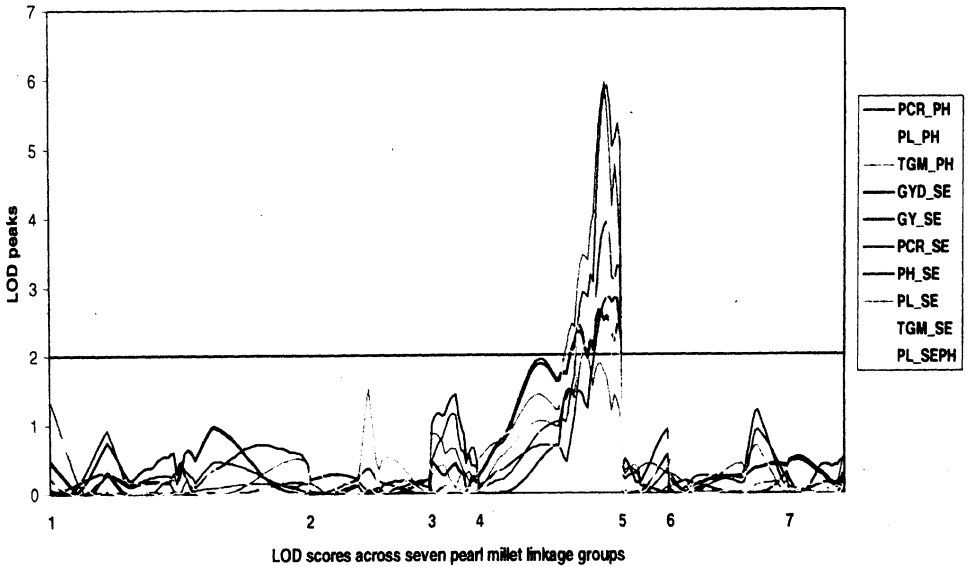
Legend: PCR- panicle circumference; PL- panicle length; TGM- thousand-grain mass; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 4.1: QTL LOD peaks for various traits using plant height and time to 50% stigma emergence as a predictor of square root-transformed values from Bhavanisagar yield trial.



Legend: GYD- grain yield per day; GY- grain yield; PCR- panicle circumference; PH- plant height; PL- panicle length; TGM- thousand grain mass; _SE- time to 50% stigma emergence as predictor

Figure 4.2: QTL LOD peaks for various traits using days to 50% stigma emergence as a predictor of square root-transformed values from Bhavanisagar yield trial.



Legend: PCR- panicle circumference: PL- panicle length: TGM- thousand-grain mass: GYD- grain yield per day; GY- grain yield; PH- plant height; _PH- plant height as predictor: _SE- time to 50% stigma emergence as predictor: _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 4.3: Comparison of QTL LOD peaks for various traits using different types of predictors of square root-transformed values from Bhavanisagar yield trial

Table 15: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height as a predictor of log-transformed values of other traits at Bhavanisagar.

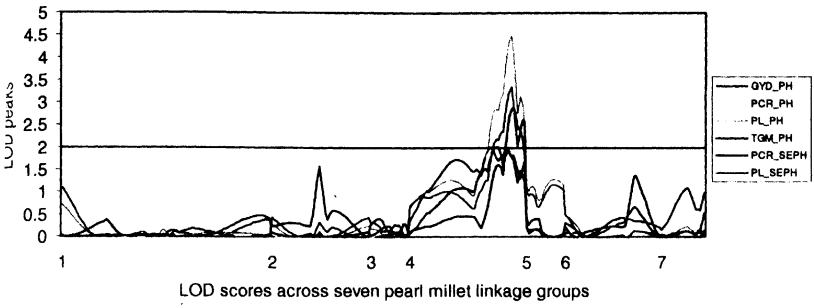
Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Grain yield per day	*Xpsm*306-*Xpsm*421c	4	2.0	2.0	7.6	0.4530	2.8 g
Panicle circumference	*Xpsm*512-*Xpsm*344	4	2.0	3.94	15.1	0.6236	4.2 cm
Panicle length	*Xpsm*512-*Xpsm*344	4	0.0	4.5	15.8	-0.6170	0.2 cm
Thousand-grain mass	*Xpsm*306-*Xpsm*421c	4	8.0	2.0	7.3	0.4390	2.8 g

Table 16: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using time to 50% stigma emergence as a predictor of log-transformed values of other traits at Bhavanisagar.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Grain yield per day	*Xpsm*568-*Xpsm*512	4	4.0	2.71	10.7	0.5208	3.3 g
Grain yield	*Xpsm*568-*Xpsm*512	4	4.0	2.68	10.6	0.5177	3.3 g
Panicle circumference	*Xpsm*512-*Xpsm*344	4	2.0	5.89	21.4	0.7378	5.5 cm
Plant height	*Xpsm*512-*Xpsm*344	4	4.0	2.83	9.9	0.5148	3.3 cm
Panicle length	*Xpsm*512-*Xpsm*344	4	0.0	5.91	20.1	-0.6947	0.2 cm

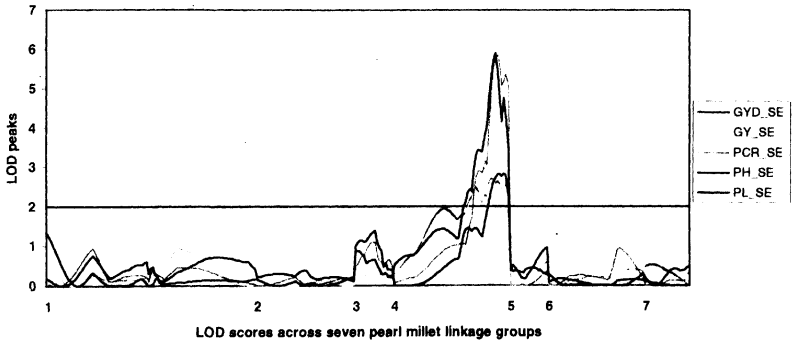
Table 17: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height together with time to 50% stigma emergence as a predictor of log-transformed values of other traits at Bhavanisagar.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*512-*Xpsm*344	4	2.0	2.90	11.3	0.5391	3.5 cm
Panicle length	*Xpsm*512-*Xpsm*344	4	0.0	3.36	12.0	-0.5381	0.3 cm



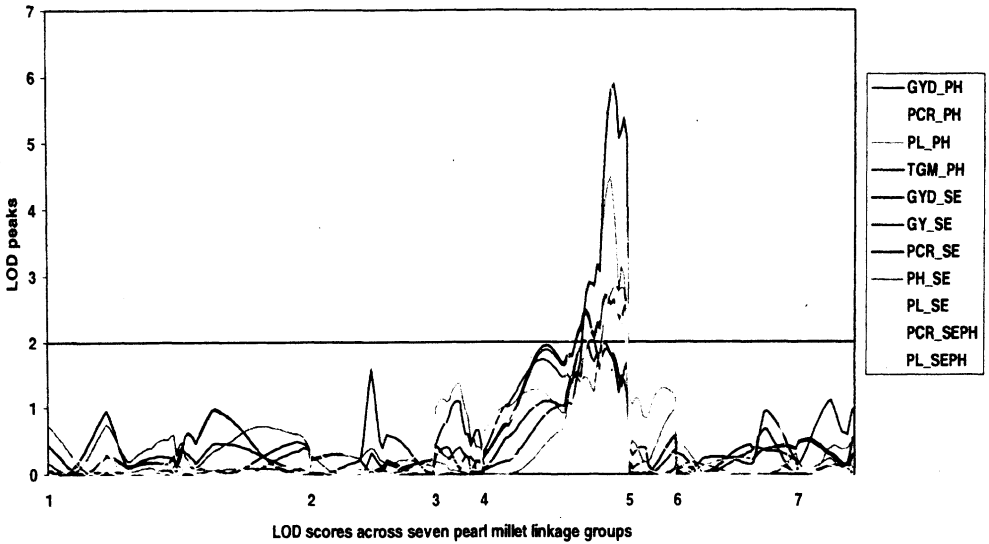
Legend: GYD- grain yield per day; PCR- panicle circumference; PL- panicle length; TGM- thousand-grain mass; _PH- plant height as predictor; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 5.1: QTL LOD peaks for various traits using plant height and time to 50% stigma emergence as a predictor of log-transformed values from Bhavanisagar yield trial.



Legend: GYD- grain yield per day; GY- grain yield; PCR- panicle circumference; PH- plant height; PL- panicle length; _SE- time to 50% stigma emergence as predictor

Figure 5.2: QTL LOD peaks for various traits using time to 50% stigma emergence as a predictor of log-transformed values from Bhavanisagar yield trial.



Legend: GYD- grain yield per day; PCR- panicle circumference: PL- panicle length; TGM- thousand grain mass: GY- grain yield; PH- plant height; _PH- plant height as predictor: _SE- time to 50% stigma emergence as predictor: _SEPH- time to 50% stigma emergence and plant height as predictors

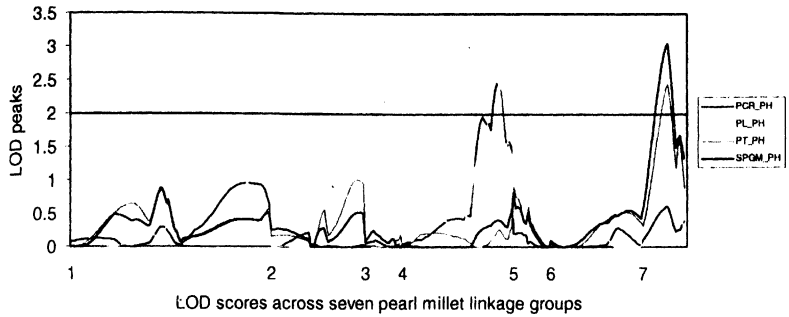
Figure 5.3: Comparison of QTL LOD peaks for various traits using different types of predictors of log-transformed values from Bhavanisagar yield trial

Table 18: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height as a predictor of square root-transformed values of other traits at across-locations.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*568-*Xpsm*512	4	6.0	2.46	10.5	0.5112	0.3 cm
Panicle length	*Xpsm*568-*Xpsm*512	4	6.0	2.51	9.4	-0.4836	0.2 cm
Productive tiller number	*Xrm*9-2b-*Xpsm*618	7	0.0	2.45	9.2	-0.4174	0.2
Single-panicle grain mass	*Xrm*9-2b-*Xpsm*618	7	0.0	3.06	11.2	0.4607	0.2 g

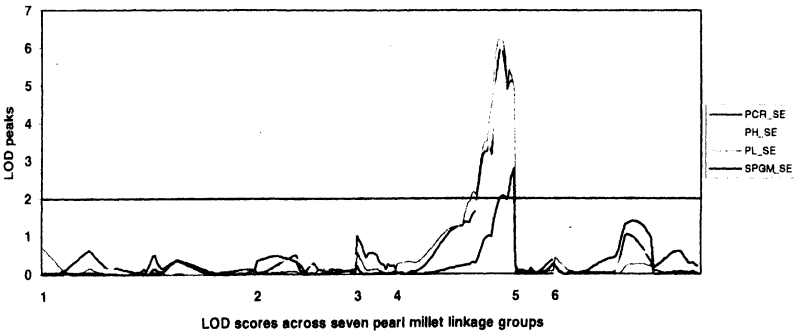
Table 19: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using time to 50% stigma emergence as a predictor of square root-transformed values of other traits at across-locations.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*512-*Xpsm*344	4	0.0	5.97	21.0	0.7059	0.5 cm
Plant height	*Xpsm*512-*Xpsm*344	4	4.0	6.48	22.5	0.7752	0.6 cm
Panicle length	*Xpsm*568-*Xpsm*512	4	6.0	6.22	22.1	-0.7406	0.5 cm
Single-panicle grain mass	*Xpsm*84-*Xpsm*612	4	4.0	2.78	9.9	0.4812	0.2 g



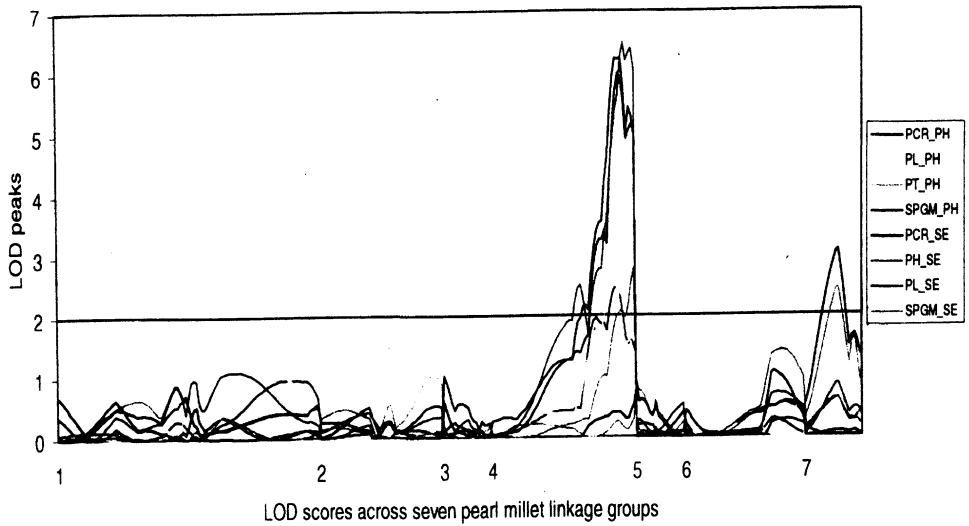
Legend: PCR- panicle circumference; PL- panicle length; PT- productive tiller number; SPGM- single-panicle grain mass; _PH- plant height as predictor

Figure 6.1: QTL LOD peaks for various traits using plant height as a predictor of square root-transformed values from across-locations



Legend: PCR-panicle circumference; PH- plant height; PANICLE LENGTH- panicle length; SPGM- single-panicle grain mass; _SE- time to 50% stigma emergence as predictor

Figure 6.2: QTL LOD peaks for various traits using time to 50% stigma emergence as a predictor of square root-transformed values from across-locations



Legend: PCR- panicle circumference: PL- panicle length; PT-productive tiller number: SPGM- single-panicle grain mass; PH- plant height; _PH- pla height as predictor; _SE- time to 50% stigma emergence as predictor

Figure 6.3: Comparison of QTL LOD peaks for various traits using different types of predictors of square root-transformed values from across-locations

Table 20: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height as a predictor of log-transformed values of other traits at across-locations.

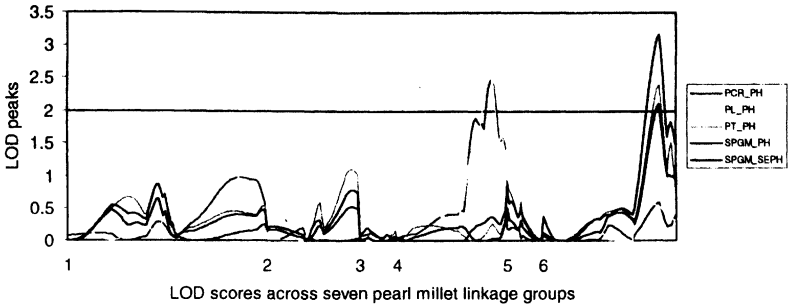
Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm* 568-*Xpsm*512	4	6.0	2.45	10.4	0.5093	3.2 cm
Panicle length	*Xpsm*568-*Xpsm*512	4	6.0	2.5	9.4	-0.4826	0.3 cm
Productive tiller number	*Xrm*9-2b-*Xpsm*618	7	0.0	2.4	9.0	-0.4123	0.4
Single-panicle grain mass	*Xrm*9-2b-*Xpsm*618	7	0.0	3.17	11.6	0.4681	2.9 g

Table 21: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using time to 50% stigma emergence as a predictor of log-transformed values of other traits at across-locations.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Panicle circumference	*Xpsm*512-*Xpsm*344	4	0.0	5.98	21.0	0.7099	5.1 cm
Plant height	*Xpsm*512-*Xpsm*344	4	4.0	6.38	22.2	0.7697	5.9 cm
Panicle length	*Xpsm*512-*Xpsm*344	4	2.0	6.14	21.4	-0.7377	0.2 cm
Single-panicle grain mass	*Xpsm*84-*Xpsm*612	4	4.0	2.75	9.8	0.4790	3.0 g

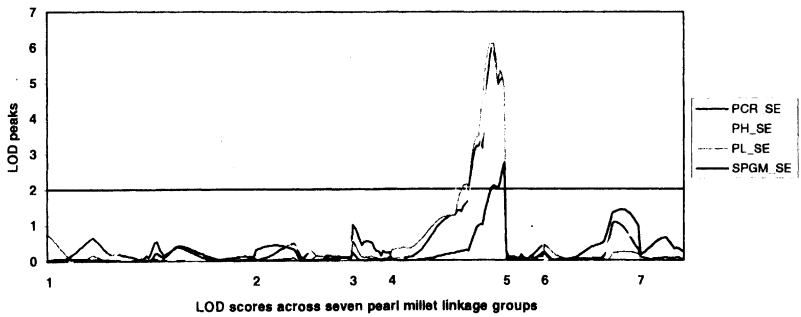
Table 22: QTL associated with grain yield-determining traits of pearl millet mapping progeny testcross hybrids using plant height together with time to 50% stigma emergence as a predictor of log-transformed values of other traits at across-locations.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	Back-transformed additive effects
Single-panicle grain mass	*Xrm*9-2b-*Xpsm*618	7	0.0	2.1	8.3	0.3963	2.5 g



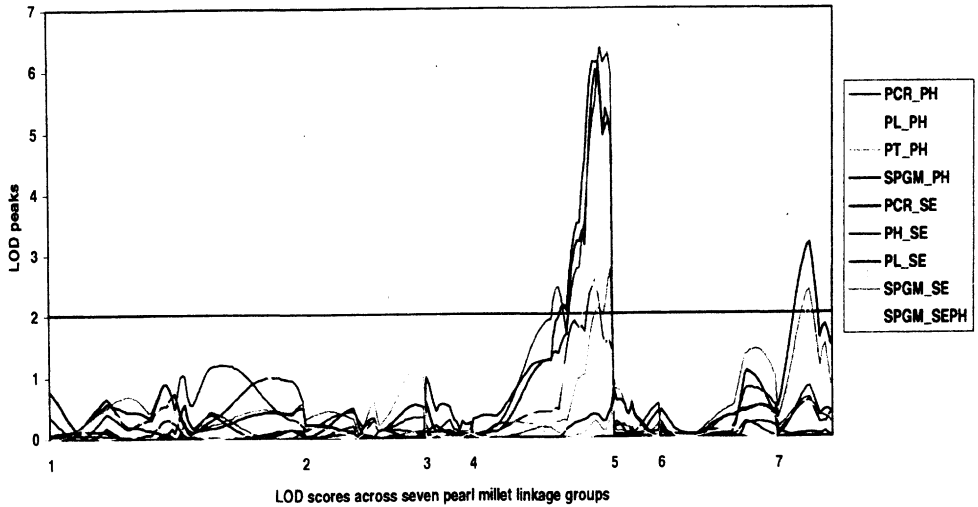
Legend: PCR- panicle circumference; PL- panicle length; PT- productive tiller number; SPGM- single-panicle grain mass; _PH- plant height as predictor; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 7.1: QTL LOD peaks for various traits using plant height and time to 50% stigma emergence as a predictor of log-transformed values from across-locations



Legend: PCR- panicle circumference; PH-plant height; PL- panicle length; SPGM- single-panicle grain mass; _SE- time to 50% stigma emergence as predictor

Figure 7.2: QTL LOD peaks for various traits using time to 50% stigma emergence as a predictor of log-transformed values from across-locations



Legend: PCR- panicle circumference; PL- panicle length; PT- productive tiller number; SPGM- single-panicle grain mass; PH- plant height; _PH- plant height as predictor; _SE- time to 50% stigma emergence as predictor; _SEPH- time to 50% stigma emergence and plant height as predictors

Figure 7.3: Comparison of QTL LOD peaks for various traits using different types of predictors of log-transformed values from across-locations

4.7. QTLs for downy mildew resistance

Data on total and diseased plant counts per plot were converted to disease incidence (%) and arc-sin of this number in radians. These values were used for mapping QTLs for downy mildew resistance. A total of five QTLs were obtained from Coimbatore, Bhavanisagar and across-locations. LOD peaks are shown in Figures 8.1-8.3.

Coimbatore

Two QTLs were mapped using downy mildew screening results from Coimbatore. Both mapped to LG 2 at different intervals. The QTL located between the marker loci *Xpsm708a* and *Xpsm706* had the maximum LOD score (4.77) and explained as much as 48.9% of the observed phenotypic variance. This was obtained from arc-sin transformed values. The P 1449-2 allele at this locus mean was associated with lowering of disease incidence by 2%. This QTL for disease resistance behaved largely as if it was dominantly inherited (Table 23).

Bhavanisagar

This location also had two QTLs but situated on different linkage groups, *i.e.* on LG 1 and on LG 4. The QTL at LG 4 had a higher LOD score (3.69) and explained a greater portion of the observed phenotypic variance (41.5). This QTL is mapped between marker loci *Xpsm464* and *Xpsm716*, and was inherited recessively. The P 1449-2 parental allele at this QTL had an additive effect of 2% mean disease incidence (Table 24).

Across-locations

Totally two downy mildew resistance QTLs were identified for across-locations data. One was similar to that of the QTL found both at Coimbatore and Bhavanisagar, which

was located in LG 2. Another QTL, a new one was mapped from across-locations data, which was not found in individual locations. This QTL was identified using multiple QTL model by fixing the previously mapped QTL, which located between the marker loci *Xpsm708a* to *Xpsm706*. The command "sequence [*Xpsm708a- Xpsm706*:additive] [all]" was used to get this new QTL. The program fixed first QTL at this location and identified the second QTL with the LOD value of 4.67, which was more than 2 to that of the fixed QTL (Table 25).

Table 23: QTL associated with downy mildew incidence of pearl millet mapping progeny F_5 self-bulks using disease resistance percentage and their arcsin-transformed values at Coimbatore.

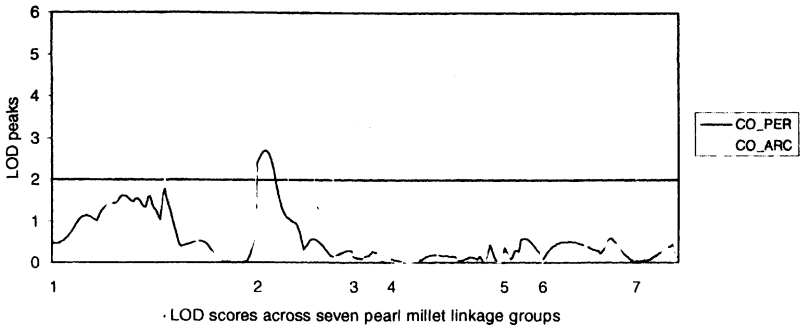
Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	4 x Dominant effects
Percentage	*Xpsm*708a-*Xpsm*706	2	8.0	2.7	40.9	-11.3820	-129.12
Arc-sin transformed	*Xpsm*708a-*Xpsm*706	2	10.0	4.8	48.9	-0.2857	-1.98
	*Xpsm*321-*Xpsm*708b	2	8.0	2.8	37.2	-0.4040	-1.74

Table 24: QTL associated with downy mildew incidence of pearl millet mapping progeny F_5 self-bulks using disease resistance percentage and their arcsin-transformed values at Bhavanisagar.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	4 x Dominant effects
Percentage	*Xpsm*341-*Xpsm*386	1	0.0	2.2	7.7	5.6150	-41.90
Arc-sin transformed	*Xpsm*341-*Xpsm*386	1	0.0	2.7	9.0	0.0837	-0.63
	*Xpsm*464-*Xpsm*716	4	16.0	3.7	41.5	0.2849	-1.80

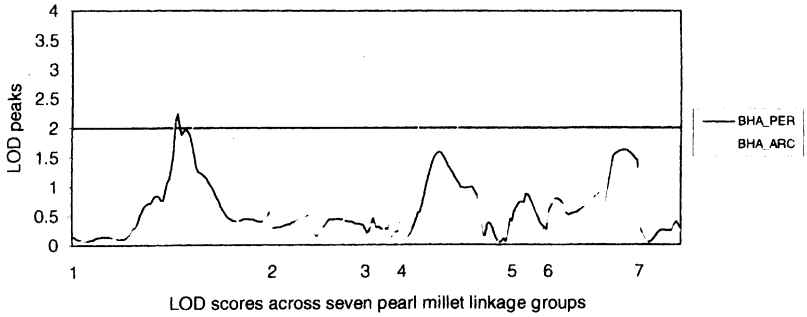
Table 25: QTL associated with downy mildew incidence of pearl millet mapping progeny F_5 self-bulks mean disease resistance percentage and their arcsin-transformed values at across-locations. Estimates of total LOD values and R^2 values in bold letters are those obtained using the combined model.

Trait	Marker interval	Linkage group	Position	LOD	R^2	Additive effects	4 x Dominant effects
Arc-sin transformed	*Xpsm*708a-*Xpsm*706	2	8.0	2.3	41.4	-0.1017	-0.99
	***Xpsm*618-*Xpsm*717**	**7**	**0.0**	**4.7**	**54.2**	**-0.0532**	**-0.25**



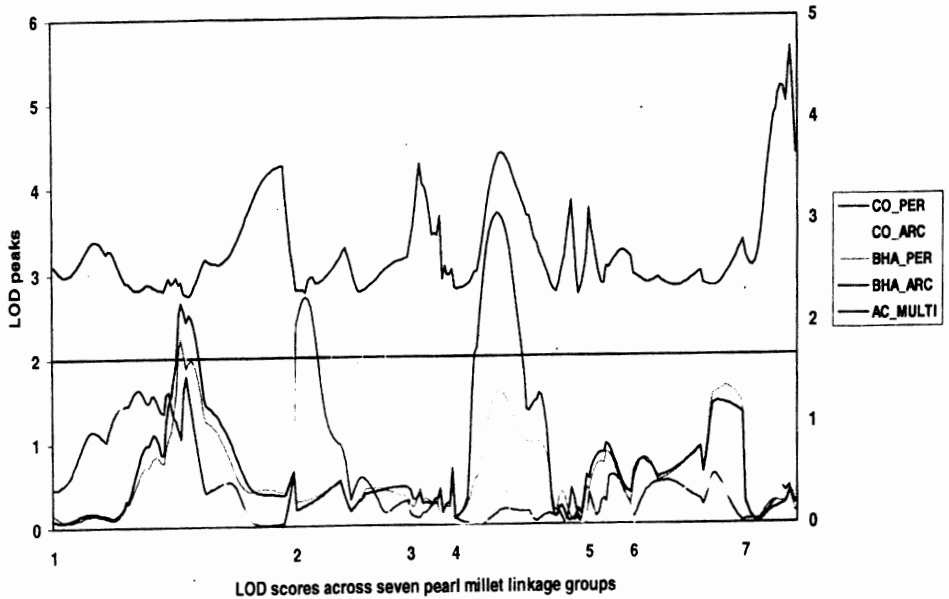
Legend: CO- Coimbatore location; _PER- percentage; _ARC- radians of arc-sin

Figure 8.1: QTL LOD peaks for downy mildew resistance from Coimbatore trial



Legend: BHA- Bhavanisagar location; _PER- percentage; _ARC- radians of arc-sin

Figure 8.2: QTL LOD peaks for downy mildew resistance from Bhavanisagar trial



Legend: CO- Coimbatore location; BHA- Bhavanisagar location; _AC- across locations; _PER- percentage; _ARC- radians of arc-sin; _MULTI- multiple QTL model for radians of arc-sin values

Figure 8.3: Comparison QTL LOD peaks for downy mildew resistance from Coimbatore, Bhavanisagar and across-locations

Discussion

5. DISCUSSION

Mean performance of the mapping population testcross hybrids

Mapping population testcross hybrids were raised at two locations in Tamil Nadu, namely Coimbatore and Bhavanisagar. The mean performance for all eleven observed traits was more or less similar at both locations. Analysis of variance study indicated high significant variation for the mean performances of individual entries for all the traits under study at both test locations, but that the interaction between these two locations and the individual entries (genotypes) was not significant for any of the traits. This may be due to the physical closeness of the places where trials were conducted and the similarity in sowing dates and agronomic practices used for the two trials. The first trial location Coimbatore, located at 11° latitude and 77° longitude and the second trial location Bhavanisagar, located at 11° 08' latitude and 77° 29' longitude. The soil types and packages of agronomic practices employed were similar, so that the environment may not have had much opportunity to differentially influence the mean performance of the mapping population testcrosses.

Trials were conducted at different locations to identify or elucidate the effect of the environment and assess the relative importance of genotype × environment interaction effects and genotype effects, because differential expression of a phenotypic trait by genotypes across environments, or genotypic × environment interaction is an old problem of primary importance for quantitative genetics and plant breeding trials (Eberhard and Russel, 1966; Falconer, 1981; Via and Lande, 1987; Tiret *et al.*, 1993).

Though the mean performances were similar for the two test locations, data from Bhavanisagar showed a slight increase in mean performance for the following traits: plant

height, panicle circumference, productive tiller number, grain yield per season, grain yield per day, single-panicle grain mass and single-panicle grain number. Other traits like time to 50% stigma emergence, panicle length and grain number per unit area registered very similar mean values at the two test locations.

Though the data from two location trials were not significantly different, the existing variation between two location trials may give different results on QTL mapping. With this precaution, QTL analysis was done for individual locations entry means as well as pooled means across-locations.

It is important to realize that heritability is a property not only of a character but also of the population in which this character was measured and of the environmental circumstances to which individuals are subjected prior to this measurement (Falconer, 1960). Also estimating heritability for a particular trait is the prime-most concern for even a simple selection scheme. This is applicable for QTL mapping also. The reliability of the QTL mapping depends very highly on the heritability of the individual traits (Kearsy and Farquhar, 1998).

Heritability (plot basis) studies from the individual location data sets revealed that all the traits registered heritability (plot basis) values greater than 50% excluding for single-panicle grain number and grain number per unit panicle surface area. At Coimbatore thousand-grain mass registered the highest heritability (plot basis) value of 93% followed by panicle circumference (75%). Other traits namely time to 50% stigma emergence, plant height, panicle length, productive tiller number, grain yield per season, grain yield per day and single-panicle grain mass showed moderate heritability values ranging from 40 to 70%. Single-panicle grain number and grain number per unit panicle

surface area were the two traits having poor heritability values. At Bhavanisagar plant height recorded the highest heritability (plot basis) value of 95% followed by thousand-grain mass, which had a heritability value of 93%. When compared to Coimbatore location, the heritability values for all the traits were higher in Bhavanisagar. Broad sense heritability values for pooled locations were higher for all the traits than plot basis heritability values obtained from individual locations data sets. Single-panicle grain number and grain number per unit panicle surface area too had higher broad-sense heritability values (>50%) from the pooled data sets.

Correlation studies

Correlation studies provide indications of the extent of linkage and pleiotropism of genes controlling the different traits. Grain yield per season was taken as the dependant variable and the other traits were correlated with this. Plant height, panicle circumference, thousand-grain mass, grain yield per day, single-panicle grain mass, single-panicle grain number and grain number per unit panicle surface area were the traits positively correlated with grain yield per season. Among these traits, grain yield per day registered the highest significantly positive correlation with grain yield per season at individual locations as well as with entry means from pooled analysis of data. This was followed by single-panicle grain mass, thousand-grain mass, panicle circumference and plant height which had similar values towards the contribution to grain yield per season. Improvement of grain yield per season may be achieved by increasing values of these positively correlated traits.

Time to 50% stigma emergence, productive tiller number and panicle length were the three traits associated negatively with grain yield per season. Among these traits time

to 50% stigma emergence had the strongest negative correlation with grain yield per season. Selection of early flowering lines may enrich the hybrid yield potential of pearl millet genotypes in this mapping population.

When time to 50% stigma emergence was taken as a dependent variable, productive tiller numbers and panicle length were associated positively with this trait. All other traits exhibited negative relationships with time to 50% stigma emergence. Plant height was also considered as a dependent variable and its relationship with other traits was assessed. Grain yield per day and panicle circumference had higher positive associations with plant height. Time to 50% stigma emergence, productive tiller numbers and panicle length were the three traits associated negatively with plant height.

From these correlation studies it can be concluded that time to 50% stigma emergence, productive tiller numbers and panicle length had strong associations with one another. This may be due to the linkage or pleiotropism among these traits. As they have negative associations with grain yield per season, it can be concluded that selection of early flowering types with shorter panicles and less number of panicles per plant will improve the total grain yield of hybrids produced by crossing PT 4450 with progeny from the cross of PT 732B and P 1449-2. Correspondingly, selection of tall genotypes having good panicle circumference, single-panicle grain mass and thousand-grain mass would likely directly improve the grain yield potential of hybrids on this mapping population.

Mean performance of downy mildew screening trials

Mean disease incidence values were converted into disease incidence percentages and radians of arc-sin transformed percentage values. The disease incidence percentage was

more severe at Bhavanisagar than at Coimbatore. Both locations registered significant differences for disease incidence percentages between mapping population progenies, as well as for radian values. Heritability (plot basis) values for these two measures of disease reaction were also high, giving confidence of success in mapping QTLs conferring resistance against downy mildew disease.

Mapping QTLs

Knowledge gained from QTL mapping experiments is of greatest interest to plant breeders if the results are directly applicable to practical breeding programs. Therefore, when such experiments are initiated, one of the most important questions is the choice of population for phenotyping experimental materials. For field trials, we used testcross progenies related to a commercially important hybrid, looking for opportunity to improve upon this combination, in a manner that comes closest to the applied plant breeder's situation. In applied breeding programs, the tester is often an elite inbred line chosen because of its use as a commercial hybrid parent. Therefore mapping of QTL for such testcrosses promises (i) an insight into the relative importance of additive effects with regard to testcross performance and their underlying genetic factors and (ii) the design of a more efficient breeding strategy (Schön *et al.*, 1994). So, this study formulated to identify opportunities for favourable contributions in terms of additive effects of the inbred P 1449-2 towards improvement the hybrid performance of seed parent PT 732 in combination with PT 4450.

Grain yield in cereals is generally controlled by a number of quantitative trait loci (QTLs) and is affected by environmental factors, making it difficult to manipulate and improve in plant breeding programs. Grain yield can be dissected into a number of

component traits such as thousand grain number, productive tiller number, panicle length, etc depending upon the crop concerned. These component traits are also under the control of QTL and the effects of individual QTLs on phenotypic variation are relatively small. Some QTLs however are less environmentally sensitive and have high heritabilities than grain yield itself (Bezant *et al.*, 1997). Further, the standard relationship between various yield component traits are not found for all QTLs, so it should be possible to identify specific QTLs that can be manipulated without adversely affecting otherwise correlated traits (Hash, 2000; Yadav *et al.*, 2002b). Therefore, while looking for QTLs controlling grain yield, QTLs for yield components should also be determined to provide more useful information.

QTL for time to 50% stigma emergence

From the MAPMAKER program, a single QTL was identified on linkage group (LG) 4 for time to 50% stigma emergence (Figure 9.1). This QTL had an additive effect of 0.5 days with the earlier flowering allele inherited from parent P 1449-2. So this parent contributed early flowering to the testcross hybrids with elite pollinator PT 4450. Using this genomic region it may be possible to transfer the early flowering allele from P1449-2 to PT 732A/B. Yadav *et al.*, (2002a) mapped two QTLS for time to 50% stigma emergence in pearl millet with one situated near the bottom of LG 4 and the other mapped to the bottom of LG 6.

QTL for plant height

A single QTL was mapped for mapping population testcross hybrids plant height. This QTL mapped to the bottom of LG 4 (Figure 9.1). A significant LOD score of 6.95 was

recorded from the Coimbatore data set. The allele from P 1449-2 had s positive additive effect of 6 cm for this trait, and increased the plant height of hybrids. This particular QTL explained 24% of the observed phenotypic variance for testcross hybrids plant height at Coimbatore.

This QTL is likely to be considered as d_2 dwarfing gene (Azhaguvel, 2001), for which mapping population parents PT 732B and P 1449-2 have the dwarf and tall alleles, respectively. Dwarf phenotypes can be considered as a consequence of mutations that occur in genes involved in plant height expression (Lin *et al.*, 1995). Dwarf mutants of pearl millet have been studied (Kadam *et al.*, 1940; Burton and Forston, 1966; Appa Rao *et al.*, 1986) and at least four single recessive genes have been reported *i.e.*, d_1 and d_2 (Burton and Forston, 1966) and d_3 and d_4 (Appa Rao *et al.*, 1986), with possible presence of additional modifying factors. The d_2 dwarfing allele may have a pleiotropic effect since d_2 near-isogenic lines have longer and narrower panicles, wider leaves and smaller seeds then their tall counterparts (Rai and Hanna, 1990).

The d_2 dwarfing gene mapped to the bottom of the LG 4 in the mapping population IP 18293 × Tift 238D1 (Azhaguvel, 2001). The d_2 dwarfing was inherited from the parent IP 18293. The parent Tift 238D1 had one more dwarfing gene, d_1, which mapped to LG 1. From the current study, it was clear that the d_2 dwarfing gene from PT 732B had a significant contribution to height reduction in the testcross hybrids with elite pollinator PT 4450, suggesting that PT 4450 and its hybrid with PT 732B, i.e., CoHCU-8 are infact also genetically dwarf at this locus.

QTL for panicle circumference

For panicle circumference, one QTL was identified in the middle of LG 4. This QTL was detected using different transformations and using Coimbatore, Bhavanisagar and across-locations data sets, after regressing out the effects of time to 50% stigma emergence, plant height and time to 50% stigma emergence together with plant height as predictors of panicle circumferences. The highest LOD peak was obtained from the Coimbatore data set using time to 50% stigma emergence as a predictor. At this LOD score, the QTL had an additive effect of 0.9, with increased panicle circumference inherited from parent P 1449-2. This QTL has a favourable effect on hybrid performance so it will be useful to introgress this genomic segment from P 1449-2 into PT 732B.

QTL for panicle length

Panicle length was also observed to be largely under the control of a single QTL and this QTL also mapped to LG 4. Nearly all the predictors allowed detection of this QTL in the interval between markers *Xpsm568* and *Xpsm512*. The P 1449-2 allele for this QTL decreased the panicle length of hybrids, in agreement with the observations of Rai and Hanna (1990) on the effect of the tall allele at the nearby d_2 dwarf gene locus on this character.

QTLs for productive tiller number

For productive tiller number, up to four QTLs were obtained, which were mapped to four different linkage groups (Figures 9.1 and 9.2). All four QTLs explained similar portions of phenotypic variation, but the QTL located on LG 2 explained a comparatively higher

portion (15 4%) For this QTL the allele from P 1449-2 reduced the number of productive tillers

QTLs for thousand-grain mass

Two linked QTLs on LG 4 were mapped for thousand-grain mass These two QTLs were detected using Bhavanisagar data and both types of transformation The QTL situated in the marker interval between *Ypsm568* and *Xpsm512* explained the higher proportion (11 6%) of observed phenotypic variation The additive effect of this QTL is 0 3 g, which is inherited from the tall parent P 1449-2 Usually dwarf plants, reduced the grain mass in the hybrids lead to reduction in yield Despite lower grain mass and grain yield in the dwarf plants, it is possible to produce dwarf hybrids with yields equal to the tall hybrids by selection of suitable pollinator Breeding programs on dwarf pearl millet should be successful if they are designed to take advantage of positive interactions between the dwarf habit and specific genetic background (Bidinger and Raju, 1990)

QTL for grain yield per season

For grain yield per season a single QTL was mapped near the bottom of LG 4 It explained 10% of observed phenotypic variance The favourable allele for this QTL was inherited from parent P 1449-2, which had the additive effect of 0 51 that is equal to a grain yield increment of 3 3 gm^2 At the plant population density used in this study (50,000 plants/ha) this corresponds to a yield advantage of 3,300 g/ha = 33 kg/ha Transfer of this genomic segment may be useful to improve the grain yield of the hybrid of PT 732A x PT 4450 = CoHCU-8, but would clearly be associated with an increase in

plant height due to the strong linkage of this QTL to the tall allele at the d_2 dwarfing gene locus.

QTLs for grain yield per day

Grain yield per day can be considered to be an important trait, where it explains the source and sink relationship after flowering. Up to three QTLs were found to be associated with grain yield per day in this study. These QTLs were distributed on LG 4 at different positions. Of these QTLs one was detected from the Coimbatore data set and the other two were detected from the Bhavanisagar data set. QTLs from Bhavanisagar using log transformation and time to 50% stigma emergence as a predictor, explained a higher portion of the observed phenotypic variances (10.7%). The corresponding additive effect for this locus was 0.52, *i.e.*, 0.3 g per day of grain yield with the favorable allele inherited from the parent P 1449-2.

QTLs for single-panicle grain mass

Four QTLs for single-panicle grain mass were obtained on LG 4 (2 QTLs), LG 6 (1 QTL) and LG 7 (1 QTL) (Figures 9.1 and 9.2). Data from Coimbatore detected all the three QTLs, where as across-locations data produced only two QTLs, *i.e.*, those mapping to LG 4 and LG 7. In all the cases, the QTLs for single-panicle grain mass co-mapped with QTLs for productive tiller number, with the parental alleles associated with increased single-panicle grain mass appearing to have negative pleiotropic effects on productive tiller number. This negative relationship between the two traits is commonly observed in pearl millet.

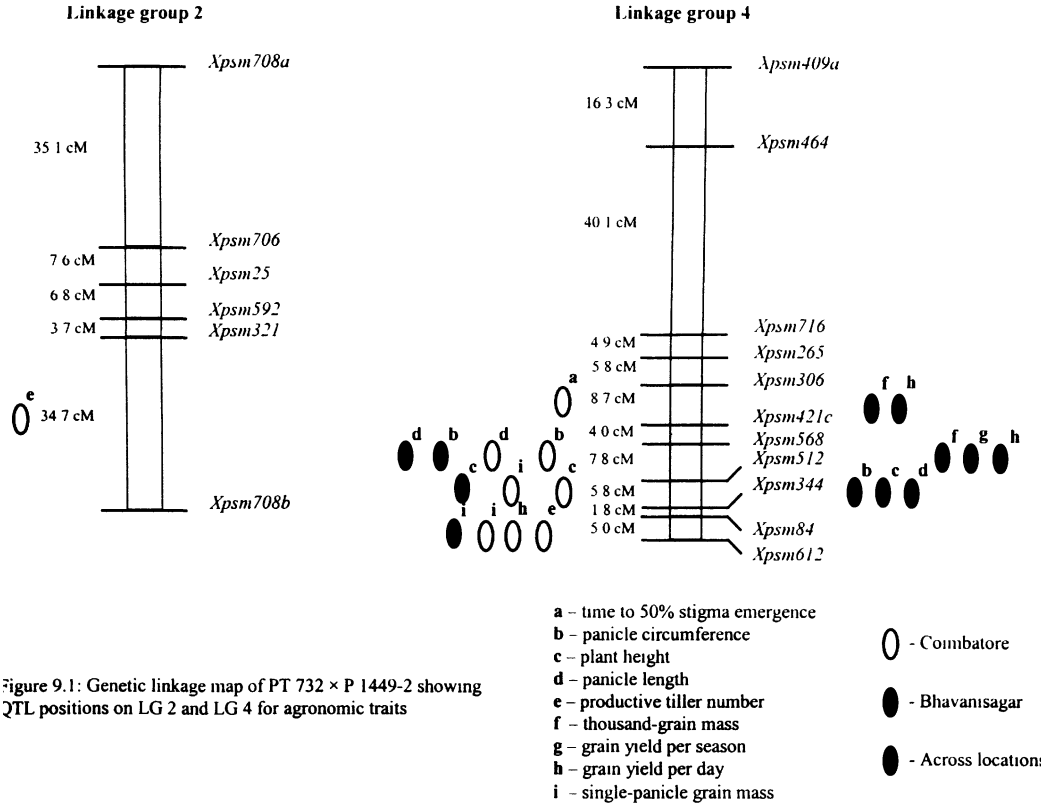


Figure 9.1: Genetic linkage map of PT 732 × P 1449-2 showing QTL positions on LG 2 and LG 4 for agronomic traits

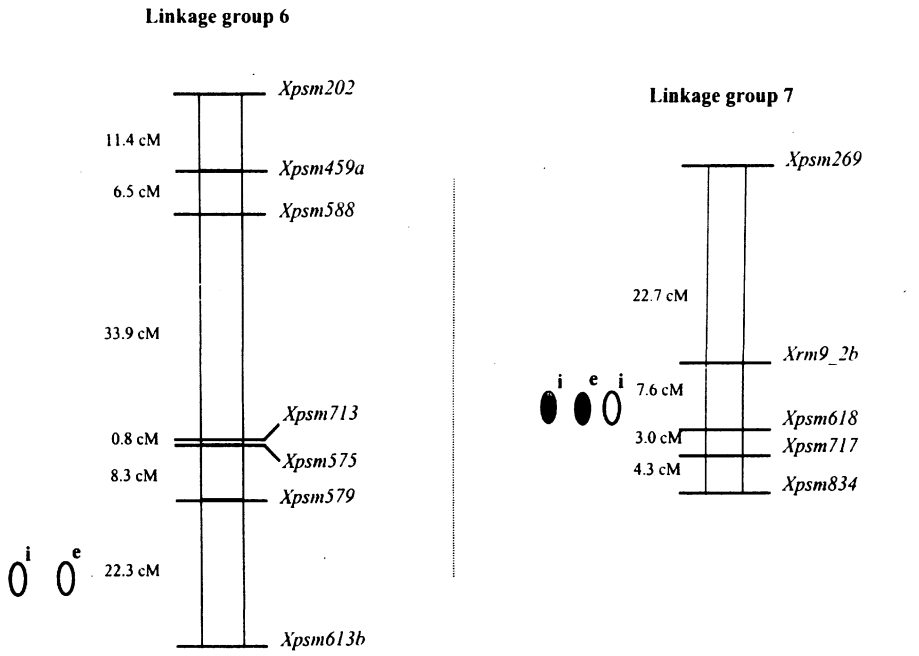


Figure 9.2: Genetic linkage map of PT 732 × P 1449-2 showing QTL positions on LG 6 and LG 7 for agronomic traits

QTLs for downy mildew resistance

Pearl millet downy mildew has historically been considered to be a quantitative trait, significantly affected by the environment (which is often confounded with pathogenic variability differences) Host plant resistance against downy mildew was continuously distributed in the F_2 F_3 progenies used in this study as has been found in most previous studies on the genetics of pearl millet downy mildew resistance (Singh *et al*, 1980, Basavaraju *et al*, 1981, Dass *et al*, 1984, Shinde *et al*, 1984, Jones *et al*, 1995)

At least five different QTLs were mapped for downy mildew resistance on four linkage groups using disease incidence percentage and radian values (Figures 10 1 and 10 2) Of these, two QTLs were identified from Coimbatore data mapped to LG 2, two QTLs from Bhavanisagar data mapped to LG 1, and LG 4 and one QTL from across-locations data mapped to LG 7

QTLs from the Coimbatore data and across-locations means were inherited in an additive fashion Alleles from P 1449-2 contributed this resistance However, the two QTLs detected from Bhavanisagar were inherited recessively and parent PT 732 was the contributor of this resistance The majority of previous research on the genetics of downy mildew resistance in pearl millet has found dominance to be an important component of resistance (Appadurai *et al*, 1975, Gill *et al*, 1978, Pethani *et al*, 1980, Basavaraju *et al*, 1981, Shinde *et al*, 1984, Mehta and Dang, 1987) and over dominance has also been detected (Singh *et al*, 1978, Basavaraju *et al*, 1981, Dass *et al*, 1984) However, the inheritance of downy mildew resistance in pearl millet is at least occasionally found to be recessive (Singh *et al*, 1978) and recessive resistance genes, although uncommon, have been found in other plant-pathogen systems (Day, 1974, De Wit, 1992)

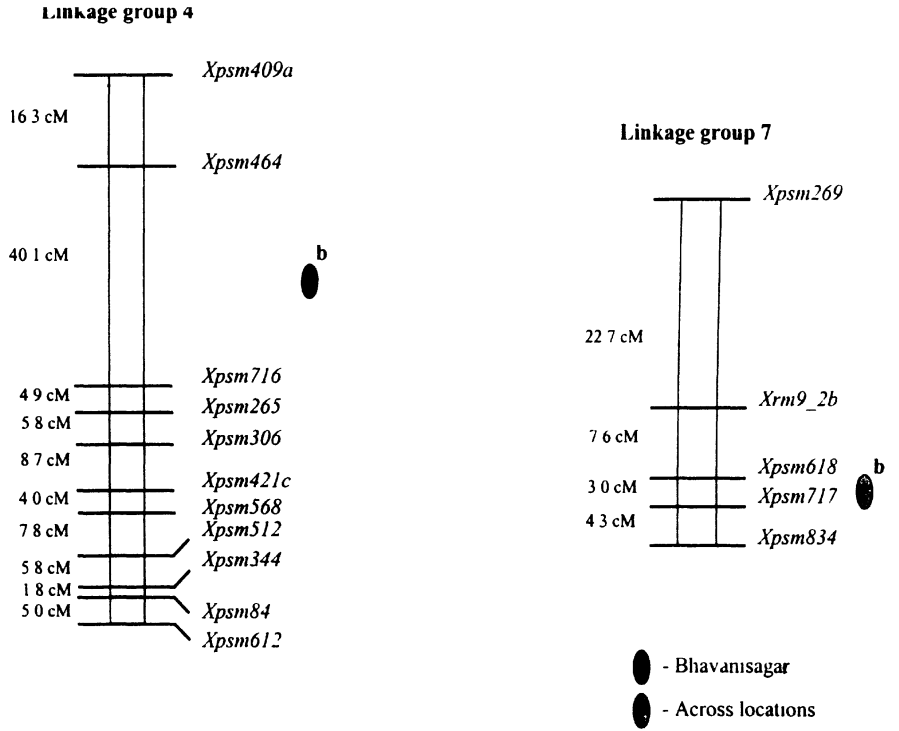


Figure 10.2: Genetic linkage map of PT 732 × P 1449-2 showing QTLs positions on LG 4 and LG 7 for downy mildew resistance

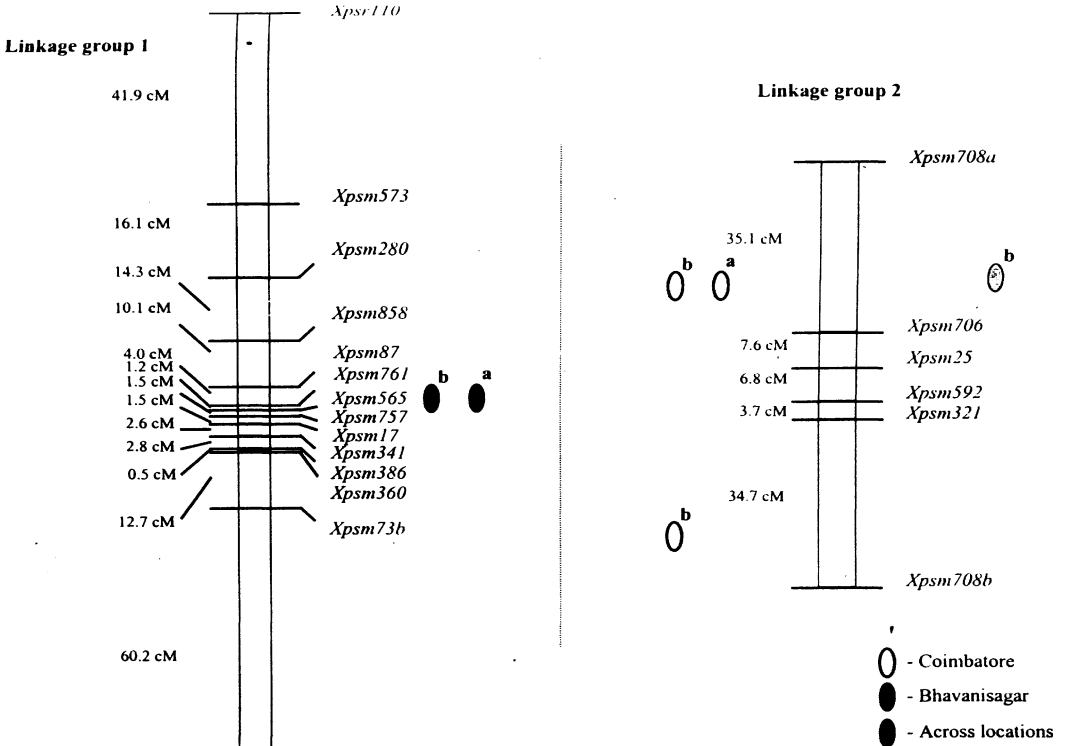


Figure 10.1: Genetic linkage map of PT 732 × P 1449-2 showing QTLs positions on LG 1 and LG 2 for downy mildew resistance

Unfortunately, while useful in hybrid seed production plots, such recessively inherited resistance is unlikely to contribute positively to hybrid performance in farmers field

General discussion

A total of 18 QTLs were obtained from this study, using square root transformation and log transformation of agronomic data sets from Coimbatore, Bhavanisagar and across-locations Between these two transformations, the square root transformation gave 24 QTLs and log transformation gave 23 QTLs (Table 26) Out of three predictors (*i e*, time to 50% stigma emergence, plant height and time to 50% stigma emergence together with plant height), time to 50% stigma emergence produced 34 QTLs followed by plant height which revealed 21 QTLs and time to 50% stigma emergence together with plant height mapped 6 QTLs Out of these 18 QTLs, only seven genomic intervals were responsible for all the QTLs controlling agronomic traits (*i e*, some of the genomic regions were responsible for controlling more than one trait) Out of these seven genomic regions, LG 4 had four and LG 2, LG 6 and LG 7 each had one genomic region contributing to the detected QTLs

In LG 4, the interval flanked by marker loci *Xpsm568* and *Xpsm512* had the control over five traits, including grain yield per season The other traits controlled by this genomic region were panicle circumference, panicle length, thousand-grain mass and grain yield per day Marker interval *Xpsm84* to *Xpsm612*, which is also on LG 4 controlled three traits, *i e*, productive tiller number, grain yield per day and single-panicle grain mass It seems highly likely that genes or gene blocks in these two regions may have pleiotropic effects on these traits So transferring these particular

Table 26 LOD scores and percentage of observed phenotypic variance explained by best QTL models for different types of data transformations and traits used for regression for different locations data sets

Trait	Location	Type of transformation used	Traits used for regression	LOD score	R^2
Days to 50% stigma emergence	Coimbatore	Log	Plant height	2 1	7 8
Panicle circumference	Coimbatore	Square root	Plant height	2 48	10 5
	Coimbatore	Square root	Time to 50% stigma emergence	7 38	26 4
	Coimbatore	Log	Plant height	2 46	10 1
	Coimbatore	Log	Time to 50% stigma emergence	7 46	26 6
	Bhavanisagar	Square root	Plant height	3 94	15 2
	Bhavanisagar	Square root	Time to 50% stigma emergence	5 94	21 6
	Bhavanisagar	Log	Plant height	3 94	15 1
	Bhavanisagar	Log	Time to 50% stigma emergence	5 89	21 4
	Bhavanisagar	Log	Plant height and time to 50% stigma emergence	2 90	11 3
	Across-locations	Square root	Plant height	2 46	10 5
	Across-locations	Square root	Time to 50% stigma emergence	5 97	21 0
	Across-locations	Log	Plant height	2 45	10 4
	Across-locations	Log	Time to 50% stigma emergence	5 98	21 0
Panicle length	Coimbatore	Square root	Plant height	2 00	8 3
	Coimbatore	Square root	Time to 50% stigma emergence	6 47	22 5
	Coimbatore	Log	Plant height	2 14	8 4
	Coimbatore	Log	Time to 50% stigma emergence	6 52	22 7
	Bhavanisagar	Square root	Plant height	4 49	15 7
	Bhavanisagar	Square root	Time to 50% stigma emergence	5 96	20 3
	Bhavanisagar	Square root	Plant height and time to 50% stigma emergence	3 33	11 9
	Bhavanisagar	Log	Plant height	4 50	15 8
	Bhavanisagar	Log	Time to 50% stigma emergence	5 91	20 1
	Bhavanisagar	Log	Plant height and time to 50% stigma emergence	3 36	12 0
	Across-locations	Square root	Plant height	2 51	9 4
	Across-locations	Square root	Time to 50% stigma emergence	6 22	22 1
	Across-locations	Log	Plant height	2 50	9 4
	Across-locations	Log	Time to 50% stigma emergence	6 14	21 4

Contd.

Trait	Location	Type of transformation used	Function used for regression	LOD score	R^2
Plant height	Coimbatore	Square root	Time to 50% stigma emergence	6.95	23.9
	Coimbatore	Log	Time to 50% stigma emergence	6.91	23.7
	Bhavanisagar	Square root	Time to 50% stigma emergence	2.84	10.0
	Bhavanisagar	Log	Time to 50% stigma emergence	2.83	9.9
	Across-locations	Square root	Time to 50% stigma emergence	6.48	22.5
	Across-locations	Log	Time to 50% stigma emergence	6.38	22.7
Productive tillers	Coimbatore	Square root	Time to 50% stigma emergence	2.56	9.0
	Coimbatore	Square root	Time to 50% stigma emergence	2.87	13.6
	Coimbatore	Square root	Plant height and time to 50% stigma emergence	2.19	14.8
	Coimbatore	Log	Time to 50% stigma emergence	2.62	9.2
	Coimbatore	Log	Time to 50% stigma emergence	2.92	13.8
	Coimbatore	Log	Plant height and time to 50% stigma emergence	2.19	15.4
	Across-locations	Square root	Plant height	2.45	9.2
	Across-locations	Log	Plant height	2.4	9.0
Grain yield	Bhavanisagar	Square root	Time to 50% stigma emergence	2.60	10.3
	Bhavanisagar	Log	Time to 50% stigma emergence	2.68	10.6
Grain yield per day	Coimbatore	Log	Time to 50% stigma emergence	2.0	7.1
	Bhavanisagar	Square root	Time to 50% stigma emergence	2.65	10.5
	Bhavanisagar	Log	Plant height	2.0	7.6
	Bhavanisagar	Log	Time to 50% stigma emergence	2.71	10.7
Thousand grain number	Bhavanisagar	Square root	Plant height	2.0	7.4
	Bhavanisagar	Square root	Time to 50% stigma emergence	3.0	11.6
	Bhavanisagar	Log	Plant height	2.0	7.3

Contd.

Trait	Location	Type of transformation used	Function used for regression	LOD scores	R^2
Single panicle grain mass	Coimbatore	Square root	Plant height	2.41	9.0
	Coimbatore	Square root	Time to 50% stigma emergence	3.58	12.4
	Coimbatore	Square root	Time to 50% stigma emergence	2.73	12.3
	Coimbatore	Log	Plant height	2.46	9.1
	Coimbatore	Log	Time to 50% stigma emergence	3.58	12.4
	Coimbatore	Log	Time to 50% stigma emergence	2.76	12.9
	Across-locations	Log	Time to 50% stigma emergence	2.19	15.4
	Across-locations	Square root	Plant height	3.06	11.2
	Across-locations	Square root	Time to 50% stigma emergence	2.78	9.9
	Across-locations	Log	Plant height	3.17	11.6
	Across-locations	Log	Time to 50% stigma emergence	2.75	9.8
	Across-locations	Log	Plant height and time to 50% stigma emergence	2.1	8.3

regions, which are controlling major traits, to the parent PT 732 may be advantageous and reasonable since in most cases P 1449-2 was found to contribute the favorable allele

But from the correlation studies it was found in the material studied that panicle length and productive tiller numbers were associated with each other and both are having negative relationship with yield So refinement of these genomic regions may provide more information about individual traits, which may be controlled by different QTLs Of course, refinement of the map positions of QTLs controlling these traits will require genotyping and phenotyping a substantially larger mapping population So further analysis of the existing data sets may be required to justify the substantial costs that this refinement would require

Although different QTLs were obtained from the two different test locations, it is better to restrict discussion of application to the QTLs from the across-locations due to statistical constraints Across-locations data set produced only six QTLs, which mapped on LG 4 and LG 7 and only four genomic regions [three on LG 4 (*Xpsm568-Xpsm512*, *Xpsm84-Xpsm612* and *Xpsm512-Xpsm344*) and one on LG 7 (*Xrm9-2b-Xpsm618*)] were responsible With respect to traits, plant height, panicle circumference, panicle length, productive tiller number and single-panicle grain mass were the traits for which QTLs could be mapped from analysis of the across-locations entry means

Traits such as plant height, panicle circumference and single-panicle grain mass were positively correlated with grain yield and grain yield per season and these regions were controlled by three different regions (two controlling single-panicle grain mass and the other controlling plant height, panicle length, and panicle circumference) So

transferring these genomic regions may offer the chance to improve grain yield performance of hybrids of PT 732B.

Opportunities for Marker-Assisted Selection (MAS) to improve CoHCU-8

The advent of molecular-marker based techniques has had a large impact on quantitative genetics. Marker-based methods applied to segregating populations have provided us with a means to locate quantitative trait loci (QTLs) to chromosomal regions and to estimate the effects of QTL allele substitution (Lander and Botstein, 1989). The ability to estimate gene effects for a quantitative trait can be very useful for the design and application of new, more efficient, breeding strategies. A new selection strategy, marker-assisted selection (MAS), has been proposed by many authors as a way to increase gains from selection for quantitative traits (Tanksley, 1993; Lee, 1995; Kearsey and Pooni, 1996). In backcross breeding programs, it has been shown that MAS can be effective in reducing linkage drag and optimizing population sizes, by permitting effective selection against the donor genome except for allele(s) in the genomic region to be introduced from the donor. MAS can also improve selection for quantitative traits by selecting for the presence of specific marker alleles that are linked to favorable QTL alleles that would be otherwise difficult to select for phenotypically (Berloo and Stam, 1998). Published reports of successful application of this strategy to improve hybrid yield performance are just beginning to appear.

From this study, it is clear that different regions of pearl millet genome are specifically associated with grain yield component traits such as plant height, panicle circumference and single panicle-grain mass when across-locations data was considered. These traits were positively correlated with grain yield and grain yield per season. These

three genomic regions also explained significant portions of observed phenotypic variance for their respective traits and these traits all had high heritability values. Soller and Beckmann (1990) stated that MAS for quantitative traits with high h^2 would not necessarily be as efficient as conventional breeding. However, for a quantitative trait with high h^2, MAS could still be effective after major QTL controlling the trait are fixed and the h^2 of remaining genetic variation is reduced (Paterson *et al.*, 1991).

The parent PT 732A serves as the female parent for producing commercial hybrids (hybrids X6 and CoHCU-8) that have been released from Tamil Nadu Agricultural University. So improving the yield potential of PT 732B (maintainer of PT 732A) may usher in new ways for increasing the grain yield of hybrids that can be produced for Tamil Nadu using this seed parent. The QTLs identified from this study can be used in a marker-assisted backcrossing program for hybrid parental line improvement because their effects have already assessed in testcrosses to the best available hybrid produced from crosses onto PT 732A. So marker-assisted backcrossing of P 1449-2 alleles for putative QTLs on LG 4 and LG 7 into the PT 732B background may be effective to improve the yield potential of hybrids produced on PT 732A. Yadav *et al.* (2002a) suggested a similar strategy in pearl millet to transfer the drought tolerance QTLs into elite pollinator inbred H 77/833-2 (male parent of early-flowering released hybrid HHB 67) using marker-assisted backcrossing to introgress genomic segments from donor PRLT 2/89-33.

To obtain durable resistance for downy mildew there are two ways. One is pyramiding genes from all known sources (Jones *et al.*, 1995) and the second possibility is the production of hybrids that are genetically heterogeneous for disease resistance, thus mimicking the durable resistance of open-pollinated cultivars (Witcombe and Hash,

2000) This could be accomplished by producing a set of backcross lines, each differing for a single resistance gene, and allowing these lines to recombine during multiplication of the male sterile line breeder seed in the hybrid seed production chain Incorporating more than one dominant gene effective against pathogen population into each component line may be expected to increase resistance durability

Hash *et al* (2000) discussed an alternative procedure of marker-assisted transfer of QTLs in pearl millet The first successful application of marker-assisted selection for pearl millet has been enhancement of downy mildew resistance of inbred pollinator H 77/833-2 (male parent of popular early-maturing pearl millet hybrid HHB 67) Several improved versions of this pollinator have been developed at ICRISAT using this "fast track" marker-assisted backcross procedure (Sharma, 2001) This has been demonstrated to be a time and cost efficient route for the application of marker-based downy mildew resistance breeding in this crop Such approaches may be warranted to improve the disease resistance of elite hybrid parental line PT 732B and its male-sterile counterpart PT 732A

We have identified the precise location of QTLs by ordinary linkage mapping, which has become a standard starting point for map-based cloning (Tanksley *et al*, 1995) In plants, several economically important genes have been isolated by map-based cloning, including a photoperiod-sensitive gene (flowering gene) in *Arabidopsis* (Puttemill *et al*, 1995) However, it would be reasonable now to confirm these QTL locations using CIM (Composite-interval mapping) methods as implemented in the QTL Cartographer and PLABQTL software packages

Most geneticists and breeders consider QTLs to be chromosomal locations of individual genes or groups of genes that influence complex traits (Stuber *et al.*, 1999). Although it is often tacitly assumed that a QTL represents a single genetic determinant (or factor), there are examples of individual QTLs that have been resolved into multiple genetic factors by recombination (Graham *et al.*, 1997; Yamamoto *et al.*, 1998). For the manipulation of the vast majority of QTLs in plant breeding programs, it may not be important to determine whether the QTL represents a single genetic factor or a cluster of tightly linked genes. However, if cloning of specific QTLs is paramount to their utilization, then the chromosomal location must be limited to a manageable piece of DNA (Paterson, 1998).

Recent advances in molecular genetics have promised to revolutionize agricultural practices. As stated by Lande and Thompson (1990) "There are, however, several reasons why molecular genetics can never replace traditional methods of agricultural improvement, but instead should be integrated with conventional methods to obtain the maximum improvement in the economic value of domesticated populations." Their analytical results, as well as the more recent computer simulations and the limited empirical results, however, are encouraging and support the use of DNA-based markers to achieve substantial increases in the efficiency of artificial selection.

Summary

6. SUMMARY

Pearl millet [*Pennisetum gluacum* (L.) R. Br.] is an important staple food crop for millions of rural people living in semi-arid regions of tropical and sub-tropical Asia and Africa. In parts of USA, South America and Southern Africa it is cultivated for feed and forage purposes. Pearl millet is a crop that can be grown in adverse agro-climatic conditions like drought, heat and infertile soil. It is the only crop that gives assured harvest to the farmers whose subsistence is totally dependant on farming in hot, dry marginal environments. Among the diseases affecting pearl millet, downy mildew is the most devastating. This is caused by the pseudo fungal pathogen *Sclerospora graminicola* (Sacc.) J. Schröt..

Improvement of yield and breeding for resistance to pests and diseases are the prime concerns of the breeders. This study was designed to identify genomic regions from donor P 1449-2 with the potential contribute to yield increments in the genetic background of released hybrid CoHCU-8 and also for downy mildew disease resistance.

One hundred and thirty-six F_2-derived F_4 mapping population progenies of a pearl millet mapping population (skeleton-mapped F_2 individuals) obtained from a cross of PT 732B and P 1449-2 were used as a source population for this study. PT 4450 is an elite pollinator line that produces an agronomically superior released hybrid (CoHCU-8) when crossed to PT 732A. It was used as a pollen source for crossing with these F_4 self-bulks. Testcross hybrids produced from these crosses were raised for the purpose of phenotyping during the rainy season (October, 2001) at two locations in Tamil Nadu: Tamil Nadu Agricultural University, Coimbatore and Regional Research Station,

Bhavanisagar. For downy mildew screening, selfed seeds from the F_4 self-bulks were raised at the two above-mentioned locations during the rainy season of 2001.

Results from the yield trials showed that there was significant variation for all observed traits within the set of mapping population testcrosses at each location, and there was no significant genotype × environment interaction for any of the 11 agronomic traits considered in this study. Heritability estimates for individual traits from the yield trials at two different locations and pooled data across these two locations had reasonably high values (>50%), which were sufficient to permit QTL mapping procedures to identify genomic regions contributing to the observed variability. Grain yield per season was positively correlated with most of the observed traits including plant height. But time to 50% stigma emergence, productive tiller number and panicle length were associated negatively with grain yield per season.

From the downy mildew screening trials, the data set from the two locations each exhibited significant genetic differences, but there was also significant genotype × environment interaction indicating that the virulences constitutions of the pathogen populations at these two locations were different. The heritability (plot basis) values were also high enough to do the QTL analysis.

Yield trial data from the two locations were subjected to two types of transformations namely, square root and log, so as to minimize the heterogeneity in the data sets. Improvement of yield is a complex process. To minimize this complexity and facilitate identification of QTLs that did not directly correspond to major genes affecting plant height and flowering time (which are relatively simply inherited traits known to grain yield and its components), plant height, time to 50% stigma emergence and plant

height together with time to 50% stigma emergence were used as predictors *i e*, all the agronomic traits were regressed with these predictors, and the residuals from these regressions for each agronomic trait were subjected to QTL mapping

A previously constructed linkage map using 60 RFLP markers for the [(PT 732B × P 1449-2)- based] mapping population were used for locating QTLs QTL analysis with the MAPMAKER/QTL program showed different QTL position for different traits In total, 18 QTLs were obtained for nine different traits from the Coimbatore, Bhavanisagar and across-locations data sets Among these nine traits, time to 50% stigma emergence, panicle circumference, plant height, panicle length and grain yield per season each registered one QTL, thousand-grain mass registered two QTLs, grain yield per day registered three QTLs and single-panicle grain mass registered four QTLs However, these 18 QTLs were under the control of only seven genomic regions, suggesting roles of tight linkage and/or pleiotropism in the inheritance of these often correlated traits Of these seven genomic regions, LG 4 had four regions, LG 2, LG 6 and LG 7 each had one genomic region contributing QTLs In LG 4 the region flanked by marker loci *Xpsm568* and *Xpsm512* contributed to control over five traits including grain yield per season

Across-locations data produced six QTLs for agronomic traits studied They were on LG 4 and LG 7 Totally four genomic regions *viz*, three on LG 4 and one on LG 7 shared these six QTLs The traits controlled by these QTLs included plant height, panicle circumference, panicle length, productive tiller number and single-panicle grain mass

For downy mildew, five different QTLs were mapped on four linkage groups by using disease incidence percentages and their arc-sin transferred radians values Of these,

two QTLs were detected from the Coimbatore data set on LG 2, two QTLs from the Bhavanisagar data set on LG 1 and LG 4, and one QTL from across-locations on LG 7

Marker-assisted selection provides an opportunity to improve the effectiveness of quantitative traits by selecting for the presence of specific marker alleles that are linked to favorable QTL alleles From this study, if we considered only the across-locations data set, different regions of pearl millet genome were detected as specifically associated with grain yield per season, plant height, panicle circumference and single-panicle grain mass QTLs for these traits also explained significant portions of observed phenotypic variation So marker-assisted backcrossing from P 1449-2 to move putative QTLs on LG 4 and LG 7 into the PT 732A/B background may be effective to improve the yield potential of hybrids of elite seed parent PT 732, at least those hybrids produced with elite pollinator PT 4450

For improving the resistance against downy mildew marker-assisted transfer and\or pyramiding of the resistance genes (or QTLs) may give good results

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Appendix

Appendix 1. Genotypic data from the RFLP- based autorads for the F_2 segregating population developed from PT 732B × P1449-2

Linkage group	No	Probe	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
LG1	1	*Xpsr110*	A	B	B	B	-	H	B	H	D	B	H	A	A	A	H	H	H	H	C	B	B	A
	2	*Xpsm573*	D	B	B	D	-	D	D	D	D	B	D	D	-	-	D	B	D	D	B	B	D	D
	3	*Xpsm280*	A	B	B	H	-	H	H	H	H	H	H	A	B	A	H	B	H	H	H	H	H	H
	4	*Xpsm858*	A	B	B	H	-	H	D	C	H	H	H	A	B	H	B	B	H	H	H	H	H	H
	5	*Xpsm87*	H	H	B	H	-	H	D	H	A	H	H	A	B	H	H	B	H	H	H	H	H	H
	6	*Xpsm761*	H	H	B	H	-	H	H	H	A	H	H	A	B	H	B	B	H	H	H	H	H	H
	7	*Xpsm565*	H	B	B	H	-	H	H	C	A	H	H	A	B	H	C	B	H	H	H	H	H	C
	8	*Xpsm757*	H	B	B	H	-	H	H	-	A	D	H	A	B	H	-	B	H	H	D	D	H	D
	9	*Xpsm17*	H	B	B	H	-	H	H	H	A	H	H	A	B	H	B	B	H	H	H	H	H	B
	10	*Xpsm341*	H	B	B	H	-	H	H	H	A	H	H	A	C	H	B	H	H	H	H	H	H	B
	11	*Xpsm386*	H	B	B	H	-	H	H	H	H	D	H	A	C	C	B	C	C	C	-	-	-	B
	12	*Xpsm360*	H	B	B	H	-	H	H	H	H	H	H	A	B	H	C	H	H	H	H	H	H	B
	13	*Xpsm73b*	D	B	B	D	-	D	D	D	D	D	D	D	B	D	B	D	D	D	D	D	D	B
	14	*Xpsm347a*	B	A	B	H	-	B	H	H	D	H	B	A	H	B	B	H	H	H	A	B	H	H
	15	*Xpsm196a*	B	A	H	H	-	B	H	H	A	H	H	A	H	B	A	H	H	H	A	A	H	H
LG2	16	*Xpsm708a*	D	D	B	B	-	D	D	B	D	D	B	B	D	D	D	D	D	D	D	D	D	B
	17	*Xpsm706*	C	H	B	B	-	C	H	B	H	A	B	B	C	C	H	A	B	B	A	H	H	H
	18	*Xpsm25*	H	H	H	B	-	H	H	B	H	A	B	B	H	H	H	H	B	B	A	H	H	H
	19	*Xpsm592*	D	D	D	D	-	D	D	B	B	A	B	-	D	D	D	D	B	B	A	-	-	D
	20	*Xpsm321*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	21	*Xpsm708b*	A	H	H	H	-	H	H	H	B	H	B	H	H	H	H	H	H	B	B	H	H	H
LG3	22	*Xpsm37*	H	A	H	H	-	H	H	H	H	B	A	H	B	H	A	B	B	H	A	B	B	H
	23	*Xpsm108*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24	*Xpsm174*	H	D	H	H	-	H	H	H	H	B	A	C	B	H	D	B	B	H	A	H	B	D
	25	*Xpsm96*	H	A	H	H	-	H	H	-	H	B	A	B	B	H	A	B	H	H	A	A	B	A
	26	*Xpsm18*	H	A	H	H	-	H	H	H	H	B	A	C	B	H	H	B	H	H	A	H	B	A
	27	*Xpsm678*	H	A	H	H	-	H	H	H	H	B	A	B	B	H	H	B	H	H	A	H	B	A
	28	*Xpsm248*	H	A	H	H	-	H	H	H	H	B	H	B	B	H	H	B	H	H	A	C	B	A
	29	*Xpsm473*	H	A	H	H	-	H	H	H	H	B	A	B	B	H	H	B	H	H	A	H	B	A
	30	*Xpsm686*	H	A	H	H	-	H	H	H	H	B	H	B	B	H	H	H	H	H	A	H	B	A
	31	*Xpsm410*	H	A	H	H	-	H	H	-	H	B	H	D	B	H	C	H	H	H	A	H	B	A

Contd...

Linkage group	No	Probe	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
LG4	32	*Xpsm409a*	H	C	H	A	-	A	H	H	B	B	H	H	C	H	H	H	A	A	H	H	A	A
	33	*Xpsm464*	C	C	C	C	-	A	C	C	C	C	C	A	C	C	C	C	C	A	C	-	A	A
	34	*Xpsm716*	A	B	B	H	-	A	H	A	H	H	A	H	H	H	A	A	H	A	A	A	A	A
	35	*Xpsm265*	A	C	C	C	-	A	C	A	C	C	A	C	C	C	C	A	C	A	A	A	A	A
	36	*Xpsm306*	A	C	C	C	-	A	C	A	C	C	A	-	C	C	C	A	C	A	A	A	A	A
	37	*Xpsm421c*	A	H	H	H	-	A	H	A	H	H	H	B	H	H	H	A	H	D	B	D	C	A
	38	*Xpsm568*	A	C	C	C	-	A	C	A	C	C	C	C	C	C	C	A	C	A	A	A	C	A
	39	*Xpsm512*	A	H	-	B	-	A	B	A	B	A	C	C	C	-	C	A	C	A	A	A	C	-
	40	*Xpsm344*	A	H	H	B	-	A	H	A	H	A	H	H	H	H	H	A	H	A	A	A	B	A
	41	*Xpsm84*	A	H	D	B	-	A	H	A	H	A	H	H	H	H	H	A	H	A	A	A	B	A
	42	*Xpsm612*	A	H	H	B	-	A	H	A	H	A	H	H	H	H	H	A	H	A	A	A	B	A
LG5	43	*Xpsm815*	H	H	A	H	-	H	A	H	H	H	H	D	A	A	H	H	H	H	H	H	H	H
	44	*Xpsm318*	H	H	A	H	-	H	A	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H
	45	*Xpsm73a*	H	H	A	H	-	A	A	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H
	46	*Xrm11_1*	H	H	A	H	-	A	A	-	H	H	H	H	A	A	H	H	H	H	H	H	H	H
	47	*Xpsm749*	H	H	A	H	-	A	A	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H
	48	*Xpsm735a*	B	A	A	H	-	A	H	H	H	H	H	A	A	A	H	H	H	B	H	H	H	B
LG6	49	*Xpsm202*	B	A	H	A	-	H	B	A	A	B	A	H	A	H	A	H	H	A	B	A	H	H
	50	*Xpsm459a*	B	A	H	A	-	H	B	A	A	B	A	H	A	A	A	H	H	A	B	H	H	H
	51	*Xpsm588*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	52	*Xpsm713*	B	A	H	H	-	H	H	A	H	H	A	B	A	A	H	B	B	A	H	H	H	B
	53	*Xpsm575*	H	A	H	H	-	H	H	A	H	H	A	B	A	A	H	B	B	A	H	H	H	B
	54	*Xpsm579*	H	A	H	H	-	H	H	A	H	H	A	B	-	A	H	B	B	A	C	C	C	C
	55	*Xpsm613b*	H	A	H	H	-	H	H	A	H	H	A	B	A	A	H	B	B	A'	H	A	-	B
LG7	56	*Xpsm269*	A	H	H	H	-	A	H	H	B	B	A	B	B	A	B	H	A	H	A	-	A	A
	57	*Xrm9-2b*	D	B	D	D	-	D	D	D	B	D	D	B	B	D	D	B	D	B	D	-	D	D
	58	*Xpsm618*	A	B	A	H	-	A	H	H	B	B	H	B	B	A	H	B	A	B	A	H	A	A
	59	*Xpsm717*	C	C	A	H	-	A	H	H	B	H	H	B	B	A	H	B	A	B	A	H	A	A
	60	*Xpsm834*	H	B	A	H	-	A	H	H	B	H	H	B	B	H	H	B	A	B	A	B	A	A

Contd...

Linkage group	No	Probe	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
LG1	1	*Xpsr110*	A	A	B	H	A	B	H	A	A	B	H	H	B	H	-	H	A	B	H	B	B
	2	*Xpsm573*	D	D	B	B	D	D	D	D	D	B	D	D	B	D	D	B	D	B	B	B	D
	3	*Xpsm280*	H	A	B	B	H	H	H	A	A	B	A	H	B	A	A	H	H	B	B	B	H
	4	*Xpsm858*	H	A	H	B	B	H	H	A	B	B	A	H	B	A	A	H	H	B	B	B	A
	5	*Xpsm87*	H	A	H	B	B	H	H	H	B	B	A	A	B	A	A	H	H	B	B	B	A
	6	*Xpsm761*	H	A	H	B	B	H	H	H	B	B	A	H	B	A	A	H	H	B	B	B	A
	7	*Xpsm565*	H	A	H	B	B	H	H	H	B	B	A	H	B	A	A	H	H	B	B	B	A
	8	*Xpsm757*	H	A	D	B	B	D	D	D	B	B	D	B	B	D	D	D	D	B	B	B	D
	9	*Xpsm17*	H	A	H	B	B	H	H	H	B	B	A	B	-	H	A	D	H	B	B	B	A
	10	*Xpsm341*	H	H	H	B	B	A	H	H	B	H	A	B	B	C	A	H	H	B	B	B	A
	11	*Xpsm386*	H	H	H	B	B	H	H	H	B	H	A	B	-	-	A	-	H	H	B	B	A
	12	*Xpsm360*	H	H	H	B	B	H	H	H	B	H	A	B	B	H	A	H	H	H	B	B	A
	13	*Xpsm73b*	D	D	D	B	B	D	D	D	B	B	D	B	D	D	D	D	D	B	B	B	D
	14	*Xpsm347a*	A	A	H	B	H	B	H	B	A	H	D	B	B	B	D	A	H	H	B	B	H
	15	*Xpsm196a*	A	A	H	B	H	B	H	B	A	H	A	B	H	B	A	A	H	H	B	B	H
LG2	16	*Xpsm708a*	D	D	D	B	D	B	D	D	B	D	D	D	D	B	D	D	B	D	B	B	B
	17	*Xpsm706*	C	B	C	C	H	B	B	H	B	H	H	A	H	H	H	A	B	H	H	B	B
	18	*Xpsm25*	H	B	H	H	H	B	B	B	B	H	H	A	H	H	B	H	H	H	H	B	B
	19	*Xpsm592*	D	B	A	D	D	B	B	B	B	D	-	-	D	D	-	D	B	D	D	D	B
	20	*Xpsm321*	-	-	-	-	D	B	B	B	B	D	D	D	D	D	B	D	B	D	D	D	B
	21	*Xpsm708b*	H	B	H	H	B	B	H	B	B	H	H	H	B	H	B	H	H	H	H	H	B
LG3	22	*Xpsm37*	B	B	B	H	A	A	H	H	H	H	B	A	H	B	H	B	H	H	B	A	H
	23	*Xpsm108*	-	-	-	-	A	A	H	H	H	H	B	D	H	B	D	B	H	H	B	A	H
	24	*Xpsm174*	A	H	B	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	*Xpsm96*	A	H	B	H	A	A	H	H	H	A	B	A	H	B	H	B	H	H	B	A	A
	26	*Xpsm18*	A	H	B	H	A	A	H	H	H	H	B	A	H	B	H	B	H	H	B	A	H
	27	*Xpsm678*	A	H	B	H	A	A	H	H	H	A	B	A	H	B	H	H	H	H	B	A	H
	28	*Xpsm248*	A	H	B	H	A	A	H	H	H	H	B	A	H	B	H	H	H	H	B	H	H
	29	*Xpsm473*	A	H	B	H	D	A	H	H	H	H	B	A	D	B	H	D	H	H	B	H	H
	30	*Xpsm686*	A	H	B	H	A	A	H	H	H	H	B	A	C	B	H	H	H	H	B	H	H
	31	*Xpsm410*	A	H	B	H	A	A	H	H	H	H	B	A	H	B	H	H	H	H	H	H	H

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Linkage group	No	Probe	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
LG4	32	*Xpsm409a*	H	A	H	H	A	B	H	H	H	H	H	A	H	H	A	H	B	H	A	B	A
	33	*Xpsm464*	A	A	C	C	A	C	C	C	C	C	C	A	C	C	C	C	C	C	C	C	A
	34	*Xpsm716*	A	H	H	H	A	C	C	A	A	C	C	A	C	A	A	-	C	C	A	A	A
	35	*Xpsm265*	A	C	C	A	A	C	C	A	A	C	C	A	C	A	A	A	C	C	A	A	C
	36	*Xpsm306*	A	C	C	A	A	C	C	A	A	C	C	A	A	A	A	A	C	C	C	A	C
	37	*Xpsm421c*	A	H	H	A	A	H	H	A	A	H	H	A	A	A	A	A	H	H	H	A	C
	38	*Xpsm568*	A	C	C	A	A	C	C	A	A	C	C	A	A	A	A	A	C	C	C	A	C
	39	*Xpsm512*	-	C	C	-	H	C	C	A	A	C	H	A	A	A	A	-	H	H	H	A	H
	40	*Xpsm344*	A	H	H	A	A	H	H	A	A	H	H	A	A	A	A	A	H	H	H	A	H
	41	*Xpsm84*	A	H	H	A	A	C	C	A	A	C	C	A	A	A	A	A	C	C	C	A	C
	42	*Xpsm612*	A	A	H	A	A	H	H	A	A	H	H	A	A	A	A	A	H	H	H	A	H
LG5	43	*Xpsm815*	A	B	B	H	A	H	H	A	H	H	A	H	H	H	H	B	A	A	A	A	H
	44	*Xpsm318*	A	B	B	H	A	H	H	A	H	H	A	H	H	H	H	B	A	A	A	A	H
	45	*Xpsm73a*	A	B	B	H	A	H	H	A	H	H	A	H	D	H	C	B	A	A	A	A	D
	46	*Xrm11_1*	A	B	B	H	A	H	H	A	H	H	A	H	H	H	H	B	A	A	A	A	H
	47	*Xpsm749*	A	H	B	H	A	H	H	A	H	H	A	H	H	H	D	B	A	A	A	A	H
	48	*Xpsm735a*	A	H	B	B	A	B	H	H	B	B	A	A	-	-	H	-	A	A	A	H	H
LG6	49	*Xpsm202*	H	H	A	B	A	H	H	H	H	B	H	H	-	A	-	B	A	B	H	H	H
	50	*Xpsm459a*	H	H	A	B	H	H	H	H	H	B	A	H	H	H	H	B	A	B	H	H	H
	51	*Xpsm588*	-	-	-	-	-	-	D	H	H	B	A	H	-	H	H	-	H	B	H	H	H
	52	*Xpsm713*	H	H	H	B	H	H	A	H	H	B	H	H	H	H	H	H	H	B	H	H	H
	53	*Xpsm575*	H	H	H	B	H	H	A	H	H	B	H	H	H	H	H	H	H	B	H	H	H
	54	*Xpsm579*	H	H	H	A	H	H	A	H	H	B	A	A	H	H	A	H	H	B	H	A	H
	55	*Xpsm613b*	H	H	H	B	-	-	-	-	-	-	C	C	A	A	B	A	A	A	B	-	-
LG7	56	*Xpsm269*	H	B	B	H	A	H	H	H	H	H	H	H	A	H	-	H	D	H	B	H	D
	57	*Xrm9-2b*	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	D	B	D	B	D	D
	58	*Xpsm618*	H	H	B	H	A	A	A	H	H	H	A	H	A	H	A	H	B	B	B	H	H
	59	*Xpsm717*	B	H	B	H	A	A	A	H	H	H	A	-	A	H	A	H	B	B	B	H	H
	60	*Xpsm834*	B	B	B	H	A	A	A	H	H	H	A	H	A	B	A	H	B	B	B	H	H

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Linkage group	No	Probe	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
LG1	1	*Xpsr110*	H	A	H	B	A	B	B	B	B	A	H	A	A	A	H	B	B	A	H	H
	2	*Xpsm573*	D	D	D	B	D	B	D	B	D	D	D	D	D	B	D	B	B	B	B	B
	3	*Xpsm280*	B	A	H	B	A	B	H	B	A	H	H	A	H	H	H	A	H	A	H	H
	4	*Xpsm858*	H	A	H	B	H	B	H	B	A	B	B	H	B	H	H	A	H	A	A	H
	5	*Xpsm87*	H	A	A	H	H	B	H	B	A	B	B	B	H	B	C	A	H	-	H	A
	6	*Xpsm761*	H	A	A	H	H	B	H	B	A	B	B	H	B	H	H	A	H	D	H	A
	7	*Xpsm565*	H	A	A	H	H	B	H	B	A	B	B	H	B	H	H	A	H	H	H	A
	8	*Xpsm757*	D	D	D	D	D	B	D	B	D	B	B	H	B	D	C	A	D	D	H	A
	9	*Xpsm17*	H	A	A	H	H	B	H	B	A	B	B	H	B	H	H	A	H	H	H	A
	10	*Xpsm341*	H	A	A	H	H	H	H	B	A	B	B	H	B	H	H	A	H	H	H	A
	11	*Xpsm386*	H	A	A	H	H	H	H	H	A	B	B	H	B	-	H	D	C	-	B	A
	12	*Xpsm360*	H	A	A	H	H	H	H	H	A	B	B	H	B	H	H	A	H	H	B	A
	13	*Xpsm73b*	D	D	D	D	D	B	D	B	D	B	B	D	B	D	D	D	D	-	D	D
	14	*Xpsm347a*	B	A	A	B	B	H	B	H	H	B	A	H	B	H	D	H	H	H	H	H
	15	*Xpsm196a*	B	H	A	B	B	H	B	H	H	B	A	H	H	H	A	H	H	A	H	H
LG2	16	*Xpsm708a*	D	D	D	D	B	D	D	D	D	D	D	D	D	B	B	D	D	B	D	D
	17	*Xpsm706*	H	H	B	H	H	H	B	B	H	H	H	H	H	H	B	B	B	B	H	A
	18	*Xpsm25*	H	H	B	H	H	H	B	B	H	H	H	H	H	H	B	B	B	B	H	H
	19	*Xpsm592*	D	D	B	D	D	D	B	B	D	D	D	D	D	D	B	B	B	B	D	D
	20	*Xpsm321*	D	D	B	D	D	D	B	B	D	-	D	D	D	D	B	B	B	B	D	D
	21	*Xpsm708b*	H	H	B	H	H	H	B	H	H	B	A	H	H	H	H	H	B	B	B	H
LG3	22	*Xpsm37*	H	H	H	A	A	A	A	H	H	H	H	A	B	H	A	H	H	B	A	B
	23	*Xpsm108*	H	H	H	A	A	A	A	H	H	H	D	H	B	H	A	H	H	-	H	B
	24	*Xpsm174*	-	-	-	-	-	-	-	-	-	-	H	H	B	-	A	H	H	B	H	B
	25	*Xpsm96*	A	-	-	A	-	-	C	C	C	C	H	H	H	H	A	H	H	B	H	B
	26	*Xpsm18*	H	H	B	A	A	A	H	H	H	H	H	H	H	H	A	H	H	B	H	B
	27	*Xpsm678*	H	H	B	A	A	A	H	H	H	H	H	H	H	H	A	H	H	B	H	B
	28	*Xpsm248*	D	C	B	A	A	A	D	H	H	C	H	H	H	H	A	H	H	B	H	B
	29	*Xpsm473*	H	H	B	D	D	D	D	D	D	D	H	H	H	H	A	H	H	B	H	B
	30	*Xpsm686*	H	H	B	A	A	A	H	H	H	H	H	H	H	H	A	H	H	B	H	B
	31	*Xpsm410*	H	B	B	A	A	A	C	C	C	C	H	H	H	H	A	H	H	H	H	B

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Linkage group	No	Probe	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
LG4	32	*Xpsm409a*	A	H	B	B	A	A	H	A	H	H	A	A	B	D	H	A	A	H	B	H
	33	*Xpsm464*	A	C	C	C	A	C	C	A	C	C	C	A	C	C	A	A	C	C	C	C
	34	*Xpsm716*	C	A	C	C	C	C	C	A	C	-	H	H	A	-	A	A	H	A	A	H
	35	*Xpsm265*	C	A	C	C	C	C	C	A	C	A	C	C	C	A	A	A	C	A	A	C
	36	*Xpsm306*	C	A	C	C	C	C	C	A	C	A	C	C	C	A	A	A	C	A	A	A
	37	*Xpsm421c*	H	A	H	H	H	A	B	A	H	A	H	D	B	A	A	A	H	A	H	A
	38	*Xpsm568*	C	A	C	C	C	C	C	A	C	A	C	C	C	A	A	A	C	A	C	A
	39	*Xpsm512*	H	A	H	C	C	-	C	-	C	A	H	H	B	A	A	A	-	-	C	-
	40	*Xpsm344*	H	A	H	H	H	A	H	A	H	A	H	H	H	-	A	A	H	D	B	A
	41	*Xpsm84*	C	A	C	A	C	A	C	A	C	A	C	C	C	A	A	A	C	A	C	A
	42	*Xpsm612*	H	A	H	H	H	A	H	A	H	A	A	H	C	A	A	A	H	A	B	A
LG5	43	*Xpsm815*	H	H	A	B	H	B	H	H	H	B	H	H	B	H	H	A	A	A	A	H
	44	*Xpsm318*	H	H	A	B	H	B	H	H	A	B	H	H	B	H	H	A	A	A	A	H
	45	*Xpsm73a*	H	D	H	B	H	B	H	H	A	B	D	H	B	H	H	A	A	A	A	H
	46	*Xrm11_1*	H	H	H	B	H	B	H	H	A	B	H	H	B	H	H	A	A	A	A	H
	47	*Xpsm749*	H	H	H	H	B	B	H	H	A	B	H	H	B	H	H	A	A	A	A	H
	48	*Xpsm735a*	H	H	H	B	H	B	H	H	A	B	H	H	B	H	H	A	A	-	A	H
LG6	49	*Xpsm202*	H	H	H	B	A	A	B	H	H	H	-	-	-	-	-	-	-	-	H	A
	50	*Xpsm459a*	H	H	H	B	A	A	B	B	H	H	H	B	H	H	A	A	B	A	H	A
	51	*Xpsm588*	H	H	H	B	A	A	B	B	H	H	H	B	H	A	A	A	-	-	D	D
	52	*Xpsm713*	A	H	H	B	H	H	A	B	H	A	H	B	H	H	A	A	B	A	B	H
	53	*Xpsm575*	A	H	H	B	H	H	A	B	H	A	H	B	H	H	A	A	B	A	B	H
	54	*Xpsm579*	A	H	H	B	H	A	A	B	H	-	H	B	H	H	A	A	B	A	B	H
	55	*Xpsm613b*	-	-	-	-	-	-	-	-	-	-	H	H	C	-	B	A	B	D	B	H
LG7	56	*Xpsm269*	A	H	D	B	A	A	A	A	H	A	A	H	D	D	H	A	H	H	H	A
	57	*Xrm9-2b*	D	D	D	B	D	D	D	D	D	D	D	D	D	D	B	D	D	-	D	D
	58	*Xpsm618*	A	H	H	B	H	A	A	A	H	A	A	A	H	H	B	A	H	B	H	H
	59	*Xpsm717*	A	H	H	B	H	A	H	A	H	A	A	A	H	H	B	A	H	-	H	H
	60	*Xpsm834*	A	H	H	B	H	A	H	A	H	A	A	A	H	H	B	A	H	B	A	H

Contd...

Linkage group	No	Probe	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
LG1	1	*Xpsr110*	B	H	H	H	B	B	B	-	H	H	B	B	B	A	C	B	B	H	A	A
	2	*Xpsm573*	D	D	D	D	D	B	D	D	D	D	B	D	B	D	D	B	D	B	B	D
	3	*Xpsm280*	H	H	H	A	H	B	H	A	H	H	H	H	B	A	H	B	H	B	B	A
	4	*Xpsm858*	H	H	B	A	H	B	H	A	A	H	H	A	B	A	H	H	H	B	B	H
	5	*Xpsm87*	A	C	B	A	A	H	H	A	A	H	H	A	B	A	H	H	H	B	B	H
	6	*Xpsm761*	A	H	B	A	H	D	H	A	A	H	H	A	B	A	H	H	H	B	B	H
	7	*Xpsm565*	A	H	B	A	H	H	H	A	A	H	H	A	B	A	H	H	H	B	B	H
	8	*Xpsm757*	A	H	B	A	H	H	H	A	A	H	H	A	B	A	H	H	H	B	B	D
	9	*Xpsm17*	A	H	B	A	H	H	H	A	A	H	H	A	B	A	H	H	H	-	-	-
	10	*Xpsm341*	A	H	B	A	H	H	H	H	A	H	H	A	B	A	H	H	H	B	B	H
	11	*Xpsm386*	A	D	B	A	D	D	-	-	D	H	D	A	B	D	H	H	H	B	B	H
	12	*Xpsm360*	A	H	B	A	H	H	H	H	A	H	H	A	B	H	H	H	H	B	B	H
	13	*Xpsm73b*	D	B	B	D	D	B	B	D	D	B	D	D	B	D	D	D	D	B	B	D
	14	*Xpsm347a*	D	H	H	H	D	H	A	A	A	H	H	H	H	B	A	A	H	H	B	A
	15	*Xpsm196a*	A	H	H	H	H	H	A	A	A	H	B	B	A	B	A	A	H	H	B	A
LG2	16	*Xpsm708a*	B	D	D	B	B	B	D	B	D	D	B	D	B	D	B	D	D	D	B	D
	17	*Xpsm706*	B	H	H	B	C	B	H	H	H	H	B	H	B	A	H	B	H	H	B	H
	18	*Xpsm25*	B	H	H	B	B	B	H	B	H	H	B	H	H	H	H	B	H	H	B	H
	19	*Xpsm592*	B	D	D	B	-	B	D	B	D	D	B	D	D	D	D	B	D	D	B	D
	20	*Xpsm321*	B	D	D	B	B	B	D	-	D	D	B	D	B	D	D	B	D	D	B	D
	21	*Xpsm708b*	H	H	H	H	H	H	B	H	H	H	B	H	H	H	H	H	B	H	H	H
LG3	22	*Xpsm37*	A	B	H	A	B	H	H	D	H	H	H	H	H	H	A	A	H	H	B	A
	23	*Xpsm108*	A	B	H	H	H	H	H	-	H	H	H	H	H	H	A	A	H	A	B	A
	24	*Xpsm174*	A	B	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	A	B	A
	25	*Xpsm96*	A	B	H	H	H	H	H	-	H	H	H	H	A	H	A	A	H	A	C	A
	26	*Xpsm18*	A	B	H	H	H	H	H	H	H	H	H	H	A	H	A	A	H	A	B	A
	27	*Xpsm678*	A	B	H	H	H	H	H	-	H	H	H	H	A	H	D	D	H	A	B	A
	28	*Xpsm248*	A	B	H	H	H	H	H	-	H	H	H	H	A	H	A	A	H	A	B	A
	29	*Xpsm473*	A	B	H	H	H	H	H	D	H	H	H	H	A	H	A	A	H	A	B	A
	30	*Xpsm686*	A	B	H	H	H	B	H	H	H	H	H	H	A	H	A	A	H	A	B	A
	31	*Xpsm410*	A	B	H	H	H	H	H	B	H	H	H	H	A	H	A	A	H	C	C	A

Contd...

Linkage group	No	Probe	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
LG4	32	*Xpsm409a*	A	A	H	A	H	A	A	A	A	A	H	A	B	H	H	B	H	H	H	H
	33	*Xpsm464*	A	A	C	C	C	A	A	A	A	A	C	A	C	C	C	A	C	C	C	C
	34	*Xpsm716*	H	H	H	H	H	A	A	A	H	A	H	A	H	A	H	A	H	A	A	B
	35	*Xpsm265*	C	C	C	C	C	A	A	A	C	A	C	A	C	A	C	A	C	A	A	C
	36	*Xpsm306*	C	C	C	C	C	A	A	A	C	C	C	A	C	A	C	A	C	A	A	C
	37	*Xpsm421c*	B	H	D	-	H	A	D	D	D	H	H	A	H	A	-	A	-	A	A	A
	38	*Xpsm568*	C	C	C	C	C	A	C	A	C	C	C	A	C	A	C	A	C	A	A	C
	39	*Xpsm512*	C	-	C	C	C	A	C	A	H	A	H	A	C	A	H	A	B	C	A	C
	40	*Xpsm344*	H	H	H	H	H	A	H	A	B	A	H	A	H	A	H	A	B	H	A	H
	41	*Xpsm84*	C	C	C	C	C	A	C	A	C	A	C	A	C	A	A	A	C	C	A	C
	42	*Xpsm612*	H	H	H	H	H	A	H	A	B	A	H	A	H	A	H	A	B	H	A	H
LG5	43	*Xpsm815*	H	A	A	H	H	A	H	H	H	A	A	B	H	H	B	H	H	H	H	H
	44	*Xpsm318*	H	A	A	H	H	A	H	H	H	A	A	B	H	H	H	H	H	H	H	H
	45	*Xpsm73a*	H	A	A	D	B	A	H	H	H	H	H	B	H	H	H	H	H	D	H	H
	46	*Xrm11_1*	H	A	A	H	B	A	H	H	H	H	H	B	H	H	H	H	H	H	H	H
	47	*Xpsm749*	H	A	A	H	B	A	H	H	H	H	H	B	H	H	H	H	H	H	H	H
	48	*Xpsm735a*	H	H	A	H	B	A	H	A	H	H	A	H	H	B	H	H	B	B	H	H
LG6	49	*Xpsm202*	H	A	-	-	-	-	-	-	-	-	-	-	-	A	H	A	H	H	B	A
	50	*Xpsm459a*	H	A	H	H	H	H	H	A	B	A	H	H	H	A	H	A	H	H	H	H
	51	*Xpsm588*	H	A	D	D	H	A	D	A	B	A	D	H	H	A	B	A	H	H	H	H
	52	*Xpsm713*	H	A	H	H	H	H	H	A	H	B	A	H	H	A	H	H	A	H	H	B
	53	*Xpsm575*	H	A	H	H	H	H	H	A	H	B	A	H	H	A	H	H	A	H	H	B
	54	*Xpsm579*	H	A	H	H	H	A	H	-	A	D	A	H	H	A	H	H	A	H	H	B
	55	*Xpsm613b*	H	A	H	H	H	-	H	-	A	H	A	H	H	A	H	H	A	H	H	B
LG7	56	*Xpsm269*	B	D	D	D	A	H	H	H	H	A	A	B	H	A	B	A	A	H	H	B
	57	*Xrm9-2b*	B	D	D	D	D	D	D	D	D	D	D	B	D	D	B	D	D	D	B	B
	58	*Xpsm618*	B	H	H	H	A	A	H	H	H	A	H	B	H	H	B	H	H	H	B	H
	59	*Xpsm717*	B	H	H	H	A	A	H	-	H	A	H	B	H	H	B	H	H	H	B	A
	60	*Xpsm834*	A	H	H	H	A	A	H	D	H	A	H	B	H	H	B	H	H	H	B	A

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Linkage group	No	Probe	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103
LG1	1	*Xpsr110*	B	H	-	H	-	-	H	-	B	-	A	-	H	H	A	D	-	H	A	H
	2	*Xpsm573*	B	B	-	B	-	-	D	-	B	-	B	-	D	-	D	B	-	D	D	D
	3	*Xpsm280*	B	B	-	H	B	-	A	H	B	-	H	-	A	B	A	B	-	H	H	H
	4	*Xpsm858*	B	B	-	B	B	-	A	H	B	-	H	-	A	H	A	H	-	H	B	B
	5	*Xpsm87*	B	B	-	B	B	-	A	-	B	-	B	-	A	H	A	A	-	H	B	B
	6	*Xpsm761*	B	B	-	B	B	-	D	B	B	-	H	-	A	H	A	H	-	H	B	B
	7	*Xpsm565*	B	B	-	B	B	-	A	B	B	-	B	-	A	H	A	C	-	H	B	B
	8	*Xpsm757*	B	-	-	B	B	-	D	B	B	-	B	-	D	D	D	D	-	D	B	B
	9	*Xpsm17*	B	B	-	B	B	-	A	-	B	-	B	-	A	H	-	C	-	H	B	B
	10	*Xpsm341*	B	B	-	B	B	-	A	B	B	-	B	-	A	H	A	H	-	H	B	B
	11	*Xpsm386*	B	B	-	B	B	-	A	-	C	-	C	-	-	-	A	H	-	H	C	C
	12	*Xpsm360*	B	B	-	B	B	-	H	B	B	-	B	-	A	H	A	H	-	H	B	B
	13	*Xpsm73b*	D	D	-	B	-	-	D	-	B	-	B	-	D	D	D	B	-	D	B	B
	14	*Xpsm347a*	H	B	-	B	B	-	H	B	B	-	H	-	H	A	H	H	-	H	B	B
	15	*Xpsm196a*	H	B	-	B	B	-	H	B	B	-	H	-	B	A	H	H	-	H	B	B
LG2	16	*Xpsm708a*	D	B	-	D	D	-	D	-	D	-	D	-	D	B	D	D	-	D	B	B
	17	*Xpsm706*	B	H	-	H	H	-	H	B	A	-	A	-	H	B	H	B	-	H	B	B
	18	*Xpsm25*	C	A	-	H	H	-	H	B	A	-	A	-	H	B	H	B	-	H	B	B
	19	*Xpsm592*	-	D	-	D	D	-	D	B	D	-	D	-	D	B	B	-	-	D	D	B
	20	*Xpsm321*	B	D	-	D	-	-	D	-	D	-	B	-	D	B	B	B	-	D	B	B
	21	*Xpsm708b*	H	H	-	A	B	-	H	-	H	-	H	-	H	H	B	B	-	A	H	H
LG3	22	*Xpsm37*	H	H	-	H	B	-	A	H	H	-	A	-	A	H	B	B	-	B	H	A
	23	*Xpsm108*	H	H	-	-	-	-	A	-	H	-	A	-	A	-	C	B	-	B	H	A
	24	*Xpsm174*	H	H	-	H	B	-	A	H	H	-	A	-	A	H	B	B	-	B	H	A
	25	*Xpsm96*	C	C	A	C	C	A	A	C	C	-	A	-	A	C	C	C	-	C	C	A
	26	*Xpsm18*	H	H	-	H	B	-	A	H	H	-	A	-	A	H	B	B	-	B	H	A
	27	*Xpsm678*	-	C	-	H	-	-	-	-	H	-	A	-	A	-	C	B	-	B	H	A
	28	*Xpsm248*	H	H	-	H	B	-	A	H	H	-	H	-	A	H	B	B	-	B	H	A
	29	*Xpsm473*	D	D	-	H	-	-	D	H	H	-	H	-	A	H	B	B	-	B	H	A
	30	*Xpsm686*	H	H	-	H	C	-	A	-	H	-	H	-	A	A	B	B	-	B	H	A
	31	*Xpsm410*	C	C	A	C	C	A	A	C	C	-	C	-	A	C	C	C	-	C	C	A

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Linkage group	No	Probe	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103
LG4	32	*Xpsm409a*	B	A	-	H	H	-	H	-	H	-	B	-	A	A	H	A	-	A	H	A
	33	*Xpsm464*	C	A	-	A	C	-	C	C	C	-	A	-	A	A	C	C	-	C	C	C
	34	*Xpsm716*	A	A	-	A	A	-	H	A	H	-	D	-	A	A	H	H	-	H	H	H
	35	*Xpsm265*	A	A	-	A	A	-	C	A	C	-	C	-	A	A	C	C	-	C	C	C
	36	*Xpsm306*	A	A	-	A	A	-	C	A	C	-	C	-	A	A	C	C	-	C	C	C
	37	*Xpsm421c*	A	A	-	-	-	-	D	-	D	-	D	-	-	-	-	H	-	H	-	-
	38	*Xpsm568*	A	A	-	-	-	-	A	-	C	-	C	-	A	C	C	C	-	C	C	C
	39	*Xpsm512*	A	C	-	A	-	-	H	-	H	-	H	-	A	C	C	C	-	C	C	C
	40	*Xpsm344*	A	H	-	A	A	-	H	H	A	-	H	-	A	H	H	H	-	H	H	H
	41	*Xpsm84*	A	C	-	A	A	-	C	-	A	-	C	-	A	C	C	C	-	C	C	C
	42	*Xpsm612*	A	B	-	B	A	-	H	B	A	-	H	-	A	H	-	H	-	H	H	H
LG5	43	*Xpsm815*	H	H	-	C	C	-	H	A	A	-	H	-	A	H	A	A	-	H	H	H
	44	*Xpsm318*	H	H	-	B	B	-	H	A	A	-	H	-	A	H	A	A	-	H	H	H
	45	*Xpsm73a*	H	H	-	B	-	-	D	-	A	-	H	-	A	H	H	A	-	H	H	H
	46	*Xrm11_1*	H	H	-	B	H	-	H	H	A	-	H	-	A	H	H	A	-	H	H	H
	47	*Xpsm749*	H	H	-	B	H	-	H	H	A	-	H	-	A	H	H	A	-	H	H	H
	48	*Xpsm735a*	H	A	-	B	-	-	H	-	D	-	A	-	A	H	H	A	-	H	A	H
LG6	49	*Xpsm202*	A	A	-	H	-	-	B	-	B	-	B	-	A	A	B	A	-	A	H	H
	50	*Xpsm459a*	B	B	-	B	D	-	B	B	B	-	B	-	A	H	B	A	-	A	H	A
	51	*Xpsm588*	B	B	-	-	-	-	H	-	H	-	B	-	-	-	B	A	-	A	C	A
	52	*Xpsm713*	B	H	-	H	B	-	B	H	H	-	B	-	H	H	H	-	-	H	B	H
	53	*Xpsm575*	B	H	-	H	B	-	B	H	H	-	B	-	H	H	H	A	-	H	B	H
	54	*Xpsm579*	B	H	-	H	B	-	B	-	H	-	B	-	A	H	H	A	-	H	B	H
	55	*Xpsm613b*	A	H	-	-	-	-	C	-	-	-	H	-	-	-	D	A	-	H	D	-
LG7	56	*Xpsm269*	H	A	-	H	H	-	H	B	H	-	H	-	H	B	B	H	-	H	A	H
	57	*Xrm9-2b*	D	D	-	B	B	-	D	B	B	-	D	-	B	B	B	D	-	D	B	B
	58	*Xpsm618*	H	A	-	H	A	-	A	-	B	-	A	-	H	B	B	H	-	H	B	B
	59	*Xpsm717*	H	A	-	H	-	-	A	-	B	-	A	-	H	B	B	H	-	H	B	B
	60	*Xpsm834*	H	A	-	H	A	-	A	H	H	-	A	-	H	B	B	H	-	H	B	B

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Linkage group	No	Probe	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123
LG1	1	*Xpsr110*	A	H	H	H	A	H	A	H	A	B	A	B	H	B	A	H	-	H	A	-
	2	*Xpsm573*	B	D	B	D	D	D	D	D	D	B	D	B	D	B	B	D	-	D	D	B
	3	*Xpsm280*	B	H	H	B	H	A	H	H	A	B	A	B	H	B	B	H	-	H	H	H
	4	*Xpsm858*	B	H	H	H	H	A	B	H	A	H	A	B	H	H	B	H	-	H	H	-
	5	*Xpsm87*	B	H	H	H	H	A	B	H	A	D	A	B	H	H	B	B	-	B	H	-
	6	*Xpsm761*	B	H	H	H	H	A	B	H	A	H	A	B	H	H	B	B	-	H	H	H
	7	*Xpsm565*	B	H	H	H	H	A	B	H	A	H	A	B	H	H	B	B	-	H	B	H
	8	*Xpsm757*	B	D	D	D	D	D	B	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	*Xpsm17*	B	H	H	H	-	A	B	-	A	H	A	B	-	H	B	B	-	B	H	-
	10	*Xpsm341*	B	H	H	H	H	A	B	H	A	H	A	B	H	H	B	B	-	B	H	-
	11	*Xpsm386*	C	C	-	C	-	A	B	D	A	H	A	B	H	H	B	B	-	H	H	H
	12	*Xpsm360*	B	H	H	H	H	A	B	H	A	H	A	B	H	H	B	B	-	H	H	H
	13	*Xpsm73b*	D	D	D	D	D	D	B	D	D	B	D	B	D	D	B	B	-	B	D	-
	14	*Xpsm347a*	A	B	H	B	A	H	A	H	H	B	A	H	H	B	B	H	-	H	H	H
	15	*Xpsm196a*	H	B	H	B	A	H	A	H	H	B	A	H	B	B	B	H	-	H	H	H
LG2	16	*Xpsm708a*	D	D	D	D	B	D	D	B	D	D	B	D	D	D	B	D	-	D	D	D
	17	*Xpsm706*	A	B	B	H	B	B	H	B	B	H	H	H	H	H	H	H	-	H	B	H
	18	*Xpsm25*	H	B	B	H	B	B	H	B	B	H	B	B	H	H	H	H	-	H	B	H
	19	*Xpsm592*	D	-	B	D	D	B	D	B	B	B	B	B	D	D	B	D	-	D	B	D
	20	*Xpsm321*	D	B	B	D	-	B	D	B	B	D	B	B	D	D	B	D	-	D	B	D
	21	*Xpsm708b*	H	H	H	H	H	H	H	H	B	B	B	B	H	H	H	H	-	H	B	H
LG3	22	*Xpsm37*	H	A	B	A	H	B	A	A	A	H	A	H	H	H	A	H	-	H	A	H
	23	*Xpsm108*	H	A	B	A	D	B	H	H	H	H	A	H	H	H	A	H	-	H	A	C
	24	*Xpsm174*	H	A	B	A	H	B	H	H	A	H	A	H	H	H	A	H	-	H	A	H
	25	*Xpsm96*	C	A	C	A	C	C	C	H	H	A	A	H	H	C	H	H	-	B	H	A
	26	*Xpsm18*	H	A	B	A	H	B	H	H	H	H	A	H	H	H	H	H	-	B	H	H
	27	*Xpsm678*	H	A	B	A	-	B	D	H	H	H	A	H	H	H	H	H	-	B	H	H
	28	*Xpsm248*	H	A	B	A	H	B	H	H	H	H	A	H	H	H	H	H	-	B	H	H
	29	*Xpsm473*	H	A	B	A	H	B	D	H	H	H	A	H	H	H	H	H	-	B	H	H
	30	*Xpsm686*	H	A	B	A	H	B	H	H	H	H	A	H	H	H	H	H	-	B	H	-
	31	*Xpsm410*	C	A	C	A	C	C	C	H	H	-	A	H	H	H	H	H	-	B	H	-

Contd...

Linkage group	No	Probe	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123
LG4	32	*Xpsm409a*	B	H	B	H	H	A	A	B	A	H	A	C	C	C	H	B	-	H	H	-
	33	*Xpsm464*	C	C	C	C	C	A	A	C	A	C	A	C	C	C	C	C	-	C	C	-
	34	*Xpsm716*	A	H	H	H	H	A	A	H	H	H	A	A	H	B	H	C	-	A	A	A
	35	*Xpsm265*	A	C	C	C	C	A	A	C	C	C	A	A	C	C	C	A	-	A	A	A
	36	*Xpsm306*	A	C	A	C	C	C	A	C	C	C	A	A	C	C	C	A	-	A	A	A
	37	*Xpsm421c*	D	D	D	-	-	-	A	H	H	H	A	A	H	B	H	A	-	H	A	B
	38	*Xpsm568*	A	C	A	C	C	C	A	C	C	C	A	A	C	C	C	A	-	C	A	C
	39	*Xpsm512*	A	A	A	H	H	H	A	H	C	H	A	A	H	B	H	A	-	B	B	-
	40	*Xpsm344*	A	A	A	H	H	H	A	H	H	H	H	A	H	B	H	A	-	H	H	H
	41	*Xpsm84*	A	A	A	C	C	C	A	C	C	C	A	A	C	C	C	A	-	C	C	C
	42	*Xpsm612*	A	A	A	H	H	H	A	B	B	H	A	A	B	B	H	H	-	H	H	-
LG5	43	*Xpsm815*	H	A	H	H	A	B	A	A	A	A	H	B	H	H	B	H	-	H	B	H
	44	*Xpsm318*	H	A	H	H	A	B	A	A	A	A	H	B	H	H	B	H	-	H	B	H
	45	*Xpsm73a*	H	A	H	H	A	H	H	H	A	B	A	H	H	H	B	D	-	B	B	-
	46	*Xrm11_1*	H	A	H	H	A	H	H	H	A	A	H	H	H	H	B	H	-	B	B	-
	47	*Xpsm749*	H	A	H	H	A	H	H	H	A	A	H	H	H	H	B	H	-	B	B	D
	48	*Xpsm735a*	H	A	B	A	A	H	H	A	A	A	D	H	H	A	B	H	-	H	B	D
LG6	49	*Xpsm202*	H	H	A	H	H	H	B	H	H	C	B	A	A	B	H	C	-	C	A	H
	50	*Xpsm459a*	H	H	A	H	H	H	B	H	H	H	B	H	A	B	H	B	-	B	H	B
	51	*Xpsm588*	B	H	H	A	H	H	B	H	H	H	B	H	H	B	H	B	-	B	H	C
	52	*Xpsm713*	B	H	A	H	H	B	B	H	H	B	A	A	H	B	H	B	-	B	A	-
	53	*Xpsm575*	B	H	A	H	H	B	B	H	H	B	A	A	H	B	H	B	-	B	A	B
	54	*Xpsm579*	B	H	H	-	-	-	-	H	H	B	A	A	H	B	H	B	-	H	A	B
	55	*Xpsm613b*	D	H	A	H	-	-	-	-	H	B	-	-	-	-	-	H	-	H	A	B
LG7	56	*Xpsm269*	H	H	A	A	H	A	-	H	H	A	B	B	A	B	H	A	-	B	A	-
	57	*Xrm9-2b*	D	D	D	D	D	-	-	D	D	D	D	B	D	B	-	D	-	B	D	-
	58	*Xpsm618*	H	H	H	H	H	H	H	H	H	A	H	B	A	B	B	A	-	B	H	-
	59	*Xpsm717*	H	H	H	H	H	H	H	H	H	A	H	B	A	B	B	A	-	B	H	H
	60	*Xpsm834*	H	H	H	H	H	H	H	H	H	A	H	B	A	B	B	A	-	B	H	H

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Linkage group	No	Probe	124	125	126	127	128	129	130	131	132	133	134	135	136
LG1	1	*Xpsr110*	H	B	A	A	A	A	B	A	H	H	H	A	H
	2	*Xpsm573*	D	D	B	D	D	D	D	D	D	D	D	D	D
	3	*Xpsm280*	H	A	B	A	H	H	A	A	H	H	A	A	H
	4	*Xpsm858*	H	A	B	A	H	B	A	A	H	H	A	H	H
	5	*Xpsm87*	A	A	B	A	H	B	A	A	H	H	A	H	D
	6	*Xpsm761*	A	A	B	A	H	B	A	A	H	H	A	H	H
	7	*Xpsm565*	A	A	B	A	H	B	A	A	H	H	A	H	D
	8	*Xpsm757*	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	*Xpsm17*	H	A	B	D	-	B	A	A	H	H	-	B	H
	10	*Xpsm341*	H	A	B	A	H	B	A	A	H	H	H	B	H
	11	*Xpsm386*	H	A	C	A	D	C	A	A	H	H	C	B	D
	12	*Xpsm360*	H	A	B	A	H	B	A	A	H	H	H	B	H
	13	*Xpsm73b*	D	D	B	D	D	B	D	D	D	D	D	B	D
	14	*Xpsm347a*	A	H	H	H	H	H	H	H	H	D	H	B	D
	15	*Xpsm196a*	A	A	H	H	H	H	H	H	H	A	A	B	H
LG2	16	*Xpsm708a*	B	D	D	B	B	D	D	D	D	B	D	D	D
	17	*Xpsm706*	H	H	H	H	H	H	B	H	B	B	B	A	B
	18	*Xpsm25*	H	H	H	H	H	B	H	H	B	B	B	H	B
	19	*Xpsm592*	D	D	D	D	D	B	B	D	-	B	B	D	-
	20	*Xpsm321*	D	-	-	D	D	B	B	B	B	B	B	D	B
	21	*Xpsm708b*	H	H	H	H	H	H	H	H	H	H	H	H	H
LG3	22	*Xpsm37*	H	H	H	C	B	A	H	B	A	A	H	H	H
	23	*Xpsm108*	H	H	H	B	B	A	H	H	H	H	D	H	A
	24	*Xpsm174*	H	H	H	B	B	A	H	H	D	H	H	H	D
	25	*Xpsm96*	H	H	B	B	B	A	H	D	H	D	B	D	A
	26	*Xpsm18*	H	H	B	B	B	A	H	H	H	H	B	H	A
	27	*Xpsm678*	H	H	B	B	-	A	D	H	H	H	C	D	A
	28	*Xpsm248*	H	H	B	B	H	A	H	H	H	H	B	H	A
	29	*Xpsm473*	H	H	B	B	H	A	H	H	H	H	B	H	D
	30	*Xpsm686*	B	H	B	B	H	A	H	H	H	H	B	H	A
	31	*Xpsm410*	B	H	B	B	H	A	H	H	H	H	B	H	A

Contd...

Linkage group	No	Probe	124	125	126	127	128	129	130	131	132	133	134	135	136
LG4	32	*Xpsm409a*	H	B	A	A	A	C	B	H	B	H	A	A	H
	33	*Xpsm464*	C	C	A	A	A	C	C	A	C	C	A	A	C
	34	*Xpsm716*	A	H	A	D	A	H	H	A	B	H	A	A	A
	35	*Xpsm265*	A	C	A	C	A	A	C	C	C	C	A	A	C
	36	*Xpsm306*	A	A	A	A	A	-	C	A	C	C	C	A	C
	37	*Xpsm421c*	A	A	A	A	A	-	H	A	B	C	A	A	A
	38	*Xpsm568*	A	A	A	C	A	A	C	A	C	C	A	A	A
	39	*Xpsm512*	-	-	-	-	-	-	H	A	B	C	A	A	H
	40	*Xpsm344*	A	A	A	A	A	A	H	A	B	H	A	A	A
	41	*Xpsm84*	A	A	A	A	A	A	C	A	C	C	A	A	A
	42	*Xpsm612*	A	A	A	A	A	A	A	A	B	H	A	A	A
LG5	43	*Xpsm815*	A	H	H	H	B	H	B	A	H	H	H	B	B
	44	*Xpsm318*	A	H	H	H	B	H	B	A	H	H	H	B	B
	45	*Xpsm73a*	H	H	H	H	B	H	H	A	D	H	D	H	B
	46	*Xrm11_1*	H	H	H	H	B	H	H	A	H	H	H	H	B
	47	*Xpsm749*	H	H	H	H	B	H	H	A	H	H	H	H	B
	48	*Xpsm735a*	B	H	B	H	B	H	H	A	H	H	H	B	H
LG6	49	*Xpsm202*	H	H	H	A	A	H	A	B	H	A	B	A	H
	50	*Xpsm459a*	H	H	H	A	A	H	H	B	H	A	B	H	H
	51	*Xpsm588*	H	H	H	A	A	D	D	-	D	-	B	H	H
	52	*Xpsm713*	A	H	H	A	A	H	B	H	B	A	H	A	B
	53	*Xpsm575*	A	H	A	A	A	H	B	H	B	A	H	A	B
	54	*Xpsm579*	A	H	A	A	A	A	C	A	C	-	-	A	A
	55	*Xpsm613b*	-	-	-	-	-	-	H	-	B	H	H	A	A
LG7	56	*Xpsm269*	H	A	H	D	H	A	D	H	H	H	H	H	A
	57	*Xrm9-2b*	D	D	D	D	D	D	B	D	B	D	D	D	B
	58	*Xpsm618*	H	A	A	H	B	A	B	H	B	H	H	A	B
	59	*Xpsm717*	A	A	A	B	B	A	B	H	B	H	H	A	B
	60	*Xpsm834*	A	H	A	H	B	A	B	H	B	H	H	A	B