Genetic Diversity Analysis and QTL Mapping in Pearl Millet (*Pennisetum glaucum*) using Diversity Arrays Technology (DArT)

Report Submitted to

Chaudhary Charan Singh Haryana Agricultural University

Hisar - 125 004 (Haryana), India

By

SUPRIYA

International Crops Research Institute for the Semi-Arid Tropics

Patancheru, 502 324

Andhra Pradesh, India

icrisat@cgiar.org

June 2010

CERTIFICATE I

This is to certify that this thesis entitled, **"Genetic diversity analysis and QTL mapping in pearl millet (*Pennisetum glaucum*) using Diversity Arrays Technology (DArT)"**, submitted for the degree of **Doctor of Philosophy** in the subject of **Biotechnology and Molecular Biology** of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Supriya** under my supervision and that no part of this thesis has been submitted for any other degree.

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

(R.C. Yadav)
Major Advisor
Sr. Scientist
Deptt. of Biotech. & Molecular Biology
CCS Haryana Agricultural University
Hisar-125 004, Haryana (India)

(C.T. Hash)
Co-Major Advisor
Principal Scientist
Molecular Breeding
ICRISAT, Patancheru-502 324
Hyderabad, Andhra Pradesh (India)

ACKNOWLEDGEMENTS

I express my utmost gratitude to my Major Advisor, Dr. R.C. Yadav, Sr. Scientist, Department of BMB, CCS HAU, Hisar for inspiring and encouraging me to undertake my research programme at ICRISAT. He offered full support and cooperation all through the course of my study. He was always helpful and positive about my work which provided me great confidence to accomplish my work successfully. Thanks would be too small to express my gratitude to him for being my major motivation during course of my study.

It gives me immense pleasure in extending my deep sense of reverence and gratidue to my Co-Major Advisor, Dr.C.T.Hash, Principal Scientist, Molecular Breeding, ICRISAT, Patancheru for providing me an opportunity to work under his esteemed guidance. I am highly obliged to him for suggestion of research problem, nice research facilities, valuable consultations, sagacious advice and help rendered during course of this investigation. I take this opportunity to thank him for his positive approach, incessant encouragement and critically going through the manuscript to bring it in final form of thesis.

Fervently and modestly, I extol the genuine cooperation and inspiration offered to me by Dr. S. Senthilvel, Scientist, Biotechnology, ICRISAT for his valuable counsel, meticulous guidance, able direction and sustained encouragement. I specially thank him for providing excellent research facilities, healthy and conducive environment and space to me to work in my own way throughout the investigation. I am not having words to express my gratitude to him for his sustained and excellent support without which this work might not be in its present form.

I am very thankful to other members of my Advisory Committee Dr. Neelam R. Yadav, Sr. Scientist (BMB), for her sincere suggestions, motivation and helping hand from time to time, Dr. H.P. Yadav, Sr. Scientist (Plant Breeding) for his help in getting me this project, Dr. Veena Chawla, Sr. Scientist (Genetics) and Dr. I.J. Paruthi, Sr. Scientist (Nematology) for their excellent support, valuable suggestions and immense help during my study.

I am sincerely obliged and express my heartful gratitude and indebtedness to Mr. K.Eshwar for his kind help, cooperation, motivation, valuable suggestions and morale support.Words are not sufficient to thank and appreciate the assistance and help rendered by him in completing my work efficiently and smoothly. My warmest thanks to Mr.G. Somaraju and Ranjan Kumar Shaw for their help, cooperation and providing a good environment to work and boosting my morale.

I wish to express my sincere thanks to Dr. T. Nepolean for valuable advice and cooperation during data analysis, Dr.R.P.Thakur and Dr.Rajan Sharma, Mr.V.PandurangaRao, Mr.A. Ganapathi and other pearl millet breeding and pathology staff, ICRISAT for their timely help during glasshouse screening. I am thankful to Ms. Seetha Kannan and CSL staff for arranging the facilities for experimentation in time.

I am grateful to HOD, BMB for providing the support to undertake the research programme at ICRISAT. I also acknowledge the staff of AGL, PMB and LSU, ICRISAT, and BMB, CCS HAU, Hisar for their cooperation and timely help.

I wish to specially thank Drs. P. Ramu, Mahendar, Ashwani Kumar, Mr. Narsi Reddy, V. Rajaram, Sushil, Abhishek, G. Mallikarjun, Ms. Sravani, Roma, Pavana, Spurthi and Usha for their help and cooperation during my study.

The love and affection showered upon me by my grandparents, parents, sisters Sneh and Suman and brother Shobhit is worth mentioning and without their good wishes and morale support it would have been difficult for me to attain academic heights.

Financial assistance received from DBT and GCP for the research work, and merit stipend awarded by CCS HAU, Hisar and ICRISAT is also fully acknowledged.

Above all, I thank the ALMIGHTY for giving me strength and belief to overcome the difficulties in life for a smooth sailing.

Place: Hisar **SUPRIYA**

Date: June 16, 2010

CONTENTS

LIST OF TABLES

LIST OF FIGURES

14	Genetic linkage map of LG 1, LG 4 and LG 7 of the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population showing QTL positions for rust resistance
15 (A)	Logarithm of odds (LOD) profiles for LG 1 thru LG 4 for rust resistance QTLs segregating in the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population
15 (B)	Logarithm of odds (LOD) profiles for LG 5 thru LG 7 for rust resistance QTLs segregating in the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population

LIST OF ABBREVIATIONS

µg	:	Micro gram
µM	:	Micro molar
bp	:	Base pair
mg	:	Milli gram
V	:	Volt
cM	:	centiMorgan
DMF	:	Dimethyl formamide
dNTP	:	deoxy ribonucleotide tri-phosphate
EDTA	:	Ethylene diamine tetraacetic acid
et al	:	et alia (and others)
LB	:	Luria Bertaini
mM	:	Millimolar
ng	:	Nanogram
rpm	:	Revolution per minute
SDS	:	Sodium dodecyl sulphate
X-gal	:	5-Bromo-4-chloro-3-indolyl-β-D thiogalactoside
Pst	:	*Providencia stuartii*
Ban	:	*Bacillus aneurinolyticus*
TE	:	Tris-EDTA
TBE	:	Tris boric acid EDTA
RNase	:	Ribonuclease
S.E.	:	Standard error
C.V.	:	Coefficient of variation
MQ	:	Milli Q
BSA	:	Bovine serum albumin
R.E.	:	Restriction endonuclease
RIL	:	Recombinant inbred line
DTT	:	Dithiothreitol
SSC	:	Saline sodium citrate
ATP	:	Adenosine triphosphate
E. coli	:	*Escherichia coli*

CHAPTER – I

INTRODUCTION

Plant germplasm is a nonrenewable natural resource indispensable for the sustenance of human life on this earth. In the search for desirable genes in different crop species, plant breeders and biotechnologists depend upon crop diversity as an immediate resource to use in tailoring new varieties and hybrids or for reconstructing the existing genotypes in accordance with the requirements of time and space. Analysis of genetic relationships in crop species is an important component of crop improvement programs as it serves to provide information about genetic diversity, which can be useful for various breeding applications.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a monocot species belonging to the Poaceae family and has a relatively small diploid genome (2n=2x=14) with a DNA content of 1C =2.36 pg (Martel *et al.*, 1997). It is a highly cross-pollinated crop and possesses abundant phenotypic variation. It has a number of wild relatives (n=5, 7, 8 and 9) including a large group with 2n=14 with which it can be intercrossed (Jauhar, 1968; Jauhar, 1981; Jauhar and Hanna, 1998). Globally, pearl millet is the fifth most important food-grain crop following rice, wheat, maize and sorghum. It has the highest levels of tolerance to heat and drought among tropical cereals and is grown on more than 26 million ha in the arid and semi-arid regions of Asia and Africa and 9-10 million ha in India (Khairwal *et al*., 2007), where its grain is often a basic staple for the poorest people. In addition, it has a relatively short growing season (60-90 days) that allows double cropping after wheat, mustard, or winter legume crops have been harvested. It has received relatively little attention of researchers compared to its potential contribution to humanity and is still regarded as an 'orphan' crop. So there is a need to better understand the diversity and genetic basis of this crop.

Pearl millet has great importance as forage and stover crop also and its vegetative matter provides excellent forage because it has low hydrocyanic acid content, and is rich in protein, calcium, phosphorous and other minerals (Athwal and Gupta, 1966). Lack of adequate foliar disease resistance can dramatically reduce the livestock feeding value of pearl millet green forage or crop residues remaining after harvest of a pearl millet grain crop (Wilson *et al*., 1991). Hash *et al.* (2003) have obtained some useful experience in quantitative trait loci (QTL) mapping and marker-assisted selection (MAS) for stover yield, foliar disease resistance, and *in vitro* estimates of the nutritive value of various stover fractions for ruminants in pearl millet and sorghum. In addition to this, QTL mapping of disease resistance (Jones *et al*., 2002; Morgan *et al*., 1998; Hash and Witcombe, 2001; Breese *et al*., 2002), drought tolerance (Yadav *et al*., 2004; Bidinger *et al*., 2007) and the association of flowering time with genotype x environment interaction of grain and stover yield (Yadav *et al*., 2003) has been done; but so far there are no reports on QTL

identification for foliar disease resistance in pearl millet that is effective in Asia or Africa. Among the various foliar diseases of economic importance in pearl millet like downy mildew, rust, pyricularia leaf spot and blast; rust, caused by the fungus *Puccinia substriata* var *indica* is the worst production constraint for this crop worldwide, leading to losses of up to 76% in grain production, as well as major losses in fodder quality (Wilson *et al.*, 1996). Therefore, improving pearl millet rust resistance to reduce annual yield and quality losses has become a high priority for breeders.

Pearl millet cultivars are generated from a narrow gene pool and current breeding programs do not make use of wild pearl millets, and there is only limited use of landrace germplasm. Genetic diversity studies in *Pennisetum* germplasm offer possibilities for their use in improving pearl millet open-pollinated varieties and hybrids. These efforts require effective DNA marker-based fingerprinting strategies for rapid assessment of genetic relationships. Such DNA markers are also required for the construction of molecular linkage maps for efficient QTL mapping (the first step in the genetic dissection of target traits) and MAS for trait introgression, as molecular markers play an important role in improving our understanding in respect of the genetic basis of economically important traits and are efficient tools to speed up crop improvement (Langridge, 2005; Varshney and Tuberosa, 2007).

During the past decades, various molecular markers have been developed and applied in crop genetic diversity analysis, gene or QTL mapping, and molecular marker-aided selection. Restriction fragment length polymorphisms (RFLPs) (Botstein *et al*., 1980; Miller, 1990), random amplified polymorphic DNAs (RAPDs) (Williams *et al*., 1990; Gonzalez, 1993), microsatellites or simple sequence repeats (SSRs) (Weber and May, 1989; Akkaya *et al*., 1992; Jain *et al*., 1994), sequence-tagged-sites (STSs) (Talbert *et al*., 1994), and amplified fragment length polymorphisms (AFLPs) (Vos *et al*., 1995) are among those that have been most commonly used. These technologies can genotype agricultural crops with varying degrees of efficiency. Among these, microsatellite markers remain standard as they are highly polymorphic, require a small amount of DNA, exhibit codominant inheritance, can be easily automated, allow high throughput screening, recognise multiple alleles and are distributed throughout the genome, can be exchanged between laboratories and are highly transferable between populations (Gupta *et al*., 1999, Jeffreys *et al*., 1985; Tautz *et al*., 1986, Hernandez *et al*., 2002). Compared to better-studied cereals such as rice, wheat, maize, and barley, there has been relatively little research on the development and application of molecular genetic tools for pearl millet (Liu *et al.,* 1996, 1997; Busso *et al*., 1995; Burton and Wilson, 1995; Bhattacharjee *et al.,* 2002; Azhaguvel *et al*., 2003; Bidinger *et al*., 2007). RFLPs (Liu *et al.,* 1994 b), STSs (Gale *et al*., 2001), AFLPs (vom Brocke *et al*., 2003), genomic SSRs (Qi *et al*., 2004; Allouis *et al*., 2001; Budak *et al*., 2003), single-strand conformational polymorphisms (Bertin *et al.,* 2005), and genic SSRs (Senthilvel *et al*., 2004, 2008; Mariac *et al*., 2006; Yadav *et al*., 2007) have been developed for pearl millet.

Most of the currently available marker systems are gel-based and have various degrees of limitations associated with their capability to quickly develop and/or rapidly assay large numbers of markers. Although some of the limitations can be overcome by utilizing specialized hardware such as high-throughput capillary electrophoresis machines, which can improve allelic discrimination ability, reproducibility and speed. However, the majority of the limitations are related to the sequential nature, low reproducibility, high assay costs of the marker technologies. In addition, development of SSR, STS and single nucleotide polymorphism (SNP) markers is reliant on DNA sequence information. Thus, the available markers significantly limit the capacity of breeding programs to obtain sufficient return on investment to justify the routine use of marker-assisted breeding for many traits and particularly quantitative traits. Recently, SSRs have become the markers of choice for cereal genetic analysis and mapping (Varshney *et al*., 2007), but their suitability for high-throughput mapping does not favorably compare to the new SNP-based genotyping techniques (Kilian *et al*., 2005). Moreover, SSR-multiplexing in order to drive down marker data point costs requires extensive, additional optimization (Hayden *et al*., 2008).

Marker technologies are undergoing a transition from predominantly serial assays that measure the size of DNA fragments to hybridization-based assays with high multiplexing levels. Two hybridization-based technologies have emerged: SNPs (Chee *et al.,* 1996) and Diversity Arrays Technology (DArT) (Jaccoud *et al*., 2001; Wenzl *et al*., 2004). Several microarray-based marker methods have been developed, but most of these still require sequence information (Pastinen *et al*., 2000; Cutler *et al*., 2001; Flavell *et al*., 2003; Borevitz *et al*., 2003; Winzeler, 2003; Li *et al*., 2004; Ji *et al*., 2004). It has been established that SNP is the most abundant marker type, promising nearly unlimited supply of markers (Chee *et al.,* 1996). Although the progress in genome sequencing and SNP identification has been impressive in humans and a limited number of model organisms, the high cost of SNP marker discovery and assay development limits their applicability for many crops, especially for the 'orphan' crops and polyploid species. Consequently, a low cost, high-throughput and electrophoresis-independent technique that can generate hundreds of molecular markers that cover the entire genome in a single, simple and reliable experiment was required to improve upon those tedious and time-consuming methodologies, especially for those crops with less-developed molecular markers like pearl millet, barley, maize, potato and tobacco (Luikart *et al*., 2003). So, DArT was developed, which performs well in polyploid species and can be rapidly developed for practically any genome in contrast to SNPs and offers a practical solution to the problems as both marker discovery and routine analysis are carried out using the same, hybridization-based assay. So, it is a cost effective, solid state platform, hybridisation-based marker technology that offers a high multiplexing level being able to simultaneously type several thousand loci per assay, while being independent of sequence information (Jaccoud *et al*., 2001; Wenzl *et al*., 2004). The cost of DArT markers per data point has been reported to be 10-fold lower than the cost of SSR (Xia *et al*.,

2005). This genotyping method was developed originally for rice (Jaccoud *et al*., 2001) and has subsequently been used in genetic mapping and fingerprinting studies in many other crops including barley (Wenzl *et al*., 2006; Alsop *et al.,* 2007; Hearnden *et al*., 2007), cassava (Xia *et al*., 2005), *Arabidopsis* (Wittenberg *et al*., 2005), pigeonpea (Yang *et al*., 2006), rice (Xie *et al.,* 2006), wheat (Akbari *et al*., 2006; Crossa *et al.,* 2007; Neumann *et al*., 2010), sorghum (Bouchet *et al*., 2007; Jordan *et al*., 2007; Mace *et al*., 2008), sugarcane (Heller-Uszynska *et al*., 2007), banana (Hippolyte *et al*., 2007; Huttner *et al*., 2007; Risterucci *et al*., 2009), Festuca-Lolium complex (Kopecký *et al*., 2009), oat (Tinker *et al*., 2009) and rye (Bolibok-Bragoszewska *et al*., 2009). DArT assays generate whole-genome fingerprints by scoring the presence versus absence of DNA fragments in genomic representations generated from genomic DNA and combine a complexity reduction method (Wenzl *et al*., 2004).

Genetic linkage maps developed so far for pearl millet are mainly based on RFLPs and SSRs (Liu *et al*., 1994 b; Qi *et al*., 2004), and generally provide less than optimal genome coverage and marker density. Genetic maps produced in four different crosses of pearl millet have been integrated to develop a consensus map of 353 RFLP and 65 SSR markers (Qi *et al*., 2004). In this map, 85% of the markers are clustered and occupy less than one third of the total map length. This phenomenon is independent of the cross. Extreme localization of recombination toward the chromosome ends, resulting in gaps on the genetic map of 30 cM or more in the distal regions, is typical for pearl millet. The unequal distribution of recombination has consequences for the transfer of genes controlling important agronomic traits from donor to elite pearl millet germplasm. To date, only circa 85 PCR compatible markers have been mapped in pearl millet. The length of published linkage maps so far ranged from 280 cM (Jones *et al*., 2002) to 675 cM (Senthilvel *et al*., 2008). Hence, there is a need to fill the gaps in these maps and saturate them for further exploitation. DArT has the potential to generate hundreds of high-quality genomic dominant markers with a cost- and time-competitive trade-off (Kilian *et al*., 2005). DArT markers can be used for diversity analyses and to construct high density genetic linkage maps. The high number of DArT markers generated in a single assay not only provides a precise estimate of genetic relationships among genotypes, but also their even distribution over the genome offers real advantage for a range of molecular breeding and genomic applications.

Thus, keeping the above facts in view, the present investigation was carried out with the following objectives:

1. To develop a DArT platform for pearl millet genotyping and diversity analysis.
2. To assess utility of DArT technology in diversity analyses compared to SSR markers previously used in characterizing a genetically diverse set of parental inbreds of available pearl millet mapping populations and construction of a pearl millet genetic linkage map based on DArT markers.
3. To identify quantitative trait loci associated with rust resistance in pearl millet.

CHAPTER – II

REVIEW OF LITERATURE

Genetic diversity plays a huge role in the survival and adaptability of a species. With very little gene variation within the species, healthy reproduction becomes increasingly difficult, and offsprings suffer problems due to inbreeding. Plant genetic resource is the component of genetic diversity that provides raw material for breeding new varieties of crops better able to cope with biotic and abiotic stresses. The richness and range of the diversity of landraces is now under threat because of many factors (Myers, 1994) and future progress in crop improvement largely depends on immediate conservation of genetic resources.

2.1 Genetic diversity analysis in plants

Accurate assessment of genetic diversity can be invaluable in crop breeding for diverse applications like analysis of genetic variability in cultivars (Cox *et al.,* 1986), identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998) and introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al.,* 1998). An understanding of genetic relationships among inbreds or pure lines can be useful in planning crosses, in assigning lines to specific heterotic groups, and for precise identification with respect to plant varietal protection (Hallauer and Miranda, 1988).

A number of methods are currently available for analysis of genetic diversity. These methods have relied on pedigree data (Bernardo, 1993; Messmer *et al.,* 1993; van Hintum and Haalman, 1994), morphological data (Smith and Smith, 1992; Bar-Hen *et al.,* 1995), agronomic performance data, biochemical data obtained by analysis of isozymes (Hamrick and Godt, 1997) and storage proteins (Smith *et al.*, 1987) and recently, DNA-based markers that allow faster and more reliable differentiation of genotypes and assessment of genetic diversity (Law *et al.,* 1998; Cooke and Reeves, 1998; Donini *et al*., 2000 a, b; Koebner *et al.,* 2003; Mohammadi and Prasanna, 2003).

2.2 Genetic diversity in pearl millet

Pearl millet is a highly cross-pollinated species and genetic diversity in the species is distributed both within and among cultivars. Due to its highly outcrossing breeding behaviour, its apparent origin from several independent domestication events (Poncet *et al.*, 1998) and the wide range of stressful environments in which it has traditionally been cultivated, pearl millet exhibits a tremendous amount of polymorphism at both phenotypic and genotypic levels (Liu *et al.,* 1992, 1994 a, b). Analyzing pearl millet genetic diversity, its origin and its dynamics is important for germplasm conservation and to increase knowledge useful for breeding programs. The more complicated distribution of diversity in pearl millet, as well as the higher degree of marker

polymorphism, makes genetic diversity studies in this crop more complicated than in the other crops. Thus, the breeding behaviour of pearl millet, and the structure of genetic diversity within this species, has strong implications for the use of molecular markers in its diversity assessment.

DNA-based markers have been applied successfully to discriminate between individual genotypes in a wide range of plant species (Epplen *et al*., 1991). In pearl millet, limited efforts have been made to study genetic diversity and various markers used included isozymes (Tostain *et al*., 1987; Tostain and Marchais, 1989; Tostain, 1992, 1994), RFLPs (Bhattacharjee *et al.,* 2002), AFLPs (vom Brocke *et al*., 2003), and recently microsatellites (Budak *et al*., 2003; Mariac *et al.,* 2006; Chakauya and Tongoona, 2008; Kapila *et al.,* 2008). The availability of DNA-based markers has provided more powerful tools for the detailed assessment of genetic diversity in cultivated and wild plants (Melchinger *et al*., 1994). Karp and Edwards (1995) demonstrated that RFLP markers are polymorphic, reproducible and, because of their co-dominant nature, ideal for the discrimination of genotypes. RFLP analyses showed that genetic polymorphism in the pearl millet gene pool is very high, not only between species (Liu *et al*., 1992), but also within landraces of the cultigen (Pilat-Andre *et al.,* 1992). This is because the crop is allogamous and, most importantly, subject to frequent genetic exchange between wild and cultivated genotypes (Brunken, 1977). Bhattacharjee *et al.* (2002) assessed genetic diversity within and between a subset of core landrace accessions with a set of selected RFLP markers. A total of 51 alleles were detected using 16 different probe-enzyme combinations, revealing high within-accession variability (30.9%); however, the variability between accessions was significantly higher (69.1%) than that within accessions. Development and utilization of PCR-based markers such as SSRs is a valuable asset for estimating genetic diversity, the identification of unique genotypes as potentially important new sources of alleles for enhancing important characteristics, analyzing the evolutionary and historical development of cultivars at the genomic level in pearl millet breeding programs (Budak *et al.,* 2003; Kapila *et al*., 2008). Chandra-Shekara *et al.* (2007) indicated moderate genetic divergence among elite pearl millet germplasm, besides unraveling the genetic relationships among male-sterile lines and restorers using RAPDs and SSRs. Mariac *et al.* (2006) and Oumar *et al.* (2008) observed significantly lower numbers of alleles and lower gene diversity in cultivated pearl millet accessions than in wild accessions using SSRs.

2.3 Markers and their importance

Conventional cereal breeding is time consuming and environment dependent. Use of molecular markers can increase breeding efficiency and genetic gains from selection relative to phenotypic selection alone (Knapp, 1998; Eathington *et al.,* 1997; Lande and Thompson, 1990). Hence, over the past 30 years, molecular marker technologies have been developed and applied to plant breeding, enabling breeders to use the genetic composition or genotypes of plants as a criterion for selection and breeding progress. Although DNA sequencing is a straightforward approach for identifying variation at a locus, historically it has been expensive and laborious. A wide variety of

alternative techniques have, therefore, been developed for visualizing DNA sequence polymorphism. As full genome sequences will not be available for many species of interest in the near future, it has been important to find strategies for developing and using molecular markers when sequence resources are limited. So, scientists have been using genetic markers for indirect analysis of genetic variation, development of improved cultivars (Sharma *et al*., 2002; Varshney *et al.,* 2006; Collard and Mackill, 2008), establishment of linkage maps that allow breeders to conduct applied research to identify, characterize and use genetic variability in economically important plants (Frova *et al*., 1999; Crossa *et al.,* 1999). The main reasons supporting the utilization of molecular markers in breeding programs are the heritability of markers (theoretically 100%), and their cost, which is potentially lower than the conventional phenotypic selection (Winter and Kahl, 1995). In addition, molecular markers can be used for selection for traits with low heritability, identification of resistance or tolerance for biotic or abiotic stresses in plants, the gene introgression coming from native or exotic germplasm (Gupta *et al*., 1996), estimation of genetic relatedness among accessions, cultivar description (Smith *et al*., 1992) and the identification of QTLs that control important agronomic traits (Dudley, 1993).

DNA or molecular markers are identifiable DNA sequences, found at specific locations of the genome, transmitted by the standard laws of inheritance from one generation to the next (Semagn *et al.,* 2006 a) and reflect heritable differences (*e.g*., polymorphisms) in homologous DNA sequences among individuals. An ideal molecular marker should have some desirable properties like easy availability, rapid and easy to assay, highly polymorphic and reproducible, codominant inheritance and recurrent occurrence in genome, selectively neutral to environmental conditions or management practices, and easy exchange of data between different laboratories. However, it is really difficult to get molecular markers fully meeting the above criteria. A large number of reviews have been published on molecular markers and their application in crop improvement (Jain *et al*., 2002; Lörz and Wenzel, 2005).

2.4 Types of molecular markers

The molecular markers can be classified into different groups based on mode of transmission, mode of gene action and method of analysis. For hybridization-based markers, DNA profiles are visualized by hybridizing restriction endonuclease digested DNA fragments, to a labelled probe, which is a DNA fragment of known sequence. In constrast, PCR-based markers involve *in vitro* amplification of particular DNA sequences with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme. The amplified DNA fragments are separated by electrophoresis (Sharma *et al*., 2008).

There are various types of DNA-based molecular markers such as RFLP, RAPD, AFLP, STS, SSR, expressed sequence tag (EST), inter-simple sequence repeat (ISSR), SNP, and DArT (reviewed by Semagn *et al*., 2006 a). These may differ in a variety of ways, such as their technical requirements; the amount of time, money and labour needed; the number of genetic

markers that can be detected throughout the genome and the amount of genetic variation found at each marker in a given population (Choudhary *et al*., 2008). RFLP is the most widely used hybridization-based molecular marker. RAPD, AFLP, ISSR, EST, SSR, STS etc. are PCR-based markers and SNPs are sequence-based DNA markers. SSRs are mostly codominant markers and are indeed excellent for studies of population genetics and mapping (Jarne and Lagoda, 1996; Goldstein and Schlotterer, 1999), QTL analysis, forensics, and diagnostics (Powell *et al*., 1996; Schlotterer, 2004; Varshney *et al*., 2005). The use of fluorescent primers in combination with automatic capillary or gel-based DNA sequencers has been adapted in most advanced laboratories and SSRs are excellent markers for fluorescent techniques, multiplexing and high-throughput analysis. SNPs have enjoyed massive popularity for their high density within the genome and their ease of characterization. However, their identification requires access to reliable DNA sequence from the complete range of plants strains/varieties or ecotypes that will subsequently be used. Further, the sequential nature of the above gel-based marker systems reduces throughput, increasing costs per assay. DArT is one of the recently developed microarray hybridization-based DNA marker technique that enables simultaneous genotyping of several hundred polymorphic loci across the genome (Jaccoud *et al*., 2001; Wenzl *et al*., 2004). No molecular markers are available yet that fulfill all requirements needed by researchers. According to the kind of study to be undertaken, one can choose among the variety of molecular techniques that combines at least some desirable properties.

2.5 Diversity Arrays Technology (DArT)

The DArT technology was originally developed by A. Kilian and D. Jaccoud (Jaccoud *et al*., 2001) at the Centre for Application of Molecular Biology to International Agriculture (CAMBIA). The inventors promote it as an open source (nonexclusive) technology with a great potential for genetic diversity and mapping studies in a number of crops. It is a novel, solid-state, microarray-based, open-platform method for genomewide discovery and genotyping of genetic variation. A DArT marker is a segment of genomic DNA, the presence of which is polymorphic in a defined genomic representation. DArT markers are biallelic and behave in a dominant (present vs absent) or co-dominant (2 doses vs 1 dose vs absent) manner. DArT operates on the principle that the genomic representation contains two types of fragments: constant fragments, found in any representation prepared from a DNA sample from an individual belonging to a given species, and variable (polymorphic) fragments called molecular markers, only found in some but not all of the representations. The variable fragments called DArT markers are informative because they reflect sequence variation that determines the fraction of the original DNA sample that is included in the representation. DArT allows simultaneous scoring of hundreds of restriction site based polymorphisms between genotypes and does not require DNA sequence information or site-specific oligonucleotides. To identify the polymorphic markers, a complexity reduction method is applied on the metagenome, a pool of genomes representing the germplasm

of interest. The genomic representation obtained from this pool is then cloned and individual inserts are arrayed on a microarray resulting in a "discovery array." Labeled genomic representations prepared from the individuals can be genotyped by hybridisation to the discovery array. Polymorphic clones (DArT markers) show variable hybridization signal intensities for different individuals. The hybridization signal for each marker is measured and converted into a score. DArT fingerprints will be useful for accelerating plant breeding, and for the characterisation and management of genetic diversity in domesticated species as well as in their wild relatives. Wenzl *et al.* (2004), Syvanen (1999) and Xia *et al.* (2005) reported simultaneous analysis of hundreds of markers at once, with the added advantage of much lower cost per marker than other technologies like SNPs and microsatellites (Huttner *et al*., 2005). In contrast to current SNP technologies, DArT performs well in polyploid species such as wheat (Akbari *et al*., 2006; Wenzl *et al*., 2007), banana (Kilian, 2007) and sugarcane (Heller-Uszynska *et al*., 2007).

DArT offers low cost, quick, high throughput, electrophoresis-independent, highly reproducible and sequence-independent genotyping. The other advantages include fast data acquisition and analysis, detection of single-base changes as well as insertions/deletions, detection of differences in DNA methylation depending on the enzyme used to generate the fragments, generatation of sequence-ready clones, minimal DNA sample requirement, good transferability of markers among breeding populations, and high quality markers. The same platform is used for both discovery and scoring of markers, therefore, no assay development is required after the initial marker discovery. The system is highly automated and the data generated will have increasing value with continuing advances in bioinformatics, particularly if polymorphic clones are sequenced. The genetic scope of DArT analysis is defined by the user and easily expandable. This technique, however, has also its own limitations as DArT markers are primarily dominant and the microarray-based technique that several steps, including preparation of genomic representations for the target species, cloning, and data management and analysis. The latter requires dedicated software such as DArTsoft and DArTdb. The establishment of a DArT system, therefore, demands extensive investment both in laboratory facilities and skilled manpower. Intellectual property constraints and process variation also limit its widespread use.

2.6 Applications of DArT

Potential applications of DArT include genome profiling and genome background screening, rapid construction of high-density genetic linkage maps (Akbari *et al*., 2006; Alsop *et al.,* 2007; Mace *et al*., 2008), identification of QTLs (Alsop *et al.,* 2007; Pozniak *et al.,* 2007; Rheault *et al.,* 2007), association mapping (Bouchet *et al.,* 2007), rapid introgression of genomic regions in accelerated backcrossing programs, simultaneous marker-assisted selection for several traits, microbial diagnostics, evaluation of genetic diversity, rapid germplasm characterization and tracking genome methylation changes in a cost-effective and high-throughput manner (Jaccoud *et al.,* 2001; Wenzl *et al.,* 2004). The types of polymorphism detected by DArT (single nucleotide

polymorphisms, insertion-deletions and methylation changes) expand the potential of traditionally used markers, increasing power to ascertain the structure of germplasm collections. Wenzl *et al*. (2007) used a DArT platform for quantitative bulk segregant analysis (BSA) in barley and found that DArT-BSA identifies genetic loci that influence phenotypic characters in barley with at least 5 cM accuracy and should prove useful as a generic tool for high-throughput, quantitative BSA in plants irrespective of their ploidy level.

2.7 Availability of DArT for different species/crops

As the DArT technique proved to be efficient for marker discovery and screening in various species, it has been applied in a number of plant, animal, microbial and fungal species. It has most widely been used in plants and has proven to be successful in the various species like rice (Xie *et al*., 2006), cassava (Xia *et al.,* 2005), barley (Wenzl *et al.,* 2004, 2006), wheat (Akbari *et al.,* 2006; Semagn *et al.,* 2006 b; White *et al.*, 2008), pigeonpea (Yang *et al.,* 2006), sorghum (Mace *et al.,* 2008), *Arabidopsis* (Wittenberg *et al.*, 2005), banana (Risterucci *et al.,* 2009; Kilian, 2007), eucalyptus (Lezar *et al.,* 2004), fern and moss (James *et al.,* 2006). In addition, DArT platforms have been developed for other crop species such as lupin, potato, quinoa, rice, ryegrass, coconut, apple, lily and tomato (Wang *et al*., 2006; Kilian *et al*., 2005). Currently DArT Pty. Ltd. provides DArT genotyping services for a number of these species. Further, Triticarte Pty. Ltd., a joint venture between the Value Added Wheat CRC Ltd. and DArT Pty. Ltd., has been established to deliver genotyping services for barley and wheat breeders (Huttner *et al*., 2006).

Xie *et al*. (2006) used and validated DArT for rice genotyping in a high throughput manner and 1152 clones were re-arrayed on a slide and used to fingerprint 17 of 24 germplasms. Xia *et al.* (2005) used DArT for high-throughput genotyping of cassava and its wild relatives and detected nearly 1,000 candidate polymorphic clones using two arrays. The genetic relationships among the samples analyzed with DArT were consistent with existing information on these samples. Hurtado *et al.* (2008) compared SSR and DArT markers for assessing genetic diversity in cassava and suggested that SSR markers, while low throughput in comparison with DArTs, are relatively better at detecting genetic differentiation in cassava germplasm collections. Wenzl *et al.* (2004) used DArT for whole-genome profiling of barley and constructed a genetic map for a cross between cultivars Steptoe and Morex. Most of the DArT markers (98.8%) were incorporated into a linkage map whose quality was superior to that of an RFLP-based framework map (Wenzl *et al.*, 2004). The resulting map included 385 unique DArT markers and spanned 1,137 cM. A polymorphism-enriched *Pst*I/*Bst*NI array was produced from 1,920 candidate polymorphic clones. Wenzl *et al.* (2006) built a high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci, this comprised 2,935 loci (2,085 DArT, 850 other loci) and spanned 1,161 cM. A similar study in *Arabidopsis* confirmed the Mendelian behavior of DArT markers, and also established perfect co-linearity between the genetic and the physical maps (Wittenberg *et al.,* 2005). Hearnden *et al.* (2007) developed a high-density genetic map in

wide barley cross between cultivated barley (*Hordeum vulgare*) and wild subspecies *H. vulgare* ssp. *spontaneum*. The map comprises 1,000 loci, including 558 SSR (detected by 536 primer pairs) and 442 DArT markers. To incorporate novel alleles into cultivated barley, Alsop *et al.* (2007) crossed a wild barley accession possessing multiple disease resistance (Damon) with a malting cultivar (Harrington) and constructed a DArT marker-based linkage map for qualitative/quantitative trait analysis of disease resistance loci. A genotyping array was developed for sorghum representing approximately 12,000 genomic clones using *Pst*I+*Ban*II complexity with a subset of clones obtained through the suppression subtractive hybridisation method. Over 500 markers detected variation among 90 accessions used in a diversity analysis and an integrated linkage map was also constructed with DArT markers, which spanned 1431.6 cM (Mace *et al.,* 2008). Yang *et al.* (2006) reported the development of DArT for pigeonpea using a *Pst*I/*Hae*III array, which revealed low levels of genetic diversity in cultivated pigeonpea compared to its wild relatives. A total of nearly 700 markers were identified with the average call rate of 96.0% and the scoring reproducibility of 99.7%. Semagn *et al.* (2006 b) compared the utility of DArT with AFLP and SSR markers, in a genetic linkage map of a doubled-haploid hexaploid wheat population. The map contains a total of 624 markers with 189 DArTs, 165 AFLPs and 270 SSRs, and spans 2595.5 cM. It has been successfully used to identify novel QTLs for resistance to *Fusarium* head blight and powdery mildew. Akbari *et al.* (2006) used DArT for high-throughput profiling of the hexaploid wheat genome and generated a large number of high-quality markers in wheat (99.8% allele-calling concordance and approximately 95% call rate). Mantovani *et al.* (2008) developed a DArT platform for durum wheat. The integrated DArT-SSR map included 554 loci (162 SSRs and 392 DArT markers) and spanned 2022 cM. White *et al.* (2008) analyzed the genetic diversity of UK, US and Australian cultivars of *Triticum aestivum* measured by DArT markers. Risterucci *et al.* (2009) used DArT for high-throughput DNA analyses in *Musa* and found that DArT markers revealed genetic relationships among *Musa* genotypes consistent with those provided by the other markers technologies, but at a significantly higher resolution and speed, and reduced cost. Bonin *et al.* (2008) used a new miniature inverted repeat transposable element (MITE) based genome complexity reduction method taking advantage of the abundance of MITEs in the genome of mosquito *Aedes aegypti* and constructed a library comprising more than 6,000 DArT clones.

2.8 Molecular markers in pearl millet

The first major milestone was achieved in 1993 with the creation of a genetic linkage map of the pearl millet genome with 181 RFLP markers (Liu *et al.,* 1994 b). By 2001, hundreds of pearl millet molecular markers had been created (Liu *et al.,* 1994 b; Allouis *et al.,* 2001; Qi *et al.,* 2001), detailed marker-based genetic linkage maps produced (Liu *et al.,* 1994 b, 1996; Devos *et al.,* 2000) and using those maps QTLs for pearl millet downy mildew resistance were flagged (Jones *et al.,* 1995, 2002; Azhaguvel, 2001; Kolesnikova, 2001; Breese *et al.,* 2002). Initially,

most DNA marker-based studies in pearl millet used RFLP markers (Liu *et al.,* 1994 b). These markers were used in studies of recombination rates (Liu *et al.,* 1996) and genetic diversity (Bhattacharjee *et al*., 2002), and QTL mapping of disease resistance (Jones *et al.,* 1995, 2002; Morgan *et al*., 1998; Hash and Witcombe, 2001; Breese *et al.,* 2002), drought tolerance (Yadav *et al*., 1999, 2002, 2004; Bidinger *et al*., 2007), and the association of flowering time with genotype x environment interaction of grain and stover yield (Yadav *et al.,* 2003). The potential of DNA markers such as microsatellites, minisatellites, STS markers (Gale *et al*., 2001), AFLPs (vom Brocke *et al.,* 2003) and RAPDs was also investigated in pearl millet. Various SSR markers were developed for pearl millet (Qi *et al.*, 2001, 2004; Allouis *et al.,* 2001; Budak *et al*., 2003) but many more are required for their applied use in plant breeding. Discovery of microsatellites in ESTs provides the opportunity to develop SSR markers (EST-SSRs) in a simple and direct way, *i.e*., by electronic searches (data mining) of EST databases. Senthilvel *et al*. (2004, 2008), Mariac *et al.* (2006), Yadav *et al*. (2007) used this approach to design SSR markers for pearl millet. Mishra *et al.* (2007) isolated ESTs from subtracted cDNA libraries of pearl millet and a total of 2,494 EST sequences were clustered and assembled into a collection of 1,850 unique sequences with 224 contigs and 1,626 singleton sequences. A new marker system, single-strand conformational polymorphism (SSCP)-SNP, was developed using annotated rice genomic sequences to initially predict the intron-exon borders in millet ESTs and then to design primers that would amplify across these introns (Bertin *et al.,* 2005).

2.9 QTL mapping in pearl millet

With rapid advancement of molecular technology, it is now possible to use molecular marker information to map major QTLs on chromosomes (Paterson *et al.,* 1988, 1991; Hilbert *et al.,* 1991; Jacob *et al.,* 1991; Stuber *et al.,* 1992). Mapping is putting markers in order, indicating the relative genetic distances between them, and assigning them to their linkage groups on the basis of the recombination values from all their pairwise combinations. QTL mapping provides a means to dissect complex phenotypic characters into their component traits (QTLs), and allows the identification of molecular markers linked to desirable QTLs, so that these can be directly used in marker-assisted selection (Tanksley *et al.,* 1989; Lee, 1995; Schneider *et al.,* 1997; Mohan *et al.,* 1997; Paterson, 1996 a, b). The theory of QTL mapping was first described in 1923 by Sax and was further elaborated by Thoday (1961). QTLs are identified via statistical procedures that integrate genotypic and phenotypic data. QTL mapping studies have been reported in most crop plants for diverse traits including yield, quality, disease and insect resistance, abiotic stress tolerance and environmental adaptation. QTL mapping requires a suitable mapping population generated from phenotypically contrasting parents, a saturated linkage map based on molecular markers, reliable phenotypic screening of mapping population, appropriate statistical packages to analyze the genotypic information in combination with phenotypic information for QTL detection. A number of methods for mapping QTL and

estimating their effects have been suggested and investigated (Edwards *et al.,* 1987; Haley and Knott, 1992; Jiang and Zeng, 1995; Lander and Botstein, 1989; Jansen and Stam, 1994; Utz and Melchinger, 1994; Zeng, 1994). Methods for QTL mapping range from the simplest method of single-marker analysis (Sax, 1923) to more sophisticated methods such as interval mapping (Lander and Botstein, 1989; Haley and Knott, 1992), joint mapping (Kearsey and Hyne, 1994), multiple regression (Wright and Mowers, 1994; Whittaker *et al.,* 1996), and composite interval mapping (Zeng, 1994). Software packages for mapping include *MAPMAKER/QTL* (Lincoln *et al.,* 1993), *JoinMap* (Stam, 1993), *QTL Cartographer* (Basten *et al.,* 1994), *PLABQTL* (Utz and Melchinger, 1996), *QGene* (Nelson, 1997), and *TASSEL* (Buckler, 2007).

Genetic linkage maps in pearl millet have been constructed and QTLs identified and mapped for downy mildew resistance (Jones *et al.,* 1995, 2002; Breese *et al.,* 2002; Gulia, 2004; Gulia *et al.,* 2007), rust and blast resistance (Morgan *et al.,* 1998), drought tolerance (Yadav *et al.,* 2002, 2004), flowering time and grain and stover yield (Yadav *et al.,* 2003), and ruminant nutritional quality of straw (Hash *et al.*, 2003), for characterstics involved in domestication (Poncet *et al.,* 2000, 2002). Jones *et al.* (2002) detected the same two QTLs for resistance to downy mildew in pearl millet in two different environments *i.e.* field and glasshouse. One QTL had a major effect and explained up to 60% of the phenotypic variation, while the other had a minor effect and explained up to 16% of the phenotypic variation. Bidinger *et al.* (2007) identified QTLs that improve grain yield across the full range of postflowering moisture conditions, rather than just in drought-stressed environments. Yadav *et al.* (2002) studied QTLs associated with traits determining grain and stover yield in pearl millet under terminal drought-stress conditions and found a QTL associated with grain yield *per se* and the drought tolerance of grain yield mapped on linkage group 2, explaining up to 23% of the phenotypic variation.

2.10 Rust resistance in pearl millet

Pearl millet rust (*Puccinia penniseti Zimm=P. substriata var. indica*) can reduce yields in hybrid seed production fields, quality in forage, and occasionally grain yields. Thus, rust resistance has become a high priority for pearl millet breeders. Rust was first reported on pearl millet in 1904 (Zimmerman, 1904 in Singh and King, 1991). It was first recorded in India as being caused by *Puccnia penniseti* Zimm. (Ramakrishnan and Soumini, 1948) and in the U.S. in 1954 as *P. penniseti* Zimm. (Luttrell, 1954) and as *P. substriata* var *indica* in 1973 (Wells *et al.,* 1973). It can infect pearl millet at any stage during the growing season and resistance genes are the primary control method for rust on pearl millet. Resistance to rust has been reported in some pearl millet germplasm accessions and breeding lines (Wilson, 1993 a; Singh *et al.,* 1997). However, lines that were resistant in India are susceptible in USA indicating existence of different physiological races in India and USA (Wilson 1991, Tapsoba and Wilson, 1996). Although a number of germplasm accessions and some breeding lines have shown good level of rust resistance (Singh *et al.,* 1997), identification of resistance in elite advanced breeding lines is

likely to be more useful and effective in resistance breeding than transferring resistance from a germplasm accession, which requires more time and resources. Andrews *et al*. (1985) reported that a single dominant gene, Rpp_1, conferred rust resistance effective in India. The original source of rust resistance deployed in USA came from a wild subspecies [*P. glaucum* subspecies *monodii* (Maire) Brunken] of pearl millet from Senegal (Hanna *et al.,* 1985). This resistance gene was labeled Rr_1 and was used in the parental lines 'Tift 85DB' (Hanna *et al.,* 1987) and 'Tift 65' (Burton and Wilson, 1995), a grain hybrid (Hanna, 1993), and a forage cultivar (Hanna *et al.,* 1988). Beginning in 1992, the complete resistance conferred by Rr_1 was overcome by a new race or races of the rust pathogen (Wilson, 1993 b), and today rust can be found on all lines with this gene. However, the Rr_1 gene still confers resistance to many rust isolates purified from natural rust populations and therefore should be used in any new resistant cultivar. A second source of rust resistance comes from the Senegalese pearl millet landrace 'Sa Fe'. This resistance is believed to be controlled by more than one gene (Hanna and Wells, 1993). Screening with different isolates of *P. substriata* var. *indica* showed that the gene(s) of this second source of resistance are different from the Rr_1 gene (Tapsoba and Wilson, 1996). This resistance has been incorporated into the breeding line 'Tift 89D2' (Hanna and Wells, 1993) and is effective against most isolates virulent to the Rr_1 gene. Morgan *et al.* (1998) used RAPDs and RFLPs and mapped rust resistance genes on linkage groups 3 (Rr_1) and 4 of the pearl millet map.

Efforts to develop rust-resistant pearl millet hybrids have been impeded by variability for virulence in the pathogen population in the southeastern United States. Virulence to Rr_1 (Wilson, 1993 b) and the subsequent identification of many pathotypes (Tapsoba and Wilson, 1996) has indicated the potential response to control through the use of major gene resistance. It was hypothesized that the increased genetic heterogeneity for rust resistance resulting from intermating or its subsequent enhancement through stacking of the resistance genes within heterogeneous populations should significantly suppress disease with an accompanying yield advantage (Tapsoba and Wilson, 1999). Partial rust resistance could complement race-specific resistance and has been identified in pearl millet inbreds (Pannu *et al.*, 1996; Sokhi and Singh, 1984; Wilson, 1994). Wilson (1994) evaluated inbreds Tift 383, 700481-21-8 and ICMP 501 for partial rust resistance in comparison to susceptible inbred Tift 23DB. The partial resistance of 'ICMP 501' was of particular interest because of its high levels of expression in segregating progeny, significant dominance and additive genetic effects, and control by relatively few genes (Wilson, 1997). The expression and inheritance of partial rust resistance of pearl millet inbreds 700481-21-8 and ICMP 501 crossed to moderately susceptible Tift 383 were evaluated. The number of genes conferring partial resistance was estimated to be 2 for 700481-21-8 and 2.5 for ICMP 501 (Wilson, 2006).

CHAPTER – III

MATERIALS AND METHODS

The present investigation entitled "Genetic diversity analysis in pearl millet (*Pennisetum glaucum*) using Diversity Arrays Technology (DArT)" was conducted in the Applied Genomics Laboratory of the Global Theme-Biotechnology at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh. The details of materials used and methods adopted during the course of this investigation are described below:

3.1 MATERIALS

3.1.1 Plant material

The seeds of pearl millet (*Pennisetum glaucum*) for the present study were procured from the RS, Paroda Gene Bank and pearl millet breeding unit, Global Theme-Crop Improvement, ICRISAT, Patancheru, Andhra Pradesh.

3.1.2 Chemicals

During the course of investigation, precautions were taken to use chemicals of high purity only. All the enzymes used in the present study were from New England Biolabs (NEB), UK or Sibenzyme Ltd., Russia. Analytical grade chemicals from Qualigens, India; Sigma Chemicals Company, USA; Hi-Media, India; USB Corporation, USA; Amersham, UK and Life Technologies, USA were used for carrying out all the experiments.

3.1.3 Glasswares and plasticwares

All the glasswares used were of the borosilicate quality obtained from Borosil India. Poly-L-lysine-coated glass slides were procured from Thermo Scientific, USA and coverslips were from Corning and Menzel, Germany. Disposable petridishes, micropipette tips, centrifuge tubes, 96 and 384-well PCR plates and 384-well library storage plates were from Tarsons Products Private Ltd., India; Axygen Scientific, USA and Genetix, respectively.

3.2 METHODS

3.2.1 Development of DArT platform for pearl millet genotyping and diversity analysis

3.2.1.1 Sterilization of glassware and culture media

The glassware was washed properly and rinsed with distilled water and sterilized in hot air oven at 180°C for 2-3 hours. Scalpels, forceps, spreader were flame sterilized using 70 per cent ethanol prior to use. Centrifuge tubes, microtips, PCR plates, spatula, oakridge tubes, culture media were sterilized by autoclaving at 1 kg/cm^2 at 121°C for 15 minutes.

Stock solutions of antibiotics such as kanamycin, ampicillin and other solutions like X-gal, freezing medium and DArT spotter were filter sterilized using millipore syringe filters of pore size 0.2 µm.

3.2.1.2 Seed germination and DNA isolation

To develop the pearl millet DArT array, seeds of 95 diverse genotypes of pearl millet (Table 1) were sown in pots in a glass house and after 10 days young leaves were collected for DNA isolation. DNA was extracted using the SDS buffer method described below:

Procedure for DNA isolation

Approximately 50 mg of young tender leaves were taken and 450 µl of SDS buffer was added and the leaves were ground in a Genogrinder. The ground sample was kept in 65°C water bath for 20 minutes and the contents were mixed inbetween 2-3 times. 450 µl of phenol+chloroform (24:1) mixture was added to it and mixed gently. It was centrifuged at 5500 rpm for 10 minutes. Supernatant was collected and equal volume of chilled isopropanol was added to it and mixed gently. Then, it was kept at -20°C for 10 minutes and centrifuged at 5500 rpm for 10 minutes. Supernatant was discarded and the pellet was collected. To the pellet, 200 µl of low salt TE + 5 µl of RNase (10 mg/ml) was added and pellet was disturbed by tapping gently. Then, it was kept in 37°C incubator for 40 minutes. 200 µl of phenol+chloroform was added to it and mixed gently. Centrifugation was done at 5500 rpm for 5 minutes. Supernatant was collected and 200 µl of phenol+chloroform was added to it and mixed gently and centrifuged at 5000 rpm for 5 minutes. Supernatant was collected and 300 µl of absolute ethanol (100%) and 15 µl of 3 M sodium acetate (pH 5.2) was added per sample. It was mixed gently and kept at -20°C for 10 minutes. It was centrifuged at 6000 rpm for 10 minutes. It was decanted and then 200 µl of 70% ethanol was added and centrifuged at 5500 rpm for 5 minutes. It was decanted again and vaccum dried for 5-10 minutes. 100 µl of $T_{10}E_{10}$ was added per sample and kept at room temperature for 30 minutes.

3.2.1.3 Quantification and dilution of DNA

DNA was quantified on 0.8% agarose gel.

Gel loading orange dye (for 100 ml)

0.5M EDTA (pH=8.0)	10 ml
5M NaCl	1 ml
Glycerol	50 ml
MQ H_2O	39 ml

After adding the above components Orange G dye powder was added until the colour was sufficiently dark and mixed properly.

Procedure

To prepare gel, 0.8 g of agarose was melted in 100 ml of 1X TBE buffer in microwave oven and cooled to 50°C by keeping at room temperature for 5-10 minutes. Now, 5 µl of 10 mg/ml stock of ethidium bromide was added to it. The gel plate was sealed on both sides with tape and a comb was inserted and the gel solution was poured in it gently. Then, it was allowed to solidify for 30 minutes. After setting, the seal tapes were removed from both sides and comb was removed gently. The gel plate was placed in an electrophoresis unit and it was filled with 1X TBE buffer.

Table 1. Pearl millet genotypes used for array development

S. No.	Genotype ID	Origin	Biological status	Region	Remarks
1	IP 11841	IND	Landrace	Asia	Core collection
2	IP 11353	BFA	Landrace	Africa	Core collection, large seed (>18 g)
3	IP 15710	TZA	Landrace	Africa	Core collection
4	IP 11917	SLE	Landrace	Africa	Very late, post rainy (>125 days)
5	IP 20546	NGA	Landrace	Africa	Plant height -tall, rainy
6	IP 10759	SDN	Landrace	Africa	Purplish black seed,endosperm texture-mostly starchy
7	IP 10945	SDN	Landrace	Africa	Core collection
8	IP 6869	KEN	Landrace	Africa	Core collection
9	IP 13137	NER	Landrace	Africa	Core collection
10	IP 9286	TGO	Landrace	Africa	Core collection
11	IP 13344	SDN	Landrace	Africa	Total tillers (<2)
12	IP 8129	ICRISAT	Breeding material (BM)		Thin panicle-rainy (<11 mm)
13	IP 13121	NER	Landrace	Africa	Core collection
14	IP 19408	TCD	Landrace	Africa	Core collection
15	IP 5923	SEN	Landrace	Africa	Endosperm texture-mostly corneous
16	IP 8172	ICRISAT	BM		Top leaves yellow
17	IP 18294	ICRISAT	BM		Resistant to downy mildew (DM)
18	IP 13927	ZWE	Landrace	Africa	Sweet stalk
19	IP 21184	ICRISAT	BM		Resistant to DM
20	IP 21178	ICRISAT	BM		Resistant to DM
21	IP 21163	ICRISAT	BM		Resistant to DM
22	IP 7118	IND	Landrace	Asia	Core collection
23	IP 9981	ZMB	Landrace	Africa	Core collection
24	IP 6793	MWI	Landrace	Africa	Core collection
25	IP 7998	BWA	Landrace	Africa	Panicle shape_globose
26	IP 8823	ZWE	Landrace	Africa	Resistant to rust
27	IP 21268	ICRISAT	BM		Multiple disease resistant (ergot, smut and DM)
28	IP 7979	ICRISAT	BM		Dumb-bell; purple spike
29	IP 9597	YEM	Landrace	Asia	High fodder yield potential
30	IP 21162	ICRISAT	BM		Resistant to DM
31	IP 8074	ICRISAT	BM		Core collection
32	IP 18090	PAK	Landrace	Asia	Core collection
33	IP 9651	NGA	Landrace	Africa	High seed yield potential

34	IP 21491	ICRISAT	BM		Resistant to rust
35	IP 8041	GHA	Landrace	Africa	Zebra
36	IP 8182	ICRISAT	BM		Seed color-purple
37	IP 21172	ICRISAT	BM		Resistant to DM
38	IP 21187	ICRISAT	BM		Resistant to DM
39	IP 12644	ICRISAT	BM		Small panicle- post rainy (<6 cm)
40	IP 14926	UGA	Landrace	Africa	Core collection
41	IP 21181	ICRISAT	BM		Resistant to DM
42	IP 4021	IND	Landrace	Asia	Early-rainy (<37 days), early - post rainy (<40 days); thin panicle (<11 mm)
43	IP 17995	IND	BM	Asia	Panicle shape-dumb-bell
44	IP 2058	NGA	Landrace	Africa	Total tillers (<2)
45	IP 7795	IND	Landrace	Asia	Panicle shape-club
46	IP 22092	NER	Wild	Africa	*P. edicellatum*-resistant to DM
47	IP 21864	TZA	Wild	Africa	*P. edicellatum*-resistant to DM
48	IP 21901	TZA	Wild	Africa	*P. polystachion*-resistant to DM
49	IP 22017	SDN	Wild	Africa	*P. violaceum*
50	IP 21779	SDN	Wild	Africa	*P. mollissimum*
51	IP 22419	ICRISAT			Common-resistant to ergot
52	IP 21789	IND	Wild	Asia	*P. pedicellatum*-resistant to DM
53	IP 21694	ZWE	Wild	Africa	*P. violaceum*
54	IP 21933	USA	Wild	North America	*P. schweinfurthii*-resistant to DM
55	IP 22031	MLI	Wild	Africa	*P. violaceum*-resistant to DM
56	IP 22102	MOZ	Wild	Africa	*P. polystachion*-resistant to DM
57	Tift 23 D_2B_1-P5	ICRISAT	Mapping population-parental line (MPPL)	North America	
58	843 B	ICRISAT	Pearl millet line of interest	North America	Susceptible to DM, ergot and smut
59	Tift 383	ICRISAT	Pearl millet line of interest		
60	IP 21973	IND	Wild	Asia	*P. violaceum*-resistant to DM
61	ICMB 90111-P6	ICRISAT	MPPL		
62	P 1449-2-P1	ICRISAT	MPPL		
63	P 310-17-B	ICRISAT	MPPL		
64	IP 10437	BEN	Landrace	Africa	Core collection, very late-rainy (>151 days), large seed (>18 g)

65	IP 19125	NAM	Landrace	Africa	Forage type
66	PRLT 2/89-33	ICRISAT	MPPL		
67	H 77/833-2-P5 (NT)	ICRISAT	MPPL		
68	ICMP 451-P8	ICRISAT	MPPL		
69	PT 732B-P2	ICRISAT	MPPL		
70	ICMB 841-P3	ICRISAT	MPPL		
71	ICMP 85410-P7	ICRISAT	MPPL		
72	Tift $238D_1$-P158	ICRISAT	MPPL	North America	
73	81B-P6	ICRISAT	MPPL		
74	IP 18293-P152	ICRISAT	MPPL		
75	W 504-1-P1	ICRISAT	MPPL		
76	JBV 2	IND			
77	863B-P2	ICRISAT	MPPL		
78	IPC 804-P4	ICRISAT	MPPL		
79	WSIL-P8	ICRISAT	MPPL		
80	ICTP 8203				Check
81	LGD 1-B-10	ICRISAT	MPPL		
82	ICMR 01004	ICRISAT			
83	IP 6037				
84	IP 9453				
85	IP 11670				
86	IP 11765				
87	IP 14317				
88	IP 14811				
89	IP 15533				
90	IP 17405				
91	IP 17956				
92	IP 21155				
93	IP 22138	TZA	Wild	Africa	*P. ramosum*-resistant to DM
94	IP 21897	ZMB	Wild	Africa	*P. olystachion*-resistant to DM
95	IP 21950	IND	Wild	Asia	*P. orientale*-resistant to DM

The DNA sample was prepared by adding 5 µl of gel loading dye and 1 µl of the DNA sample and then it was loaded on agarose gel and electrophoresis unit was run at 80 V for 20 minutes and then gel was visualized under UV and photographed on the gel documentation system. After quantification of DNA, it was diluted to 50 ng/µl.

3.2.1.4 Restriction enzyme digestion and adapter ligation

DNA (50 ng/µl) of suitable genotypes was taken and combined restriction digestion and ligation reaction was carried out to reduce complexity of the genome sample by using *Pst*I restriction enzyme as a rare cutter and *Ban*II as a frequent cutter with simultaneous ligation of adapters complementary to *Pst*I overhangs and PCR amplification of intact *Pst*I fragments with adapters ligated at both ends.

To prepare the digestion/ligation reaction the following components were added to a 1.5 ml microcentrifuge tube as given below:

Component	Conc. used	Volume/reaction (µl)
10X R.E. buffer (100 mM Tris-OAc, 500 mM KOAc, 100 mM Mg $(OAc)_2$, 50 mM DTT, pH 7.8)	1X	0.70
100X BSA	1X	0.07
50 mM ATP	1 mM	0.14
*Pst*I adapter (5 µM)	0.05 µM	0.07
T_4 DNA ligase (2000 U/µl)	140 U(=2.1 Weiss units)	0.07
*Pst*I R.E. (20 U/µl)	1.4 U	0.07
*Ban*II R.E. (10 U/µl)	1.4 U	0.14
MQ H_2O	-	4.74
DNA (50 ng/µl)	50 ng	1.0
Total		7.0

After setting the reaction it was incubated at 37°C for 2 hours and stored at -80°C.

Oligo sequence of *Pst*I adapters

*Pst*I-adapter 1: 5’ CACGATGGATCCAGTGCA 3’

*Pst*I-adapter 2: 5’ CTGGATCCATCGTGCA 3’

Adapter preparation

Equal volumes of each of the two *Pst*I adapters (500 µM) were mixed and incubated at 80°C for 5 minutes. It was cooled to room temperature and spinned. 5 µM of working conc. of adapter was prepared and stored at -20°C.

3.2.1.5 PCR amplification of the genomic representation (target preparation)

PCR amplification of intact *Pst*I fragments with adapters ligated at both ends was carried out using adapter specific primer (*Pst*I+0). The various components used for PCR reaction are as follows:

Component	Conc. used	Volume/reaction (µl)
10X PCR buffer	1X	5.0
50 mM $MgCl_2$	1.5 mM	1.5
2 mM dNTPs	0.2 mM	5.0
*Pst*I+0 primer (500 µM)	0.4 µM	0.04
Taq polymerase (Sibenzyme 5 U/µl)	2 U	0.4
MQ H_2O	-	37.06
Digestion/Ligation product	-	1.0
Total		50.0

Target PCR primer (*Pst*I+0) and cycling conditions

***Pst*1+0 primer:** 5` GATGGATCCAGTGCAG 3`

Thirty cycles of amplification were carried out in a thermocycler (MJ Research). The programme consisted of the following conditions:

Cycling conditions used for Target PCR

Initial denaturation	4 min. at 94°C
Denaturation	20 s at 94°C
Annealing	40 s at 58°C
Extension	1 min. at 72°C
No. of cycles	30
Final extension	7 min. at 72°C

The amplified product was analysed on 1.5% agarose gel as described in section 3.2.1.3.

3.2.1.6 Genomic library preparation

To prepare a genomic library various steps were followed, which are described below:

3.2.1.6.1 Transformation of fragments in *E. coli* using electroporation

Products generated in PCR amplification (genomic representations = targets) were cloned using TOPO TA cloning kit of Invitrogen.

Procedure

Two µl of target PCR product was taken from each of the 95 genotypes (Table 1) and was pooled together. Four µl of this pooled product was ligated to the pCR2.1-TOPO vector by mixing 4 µl of pooled PCR product, 1 µl of salt solution (1:4 diluted) and 1 µl of pCR2.1-TOPO vector. Now,

this total 6 µl TOPO cloning reaction was incubated at room temperature for 15 minutes. TOP10 electrocompetent cells were thawed on ice and 2 µl TOPO ligation mix was added into a vial (approx. 50-60 µl) of One Shot electrocompetent *E. coli* cells and was mixed gently without pipetting up and down. Then, it was incubated on ice for 15 minutes. The cuvette was kept in the icebox of the electroporator (GIBCO-BRL Electroporator) and 30 µl product was added to it and electroporated using 800 µF capacitance, 16kΩ voltage booster, and 320 DC volts. The eletroporated product was added in to 500 µl of SOC medium and the remaining 30 µl product was also electroporated in the same way and added to the same 500 µl SOC medium. Now, this transformed product was kept for shaking on a shaker for 1 hour at 150 rpm at 30°C. After 1 hour, 25 µl of electroporated product was plated on LB plates having X-gal and ampicillin and the plates were incubated at 37°C in an incubator for 16-18 hours for blue-white screening.

Composition of LB Agar (per litre)

NaCl	5.0 g
Tryptone	10.0 g
Yeast extract	5.0 g
Agar	15.0 g
pH	7.0

The above components were weighed and added to 800 ml of distilled water and mixed on magnetic stirrer and then final volume was made to 1 litre with distilled water. Now, it was autoclaved and after cooling it to 55°C, 1 ml of ampicillin (100 mg/ml) and 2 ml of X-gal (50 mg/ml) were added to it and was poured into petriplates.

3.2.1.6.2 Colony picking in freezing medium

White colonies*, i.e.* transformed colonies, were picked with toothpicks and grown in freezing medium in 384-well storage plates for 18-24 hrs.

Freezing medium (FM) preparation (per litre)

Component solutions for long-term storage (20X stock solutions):

FM mix 1: 177.54 g of $K_2HPO_4 \cdot 3H_2O$, 36.00 g KH_2PO_4 and 18.00 g $(NH_4)_2SO_4$ were weighed and dissolved in 500 ml of MQ H_2O. Then, it was filter-sterilized (0.22 µm) and distributed into 50 ml aliquots and stored at room temperature.

FM mix 2: 10.00 g Na_3-citrate$\cdot 2H_2O$ and 2.00 g $MgSO_4 \cdot 7H_2O$ were weighed and dissolved in 500 ml of MQ H_2O. Then, it was filter-sterilized (0.22 µm) and distributed into 50 ml aliquots and stored at room temperature.

Preparation of fresh FM

14 g of premixed LB broth components were dissolved in 860 ml MQ water. Then, it was autoclaved and cooled and 50 ml of FM mix 1, 50 ml of FM mix 2, 44 ml of glycerol, 1 ml of 100 mg/ml ampicillin and 1 ml of 100 mg/ml kanamycin solution were added under sterile conditions.

It was mixed well by inversion and dispensed into 384-well storage plates and sealed with thick plastic seals and stored at -20°C.

3.2.1.7 Insert amplification using colony PCR

Different individual inserts were PCR amplified directly from overnight grown bacteria plate by doing replication with the help of 384-tooth replicator. The various components used for PCR reaction are as follows:

Component	Conc. used	Volume/reaction (µl)
10 X PCR buffer	1X	1.5
50 mM $MgCl_2$	1.5 mM	0.45
100 mM dNTPs (dATP, dCTP, dGTP, dTTP)	0.2 mM	0.03x4=0.12
M13 forward primer (500 µM)	0.1 µM	0.003
M13 reverse primer (500 µM)	0.1 µM	0.003
Taq polymerase (Sibenzyme, 5 U/µl)	0.3 U	0.06
MQ H_2O	-	11.864
Insert DNA (5 times dip by 384-well replicator)	-	1.0
Total		15.0

Colony PCR M13 primers and cycling conditions

M13 forward primer 5` GTTTTCCCAGTCACGACGTTG 3`

M13 reverse primer 5` TGAGCGGATAACAATTTCACACAG 3`

Forty cycles of amplification were carried out in a thermocycler (Applied Biosystems) and the programme consisted of the following conditions:

Cycling conditions used for colony PCR

Pre-PCR Step 1	3 min. at 95°C
Pre-PCR Step 2	35 s at 57°C
Pre-PCR Step 3	1 min. at 72°C
Denaturation	35 s at 94°C
Annealing	35 s at 52°C
Extension	1 min. at 72°C
No. of cycles	40
Final extension	7 min. at 72°C

The amplified product was analysed on 1.5% agarose gel as described in section 3.2.1.3. The plate was considered as amplified successfully and passed quality control (QC) if less than 10% of inserts failed to amplify and/or multibands amplified.

3.2.1.8 Spotting plates preparation

Composition of spotting buffer/DArT spotter (per litre)

D-sorbitol	273.255g
TEA HCl (triethonolammonium hydrochloride)	18.57g
dextran	5.0g
CHAPS	0.2g

250 ml of MQ water was taken in a 2-litre glass beaker and the above components were added to it. These were mixed by magnetic stirrer on hot plate and allowed the solution temperature to come down. Then, 500 ml of DMSO (dimethyl sulfoxide) was added after completely dissolving the salts and final volume was made to 1 litre by water. It was stored at room temperature.

Procedure

Quality control (QC) passed PCR plates were processed by drying at 37°C overnight (18 hours) by wrapping in kimwipes. After that 35 µl of 77% ethanol was added to each well and the plates were spun briefly to collect ethanol at bottom and incubated at room temperature for 1.5 hour. Then, the plates were spun at 4000 rpm for 40 minutes at 30°C. Now, ethanol was decanted by inverting the plates and the plates were kept upside down and blotted on kimwipes. The plates were dried at 37°C for 1 hour by wrapping in kimwipes. After drying the plates, 20 µl of DArT spotter was added to each well and plates were then sealed with thin film and spun briefly and vortexed. Now, the plates were left at room temperature for 2 days and in these 2 days the plates were shaken by vortexing and spun several times to dissolve DNA properly.

3.2.1.9. Printing of array on slides using MicroGrid II microarrayer

The fragments were arrayed with two replicates per fragment onto poly-L-lysine-coated glass slides using a MicroGrid II arrayer (Biorobotics, Cambridge, UK) and after printing, slides were kept at room temperature for at least 24 hours before slide processing.

3.2.1.10. Slide pretreatment/processing

Barcode labels were printed and stuck onto the slides before processing. Two beakers of 2 litre capacity were taken with 2 litre of MQ water in each and kept in microwave oven to heat the water to 92°C. To 1 litre MQ water contained in a bottle, 200 µl of 0.5 M DTT and 200 µl of 0.5 M EDTA were added, and the solution then was distributed into four containers one for each slide rack. Two slide racks were put in each beaker and rotated slowly for 2 minutes to denature the DNA. Then, the slide racks were put into four containers and slowly shaken up and down. The water was removed slowly by placing on a paper towel with kimwipes. Slide racks were spun down in a centrifuge for 10 minutes at 1000 rpm to remove water from the slides. Then, the slides were kept in a vaccum desiccator for 1-2 hours for drying.

3.2.1.11 Target precipitation/purification for hybridisation

Target PCR was performed for DNA samples from 24 pearl millet inbred lines (Table 2) for diversity analysis and two different RIL mapping populations (H 77/833 - 2 x PRLT 2/89 -33 and

Table 2. Pearl millet inbred lines used for diversity analysis

S. No.	Genotype	Origin and characteristics
1	H 77/833-2	Bred at CCS HAU, Hisar, India by selfing and selection within a Rajasthani landrace population
2	PRLT 2/89-33	Inbred derived from the ICRISAT Bold Seeded Early Composite, elite breeding population based predominantly on Iniadi landrace germplasm from West Africa
3	ICMB 841-P3	Bred at ICRISAT by pure-line selection for downy mildew resistance in seed lot of elite maintainer line MS 5141B
4	863B-P2	Bred at ICRISAT from Iniadi landrace material from Togo by selfing and selection
5	Tift $23D_2B_1$-P1-P5	Bred at the Coastal Plain Experiment Station by introducing the d2 dwarfing gene into the genetic background of elite seed parent maintainer line Tift 23B1
6	WSIL-P8	Selection from IP 18292, genetic stock developed in ICRISAT from a complex cross of diverse parental materials
7	PT 732B-P2	Tamil Nadu Agricultural University, Coimbatore, India
8	P1449-2-P1	Selection (IP 21168) made at ICRISAT from germplasm accession IP 5853 originated from Senegal
9	LGD 1-B-10	Derivative of (B70 × Tift 756)-1-4-5 based on Iniadi landrace germplasm from Togo (B70) and a breeding line from the Coastal Plain Experiment Station, USA (Tift 756)
10	ICMP 85410-P7	Derivative of (IPC 165 × IPC 220)-64 based on a germplasm from Uganda, Mali and Nigeria
11	81B-P6	Downy mildew (DM) resistant selection from gamma radiation-treated Tift $23D_2B_1$
12	ICMP 451-P8	Derived from LCSN 72-1-2-1-1, a selection made in Upper Volta from the ICRISAT Center Late Composite
13	ICMP 451-P6	Derived from LCSN 72-1-2-1-1, a selection made in Upper Volta from the ICRISAT Center Late Composite
14	H 77/833-2-P5(NT)	Sub-selection of H 77/833-2, which was originally bred at CCS HAU, Hisar, India from a Rajasthani landrace population
15	W 504-1-P1	Inbred line from the IARI, New Delhi
16	P310-17-Bk	Bred at ICRISAT by selfing and selection within germplasm accession IP 6329 originating from Mali, West Africa
17	IP 18293-P152	Isolated at ICRISAT from a segregating population from the cross IP 10399 × IP 10729
18	Tift $238D_1$-P158	Developed at the Coastal Plain Experiment Station, Tifton, USA
19	ICMB 89111-P6	DM susceptible selection from within ICMB 89111, which was bred at ICRISAT from the cross 843B × (Gero New Source × Saria Synthetic-48-40-4)-1-9-8
20	ICMB 90111-P6	DM resistant selection from ICMP 423 (IPC 94), which has the pedigree EC-S3-211-1-2
21	81B-P8	DM resistant selection from gamma radiation-treated Tift $23D_2B_1$
22	IPC 804	Developed at ICRISAT by crossing S 10LB (PAU, Ludhiana, India from a Serere Composite) and LCSN 1225-6-3-1(Kamboinse, Burkina Faso from ICRISAT's Late Composite)
23	ICMR 01004	Bred by MAB at ICRISAT using ICMP 451-P6 as donor H 77/833-2 as recurrent. Moderately susceptible to rust
24	ICMR 01007	Bred by MAB at ICRISAT using ICMP 451-P6 as donor H 77/833-2 as recurrent. Resistant to rust

81B-P6 x ICMP 451-P8) consisting of 140 F_7 and 168 F_7 RILs, respectively, as described in section 3.2.1.5 and then these targets were precipitated by adding equal volume of isopropanol to each well and mixed by pipetting. The plate was sealed and incubated at room temperature for 15 minutes and was centrifuged at 4000 rpm for 40 minutes at 30°C. The supernatant was discarded by inverting the plate and blotted on kimwipes to remove excess isopropanol. Two volumes of 77% ethanol were added to each well and the pellet was washed for 10 minutes. It was centrifuged at 4000 rpm for 40 minutes at 30°C. The supernatant was discarded by inverting the plate and blotted on kimwipes to remove excess ethanol. The plate was dried at 37°C for 1 hour.

3.2.1.12 Target labelling

Labelling mix was prepared as follows:

Component	Concentration used	Volume/reaction (µl)
MQ H_2O	-	3.5
10 X NEB2 buffer	1X	0.5
Random decamers (500 µM)	50 µM	0.5
Labelling dNTPs	2 mM dATP, 2 mM dCTP, 2 mMdGTP, 0.2 mMdTTP	0.5
Total		5.0

Five µl of labelling mix was added to the dried target purified pellet. It was spun briefly to collect the sample at bottom and denatured at 95°C for 3 minutes and was held at 25°C. Then, it was spun briefly and Cy3 and Cy5 dye mix was prepared in the following order:

Component	Concentration used	Volume/reaction (µl)
MQ H_2O	-	4.2
10X NEB2 Buffer	1X	0.5
Cy3/Cy5 (25 nmoles)	0.5 nmol	0.1
Klenow exo^- (5U/µl)	1 U	0.2
Total		5.0

Five µl of the Cy dye mix was added to the denatured target and incubated at 37°C for 3 hours by wrapping in aluminium foil as Cy dye is light sensitive.

3.2.1.13 Hybridization

Hybridization chambers were made ready by arranging the slides on them. 10 µl each of the Cy3 and Cy5 dye labelled product was taken and 50 µl of the DArT hybridizer was added to it and mixed by pipetting. This 70 µl product was kept in PCR machine at 95°C for 3 minutes and 56°C for 5 minutes. Then, it was held at 55°C and 60 µl of the target-hybridizer mix was added to the

slide in the form of small drops and then a dust free coverslip (24 x 60 mm) was put on the slide without introducing bubbles. The hybridization chamber was covered and screws were fixed tightly and it was placed into a waterbath at 63°C for overnight incubation.

3.2.1.14 DArT-hybridizer preparation (for 500 ml)

Two 96-well plates of TOPO polylinker fragment were amplified using a reaction mixture consisting of 11.9 ml H_2O, 1.5 ml 10X buffer, 30 µl of 100 mM dATP, 30 µl of 100 mM dCTP, 30 µl of 100 mM dGTP, 5 µl of 100 mM dTTP, 250 µl of 10 mM AA-dUTP, 100 µl of 500 µM M13F, 100 µl of 500 µM M13R, 1 ml of 5 U/µl Sibenzyme *Taq* polymerase and 3.75µl of 10 ng/µl pCR2.1-TOPO plasmid. The reaction mixture was distributed into two 96-well PCR plates (75 µl per well). Forty cycles of amplification were carried out in a thermocycler using 94°C for 30 s, 50°C for 30 s, 72°C for 1 minute and final extension at 72°C for 7 minutes. PCR product from the two plates were pooled together and checked on agarose gels. The total PCR product was distributed into two 50 ml centrifugation tubes and precipitated by adding 600 µl of 3 M NaOAc (pH 5.3) and mixed. Now, 15.6 ml of 100% ethanol was added to each tube and mixed and kept at -80°C for 30 minutes. It was spun at 7000 rpm for 3 hours at 4°C and after centrifugation supernatant was discarded carefully. The pellet was washed with 8.8 ml of 70% ethanol and the supernatant was discarded carefully. The pellet was dried at 37°C for 2-3 hours. Each pellet was dissolved in 400 µl H_2O. To purify and re-precipitate the PCR product, 1.5 ml of PCI (phenol-chloroform-isoamylalcohol) was added to it and vortexed. It was spun at 7000 rpm at 4°C for 30 minutes. The upper phase was transferred into fresh tube and 1.5 ml of CI (chloroform-isoamylalcohol) was added to it and vortexed and spun at 7000 rpm 30 minutes. The upper phase was transfered into a fresh tube and 38 µl of 3 M NaOAc (pH 5.3) and 2.8 ml of 100% ethanol was added and mixed well by inverting the tube several times. It was spun at 7000 rpm at 4° for 1 hour. The pellet was washed twice with 3.8 ml of 70% ethanol by spinning at 7000 rpm for 30 minutes each time. Pellet was dried at 37°C and dissolved in 1.0 ml H_2O. To chemically cross-link FAM to the TOPO polylinker, 850 mg $NaHCO_3$ was dissolved in 7.2 ml H_2O and 25 mg of 5-(and-6)-carboxyfluorescein succinimidylester (FAMSE) was dissolved in 600 µl of DMSO. 900 µl of $NaHCO_3$ solution was added to 2 ml of the DNA solution in the centrifugation tube and was mixed well by vortexing. 600 µl of FAMSE solution was added to it and mixed by inverting the tube several times. The solution was distributed into two centrifugation tubes and was left at the bench for 1 hour in a light-protected container. After 1 hour, 6.3 ml of H_2O was added to each aliquot and mixed well by vortexing. 800 µl of 3 M NaOAc (pH 5.3) was added to each of the aliquots and mixed. 19 ml of 100% ethanol was added to each of the aliquots and mixed by inversion. It was spun at 7000 rpm at 4°C for 3 hours. Supernatant was discarded carefully and the pellet (deep yellow to orange) was washed with 8.8 ml of 70% ethanol and spun for 1 hour and then dried at 37°C in a light-protected container. Each pellet was dissolved in 1.25ml of H_2O and spun at 7000 rpm at 4°C for 30 minutes to remove

undissolved particulates. Supernatant was collected in a fresh tube and pellet was again suspended in 650 µl H_2O and spun for 30 minutes and the supernatant was collected. To prepare DArT-hybridizer one bottle (500 ml) of ExpressHyb solution was melted in a 60°C incubator for 24 hours and it was shaken on a magnetic stirrer placed within the 60°C incubator. 50 ml of herring sperm DNA solution (10 mg/ml) was prepared and boiled in a microwave oven and added to the stirring ExpressHyb solution. 2 ml of 0.5 M EDTA (pH 8.0) and 2.4 ml of the FAM-labelled TOPO polylinker (the remaining 1.2 ml was stored for later use) were added and the mixture was left for stirring in the incubator for at least 30 minutes. A second magnetic stirrer was heated outside the incubator to approximately 60°C and solution was kept over it. It was filtered using vaccum filter and aliquoted into 15 ml falcon tubes and stored at -80°C.

3.2.1.15 Slide washing

Procedure

One litre of each of four washing solutions were transfered from the stock solutions into 1 litre bottles labelled as "wash 1 + DTT" to "wash 4 + DTT," respectively. 200 µl of 0.5 M DTT was added to each bottle and mixed well by inversion. One litre wash 1+DTT solution was further distributed into four black containers (250 ml each) labelled as wash 1+DTT. Similarly, the other three solutions were also distributed in the respective black containers. The hybridization chambers were taken out from the waterbath one by one and coverslips were removed from slides using a scalpel in one hand and holding the edge of slide with other hand. Then, the slides were put in slide racks that were already kept in black containers having wash 1+DTT. The racks were moved up and down for 1 minute and left for 4 minutes in the same solution. Then, the racks were transferred into wash 2+DTT and moved up and down for 1 minute and left for 4 minutes. These were transferred to wash 3+DTT and moved up and down for1 minute and kept for 1 minute. The racks were moved up and down in wash 4+DTT for 30 s. After finishing the washing procedure, 1 ml of 0.5 M DTT was added in 5 litres MQ water and racks were moved up and down by holding. These were spun briefly to remove water droplets from the slides and kept in a vaccum desiccator (covered with black cover) for 1 hour to dry.

Preparation of washing stock solutions (per litre)

Wash 1: 950 ml of MQ H_2O was taken in a bottle and 50 ml of 20X SSC and 10 ml of 10% SDS were added to it and mixed thoroughly.

Wash 2: 950 ml of MQ H_2O was taken in a bottle and 50 ml of 20X SSC was added to it and mixed thoroughly.

Wash 3: 1 litre of MQ H_2O was taken in a bottle and 10 ml of 20X SSC was added to it and mixed thoroughly.

Wash 4: 1 litre of MQ H_2O was taken in a bottle and 1ml of 20X SSC was added to it and mixed thoroughly.

20X SSC (per litre)

700 ml of MQ H_2O was taken in a flask and 175.32 g of sodium chloride, 88.23 g of sodium citrate were added to it. Then, it was mixed on magnetic stirrer to dissolve completely and final volume was made to 1 litre and autoclaved.

10% SDS (sodium dodecyl sulphate) (per litre)

800 ml of MQ H_2O was taken in a beaker and kept on hot magnetic stirrer and 100 g of SDS was added to it. It was mixed to dissolve completely and final volume was made to 1 litre and autoclaved.

0.5 M DTT (dithiothreitol) (for 10ml)

9 ml of autoclaved MQ water was taken in a beaker and 0.771 g of DTT was added to it. Then, it was dissolved completely and final volume was made to 10 ml.

3.2.1.16 Slide scanning and data extraction

Slides were scanned using a robotic microarray confocal laser scanner (Tecan LS300 scanner) and images were generated for each of the fluorescent dyes using the appropriate laser/filter combination (Cy3: 543 nm; Cy5: 633 nm; 6-FAM: 488 nm). DArTsoft, a software package developed at DArT P/L was used to automatically analyze each batch of TIF image pairs generated in the experiment. The software localized spots, rejected those with a weak reference signal, computed and normalized the relative hybridization intensities [=log (Cy3target/FAM reference)] of all spots, calculated the median value for replicate spots, identified polymorphic clones by using a combination of ANOVA and fuzzy K-means clustering at a fuzziness level of 1.5 and finally, the relative hybridization intensities of polymorphic clones in the representation hybridized to a slide are converted into present (“1”) or absent (“0”) based on the membership probability estimates computed by the clustering algorithm. Markers that showed conflicting scores between the replicates or could not be scored in either of the replicates were scored as unknown. The Polymorphism Information Content (PIC), a measure of informativeness of a genetic marker and three quality parameters were computed for each marker: the percentage of scored DNA samples (call rate), the between-cluster (‘‘0’’ vs. ‘‘1’’) variance of the relative (denominated by the reference) target hybridization intensity as a percentage of the total relative intensity variance (P value) and the multivariate equivalent of the P parameter (Q value) (Storey and Tibshirani, 2003).

3.2.1.17 Genetic diversity analysis using DArT data

The DArTsoft-generated 0–1 scores of the polymorphic DArT markers found among the inbred lines were used as input for DARwin 5.0 software http://darwin.cirad.fr/darwin) developed at Cirad and analyzed using the single data option and genetic distances were estimated using the Jaccard dissimilarity index. Diversity trees were built using the unweighted Neighbor-Joining (NJ) algorithm.

3.2.2 Pearl millet genotyping using SSR markers

3.2.2.1 PCR using SSR markers

Multiplex PCR was carried out to amplify SSR loci for RIL mapping population based on cross 81B x ICMP 451, consisting of 168 F_7 RILs. 27 fluorescently labelled SSRs and 3 M13-labelled SSRs were used (Table 3). For M13-labelled primers a three-primer strategy was used with 1:15:15 ratio for forward primer with M13 tail, regular reverse primer and universal fluorescent-labelled M13 primer, respectively. The various components used for PCR reaction are as follows:

Component	Concentration used	Volume/reaction (µl)
10X PCR buffer	1X	0.5
50 mM $MgCl_2$	1.0 mM	0.1
2 mM dNTPs	0.2 mM	0.5
Primer (2 pm)	0.4 pm	1.0
Taq polymerase (NEB 5 U/µl)	0.2 U	0.04
MQ H_2O	-	1.86
DNA (5 ng/µl)	5 ng	1.0
Total		5.0

Cycling conditions used for PCR

Forty cycles of amplification were carried out in a thermocycler (Applied Biosystems) using a touchdown programme having the following conditions:

Initial denaturation	3 min. at 94°C
	1 min. at 94°C
Touchdown step	1 min. at 56°C
	1 min. at 72°C
No. of cycles	5
Denaturation	1 min. at 94°C
Annealing	1 min. at 51°C
Extension	1 min. at 72°C
No. of cycles	40
Final extension	20 min. at 72°C

The amplified product was analysed on 1.5% agarose gel as described in section 3.2.1.3.

3.2.2.2 Analysis of PCR product on ABI 3730 DNA analyzer

Two µl PCR product was taken from each marker of the multiplex set (markers labeled with different dyes) and pooled together for simultaneous detection of the amplified alleles. 7µl of formamide and 0.2 µl of fragment size standard GeneScan™ 500 LIZ were added to the pooled PCR product and run on ABI 3730 DNA/Genetic analyzer (Applied Biosystems).

Table 3. Pearl millet SSR markers used for genotyping of 168 F_7 RILs of cross 81B-P6 x ICMP 451-P8

S. No.	Marker locus	Size	Motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
1	*Xpsmp2031*	180-200	(CCA)3(TCC)3	CACATCCGCAAGAGACACCAAAT	TTTGGGGGTGTAGGTTTTGTTG
2	*Xpsmp2231*	210-235	(TG)12GG(TA)4	TTGCCTGAAGACGTGCAATCGTCC	CTTAATGCGTCTAGAGAGTTAAGTTG
3	*Xpsmp2089*	110-130	(AC)15	TTCGCCGCTGCTACATACTT	TGTGCATGTTGCTGGTCATT
4	*Xpsmp2251*	140-200	(TG)6	TCAAACATAGATATGCCGTGCCTCC	CAGCAAGTCGTGAGGTTCGGATA
5	*Xpsmp2225*	220-240	(GT)12	CCGTACTGATGATACTGATGGTT	TGGGAGGTAAGCTCAGTAGTGT
6	*Xpsmp2255*	255-300	(TG)34	CATCTAAACACAACCAATCTTGAAC	TGGCACTCTTAAATTGACGCAT
7	*Xpsmp2266*	180-200	(GA)17	CAAGGATGGCTGAAGGGCTATG	TTTCCAGCCCACACCAGTAATC
8	*Xpsmp2208*	230-300	(GT)10	GAAAGAGCAAACTGAACAATCCC	ACTTTGCCCTGGATGATCCTC
9	*Xpsmp2248*	145-165	(TG)10	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
10	*Xpsmp2236*	210-235	(TG)4(GT)4	ATAAGTGGGACCCACATGCAGCAC	CGAAAGACTAGCAAAATTGCGCCTTC
11	*Xpsmp2249*	115-160	(GT)7imperfect	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAACTCCAAACTCCA
12	*Xpsmp2275*	260-290	(GTT)10	CCAGTGCCTGCATTCTTGGC3	GCATCGAATACTTCATCTCA
13	*Xpsmp2270*	130-155	(GA)26imperfect	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC
14	*Xpsmp2261*	165-190	(GA)16	AATGAAAATCCATCCCATTTCGCC	CGAGGACGAGGAGGGCGATT

15	*Xpsmp2227*	175-190	(GT)7	ACACCAAACACCAACCATAAAG	TCGTCAGCAATCACTAATGACC
16	*Xpsmp2219*	210-280	(GT)7	ACTGATGGAATCTGCTGTGGAA	GCCCGAAGAAAAGAGAACATAGAA
17	m13_*Xpsmp2237*	245-265	(GT)8	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA
18	*Xctm12*	310-340	(CT)12	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
19	*Xpsmp2273*	140-160	(GA)12	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACAAGACCTTCTCTCC
20	*Xicmp3027*	185-210	(GAT)6	ACACCATCACCGACAACAAA	AGTGACCTGGGGTACAGACG
21	*Xicmp3088*	150-175	(TCC)8	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG
22	*Xctm25*	255-280	(CT)34	GCGAAGTAGAACACCGCGCT	GCACTTCCTCCTCGCCGTCA
23	*Xpsmp2080*	155-190	(AC)14	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA
24	*Xpsmp2069*	210-230	(CA)26	CCCATCTGAAATCTGGCTGAGAA	CCGTGTTCGTACATGGTTTTGC
25	m13_*Xpsmp2232*	220-240	(TG)8	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
26	*Xicmp3050*	195-215	(TA)8	ATGTCCAGTGTTGACGGTGA	CGGGGAAGAGACAGGCTACT
27	m13_*Xpsmp2229*	220-280	(GT)5	CCACTACCTTCGTCTTCCTCCATTC	GTCCGTTCCGTTAGTTGTTGCC
28	*Xicmp*3032	180-200	(GCT)8(ACAT)3	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA
29	*Xpsmp2085*	155-170	(AC)11	GCACATCATCTCTATAGTATGCAG	GCATCCGTCATCAGGAAATAA
30	*Xctm10*	180-200	(CT)22	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA

3.2.2.3 Data collection and analysis

The data was collected automatically by the detection of the different fluorescences and analyzed by GeneMapper v4.0 software (Applied Biosystems).

3.2.3 Genetic linkage mapping

The scores of all polymorphic DArT (0/1) and SSR markers were converted into genotype codes ('A', 'B') according to the scores of the parents after genotyping both mapping populations with DArT array and SSR markers. Linkage analysis was accomplished using the programs: JoinMap (Stam, 1993), GMendel version 0.8b (Holloway and Knapps, 1993) and RECORD (van Os *et al*., 2005). Grouping was done by JoinMap and GMendel while order was finalized using RECORD. CentiMorgan (cM) distances were calculated using the Haldane function and the maps were developed using MapChart 2.2. (Voorrips, 2002). DArT markers were named with the prefix "PgPb" where 'Pg' stands for Pennisetum glaucum, 'P' for PstI (primary restriction enzyme used) and 'b' for BanII (secondary restriction enzyme used) followed by numbers corresponding to unique clone ID.

QTL mapping for rust resistance in pearl millet

Greenhouse screening for rust resistance

Along with susceptible (ICMB 89111, ICMB 06222) and resistant (ICML 11, ICMP 451) check entries, 167 F_7 RILs segregating for rust resistance from cross 81B-P6 (susceptible) × ICMP 451-P8 (resistant) were sown in pots (15 seeds/pot) filled with sterilized soil-sand-FYM (farmyard manure) mix (2:1:1) and placed in a completely randomized design in a glasshouse where cool air blowers were initiated when the temperature exceeded 25°C. The experiment was conducted in four replications and there were two pot replicates per genotype in each replication. Irrigation was performed daily and seedlings were thinned to 10 plants/pot. Eight days after sowing, when the seedlings were at the third leaf stage, the inoculum was prepared. Leaves from 2-3 months old infected plants were detached and wiped with a spatula to harvest spores into tap water at room temperature. The spore suspension was filtered through a piece of cloth and its concentration was assessed using a haemocytometer and then adjusted to $\approx 1.0 \times 10^5$ uredinispores per ml. To 1 litre aqueous urediniospores suspension of *Puccinia substriata* var *indica*, 1 ml of Tween 20 was added, which acts as a sticker (so that spores could remain attached on leaf surfaces) as well as a dispersing agent (so that clumps of spores were not formed in the suspension). Each pot of seedlings was sprayed with approximately 4 ml of inoculum using a hand-pumped sprayer. The pots were then covered with a moist polythene sheet to maintain a high level of humidity and incubated in dark in the growth chamber at 25°C for 20 hours to promote infection. After 20 hours, the pots were shifted to glasshouse benches and exposed to high humidity (>90% RH) under misting. Rust severity was recorded 12 days after inoculation based on percentage of infected leaf area within each pot (Fig. 1).

Statistical analysis

Analysis of variance (ANOVA)

All the analyses were performed using Genstat 12th edition from Rothamsted, UK. Analysis of variance (ANOVA) was performed using completely randomized design.

3.2.4.2.2 Parameters of variability

(i) Mean

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

$$\bar{X} = \Sigma Xij / N$$

where,

X_{ij} = any observation in the ithentry and jth replication, and

N = total number of observations

(ii) Range

Lowest and highest values for each character were recorded.

(iii) Heritability

Heritability (h^2) was calculated according to the formula given below:

$h^2 = \sigma^2 g / (\sigma^2 g + \sigma^2 e)$

where,

$\sigma^2 g$ = genotypic variance

$\sigma^2 e$ = error variance

3.2.4.3 QTL analysis

Phenotypic data was averaged for each entry and sorted to correspond with the progeny order of the genotypes (marker data). The total number of progeny individuals from the cross (81B-P6 × ICMP 451-P8) with both phenotypic and genotypic information was 146. QTL analysis was performed by Composite Interval Mapping with PlabQTL (Utz and Melchinger, 1996).

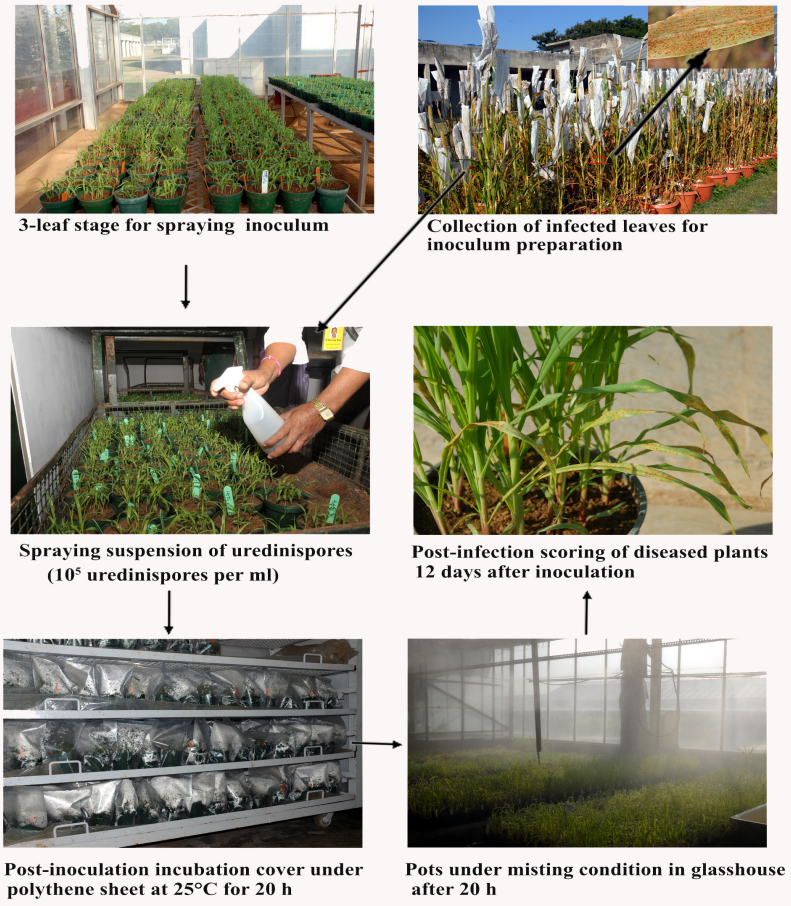

Figure 1. Screening procedure followed to evaluate rust reaction of the 167 F7 RILs, parents and control entries against the pearl millet rust pathogen

CHAPTER – IV

EXPERIMENTAL RESULTS

The present investigation aimed at genetic diversity analysis and QTL mapping in pearl millet was accomplished using Diversity Array Technology (DArT), which is a microarray-based hybridization and high-throughput DNA-genotyping technology. It was carried out in four steps - Development of the DArT array, diversity analysis of diverse pearl millet inbred genotypes using DArT markers, genotyping of two pearl millet RIL mapping populations using DArT and SSR markers followed by linkage map construction and QTL mapping for rust resistance. The results of these studies are presented in this chapter with the help of appropriate tables and suitable figures.

4.1 Development of the DArT array

4.1.1 Preparation of genomic representations

High quality genomic DNA was extracted from 95 diverse pearl millet genotypes (Table 1). This was quantified on agarose gel and diluted to 50 ng/µl (Fig. 2). A *Pst*I- based complexity reduction method was used to develop the array, where *Pst*I enzyme acts as rare cutter and a frequent cutter is used in combination with it. The intact *Pst*I fragments with adapters ligated at both ends were amplified using adapter-specific primer to produce genomic representations. Different combinations of *Pst*I with one of the following frequent-cutting restriction endonucleases: *Ban*II, *Apo*I, *Alu*I, *Bst*NI, *Hpa*II, *Taq*I, *Mse*I, and *Sty*I were tested. The products were analyzed by electrophoresis on a 1.5% agarose gel and all other combinations except *Pst*I/*Ban*II were excluded as they were showing one or more strong bands, whereas *Pst*I/*Ban*II gave a homogenous smear free from observable bands with good distribution of fragments (Figs. 3, 4) and so this genomic representation was used to construct the library.

4.1.2 Genomic library and genotyping array

*Pst*I/*Ban*II genomic representations from 95 pearl millet genotypes were pooled together and cloned using a TOPO TA cloning kit. White colonies (*i.e.* transformed colonies) (Fig. 5) were obtained with good transformation efficiency. These were picked and inserts were amplified from individual clones so that part of the polylinker region of the cloning vector was co-amplified. The amplified product was analyzed on 1.5% agarose gels and fragments of 300-1000 bp were obtained (Fig. 6). The amplified inserts were precipitated and the DNA was re-suspended in spotting buffer. The fragments were arrayed with two replicates per fragment in a randomized manner onto poly-L-lysine-coated glass slides using a MicroGrid II arrayer and uniform spots were obtained. The array consisted of 7680 clones and overall 10% missing fragments were found in the array. Thus, the array has approximately 6912 clones.

4.2 Diversity analysis of diverse pearl millet genotypes using the DArT array

4.2.1 Genotyping of diverse pearl millet genotypes using the DArT array

Genomic representations were generated from each of 24 diverse pearl millet inbred lines (Table 2) using the same complexity reduction method used for DArT library construction (*Pst*I/*Ban*II) and homogenous smears were obtained when these were analyzed on 1.5% agarose gel (Fig. 7). The fluorescent-labelled representations were hybridized to the array and the images were scanned, which showed good hybridization intensity (Fig. 8). DArTSoft was used for data analysis and 574 polymorphic clones (DArT markers) were identified from a total of 6912 clones on the array (8.3% polymorphic clones). The call rate, which reflects the total percentage of non-missing scores for a certain clone across all samples in the experiment, ranged from 80.5 to 100% with an average of 91.6% and the scoring reproducibility was 100%. DArT markers displayed high polymorphism information content (PIC) values, ranging from 0.04 to 0.50, with an average of 0.30. The P and Q values, which are measurements of variation across individuals, ranged from 47.6 to 96.9% (average 76.1%) and 47.2 to 95.6% (average 75.2%), respectively (Table 4).

Table 4. Performance of pearl millet *Pst*I/*Ban*II DArT array

Sample	Average					No. of clones on array	No. of poly morphic clones	%age of poly morphic clones
	P value (%)	Q value (%)	Call rate (%)	PIC value	Reproducibility (%)			
24 diverse inbred genotypes	76.1	75.2	91.6	0.30	100	6912	574	8.3
81B-P6 x ICMP 451-P8	81.3	80.9	89.5	0.46	100	6912	256	3.7
H 77/833-2 x PRLT 2/89-33	79.5	79.1	87.6	0.47	99.5	6912	310	4.5

4.2.2 Genetic relationship among pearl millet inbred lines revealed by DArT

A dendrogram, which displays genetic relationship among genotypes analyzed, was constructed based on 574 DArT markers for 24 pearl millet inbred genotypes. The data matrix containing the 0/1 scores (absence/presence) of the polymorphic DArT markers found among the inbred lines was analysed with DARwin 5.0 software using the single data option and genetic distances were estimated using the Jaccard dissimilarity index. A diversity tree was built using the unweighted Neighbor - Joining (NJ) algorithm. This cluster analysis discriminated well between the 24 inbred

Figure 2. Good quality pearl millet genomic DNA (50 ng/µl). M1 = marker (25 ng/µl uncut λ DNA), M2 = marker (50 ng/µl), M3 = marker (100 ng/µl) and 1-24 are pearl millet DNA samples

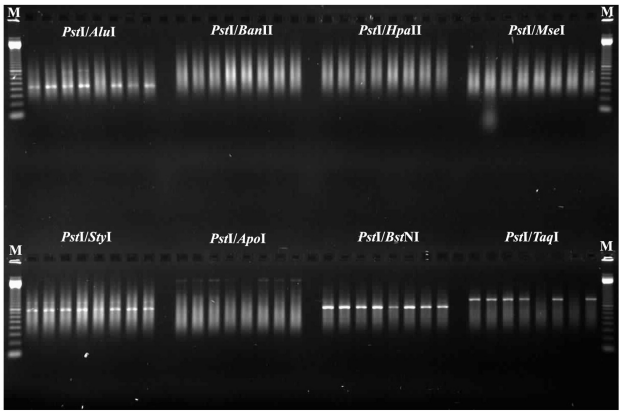

Figure 3. Different restriction enzyme combinations used for pearl millet genome complexity reduction. M: molecular size range (100 bp)

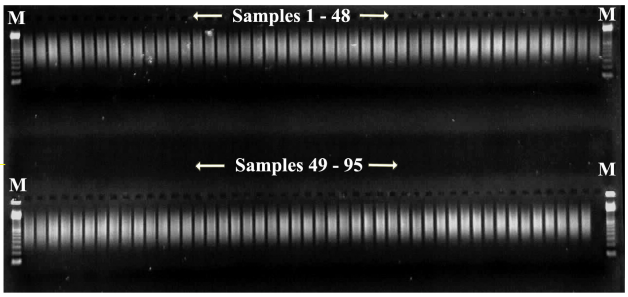

Figure 4. Genomic representations produced by *Pst*I/*Ban*II complexity reduction and used for library preparation from 95 diverse pearl millet genotypes; separated on a 1.5% agarose gel. M: molecular size range (100 bp)

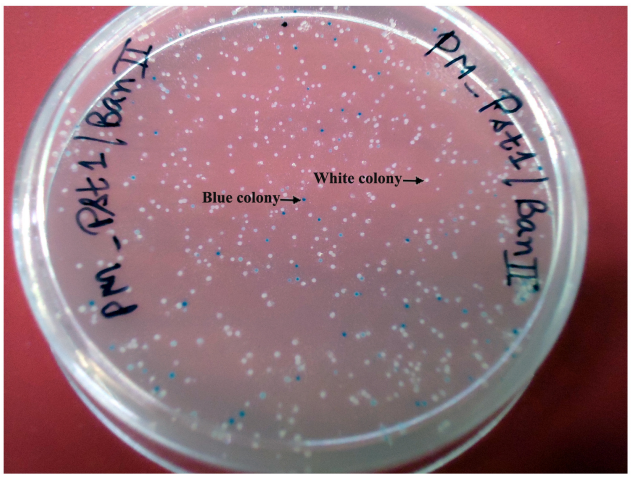

Figure 5. A view of bacterial colonies after transformation of genomic representations in *E. coli* through blue - white screening

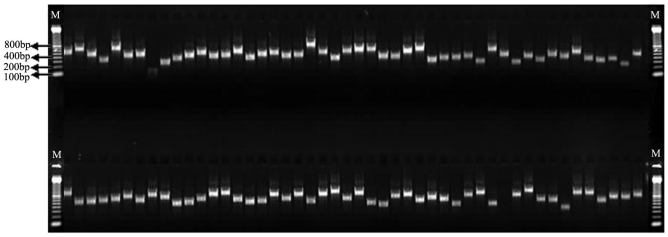

Figure 6. Amplified inserts from genomic representation of pearl millet separated on a 1.5% agarose gel. M: molecular size range (100 bp)

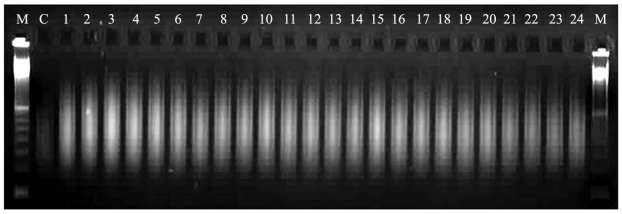

Figure 7. Genomic representations produced by *Pst*I/*Ban*II complexity reduction and used for diversity analysis from 24 diverse pearl millet inbred genotypes; separated on a 1.5% agarose gel. M: molecular size range (100 bp). C: control

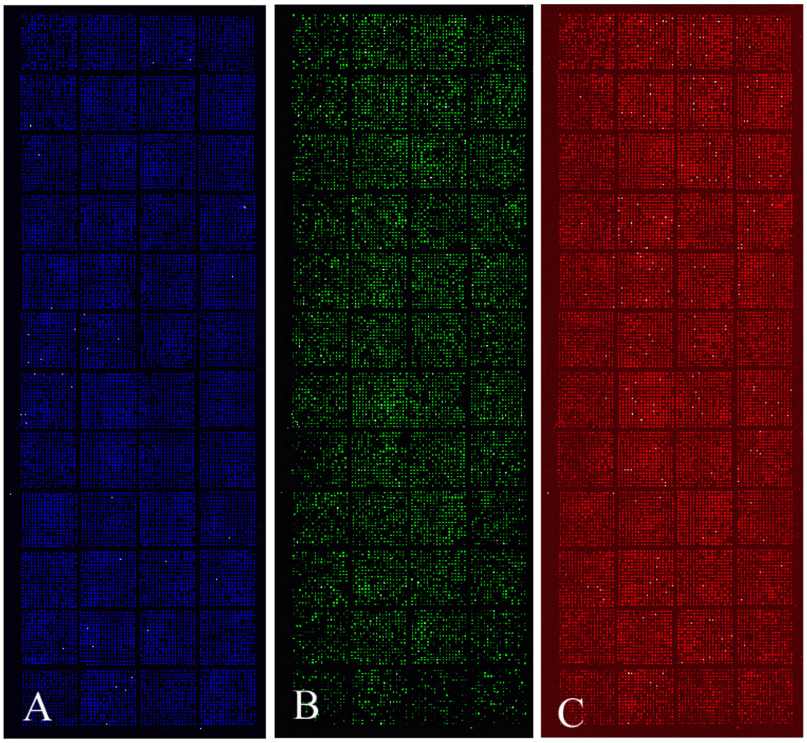

Figure 8. ***Pst*I/*Ban*II-based DArT array of pearl millet hybridized with the FAM- labelled polylinker fragment of the cloning vector that serves as a reference (A) and two targets labelled with Cy3 (B) and Cy5 (C)**

lines of pearl millet (Fig. 9) and they were grouped into two main clusters where cluster I was having 12 inbred lines, including restorer lines, downy mildew resistance sources and *Iniadi* landrace-derived lines. Cluster II contained 11 inbreds, which were mainly seed parents whereas inbred ICMB 90111-P6, derived from the ICRISAT Early Composite, was entirely separated from the two main clusters. Of twelve inbreds grouped in cluster I, four (IPC 804-P4, ICMP 85410-P7, PRLT 2/89-33, and 863B-P2) were derived from *Iniadi* landrace. IPC 804-P4 and ICMP 85410-P7 are strong male-fertility restorers for the A_1 cytoplasmic-genetic male-sterility system, whereas 863B-P2 is a maintainer. ICMP 85410-P7, PRLT 2/89-33 and 863B-P2 are downy mildew resistant parents of pearl millet mapping populations. The two inbreds P1449-2-P1 and P310-17-Bk, which are tall and downy mildew resistant mapping population parents of West African origin, were grouped together. The other six inbred fertility restorer lines (H 77/833-2, H 77/833-2-P5 (NT), ICMR 01004, ICMR 01007, ICMP 451-P8, and ICMP 451-P6) in this cluster were grouped together where H 77/833-2 is derived from a Rajasthani landrace population, H 77/833-2-P5 (NT) is a sub-selection (probably derived from an outcross) of H 77/833-2 and both are susceptible to downy mildew and rust. ICMP 451-P8 and ICMP 451-P6 grouped together as both of these are derived from the same inbred line (LCSN 72-1-2-1-1) and are moderately resistant to downy mildew and resistant to rust. ICMR 01004 is moderately susceptible and ICMR 01007 is resistant to rust, and both are resistant to downy mildew and were bred by marker-assisted backcrossing of disease resistance from donor parent ICMP 451-P6 into the genetic background of recurrent parent H 77/833-2.

In cluster II, five inbreds (ICMB 841-P3, Tift $23D_2B_1$-P5, 81B-P6 and 81B-P8, IP 18293-P152) were grouped together, four of which are d_2 dwarf lines. Out of these five inbreds, genetically tall ICMB 841-P3 and d_2 dwarf Tift $23D_2B_1$-P5, are expected to cluster together as they share genetically tall Tift $23B_1$ as a common ancestor and the two sub-selections of 81B, namely 81B-P6 and 81B-P8, were also clustered with these as 81B is a product of an induced mutation breeding program based on Tift $23D_2B_1$. Among these inbreds, ICMB 841-P3, Tift $23D_2B_1$-P5, 81B-P6 and 81B-P8 are maintainers for the A_1 cytoplasmic-genetic male-sterility system. ICMB 841-P3, 81B-P6, 81B-P8, and IP 18293-P152 are all at least moderately resistant to downy mildew while Tift $23D_2B_1$-P5 is highly susceptible to downy mildew. The other four inbreds (ICMB 89111-P6, WSIL-P8, PT 732B -P2, and LGD 1-B-10) of cluster II were grouped together and all are d_2 dwarf lines. Out of these, ICMB 89111-P6 and LGD 1-B-10 are downy mildew susceptible while WSIL-P8 is downy mildew resistant. W504-1-P1 and Tift $238D_1$-P158 were the other two inbreds in cluster II and both are downy mildew susceptible. W504-1-P1 is genetically tall and Tift $238D_1$-P158 is a d_1 dwarf fertility restorer line for the A_1 cytoplasmic-genetic male-sterility system.

4.3 Genotyping of the mapping populations using DArT and SSR markers and linkage map construction

4.3.1 Genotyping of pearl millet RIL mapping population based on 81B-P6 x ICMP 451-P8 using DArT array

High quality genomic DNA was extracted for 168 F_7 RILs from the cross 81B-P6 x ICMP 451-P8. Genomic representations were generated from DNA samples of each of these RILs as well as their parents using the same complexity reduction method used for library construction.The fluorescent-labelled representations were hybridized to the array and the images were scanned and then analysed using DArTSoft. 256 polymorphic clones (DArT markers) were identified in a total of 6912 clones (3.7% of polymorphic clones) on the array. The call rate ranged from 80.3 to 98.4% with an average of 89.5% and the scoring reproducibility was 100%. DArT markers displayed high PIC values, ranging from 0.27 to 0.50 with an average of 0.46. The P and Q values ranged from 65.1 to 92.9% (average 81.3%) and 64.8 to 92.5% (average 80.9%), respectively (Table 4).

4.3.2 Genotyping of pearl millet RIL mapping population based on 81B-P6 x ICMP 451-P8 using SSRs

The RIL mapping population was genotyped using 30 SSR primer pairs (Table 3), with capillary electrophoretic separation of fluorescent-labelled PCR products. From this, 25 SSRs (83.3%) detected reliably-scorable polymorphism (Figs. 10A and 10B).

4.3.3 Genetic linkage map construction for RIL mapping population based on 81B-P6 x ICMP 451-P8

4.3.3.1 Genetic linkage map

The scores of all polymorphic DArT and SSR markers were converted into genotype codes ('A', 'B') according to the scores of the parents ('A' = homozygous for 81B-P6 allele; 'B' = homozygous for ICMP 451-P8 allele) and heterozygotes ('H') were recorded as missing data. Out of 25 polymorphic SSRs screened, 4 markers were removed having 'D' scores (*i.e.*, not 'B', so either 'H' or 'A'); and data for 49 polymorphic IPES (ICRISAT Pearl millet EST Stress) EST-SSR markers already generated (data provided by ICRISAT) were also added for linkage map construction. So, a total of 326 polymorphic markers (256 DArT and 70 SSRs) were used for assembling the linkage map using data from 146 RILs. Out of this, 286 loci (229 DArT markers and 57 SSRs) (Table 5) were distributed across the expected 7 linkage groups using logarithm of odds (LOD) thresholds ranging from 2-10 and a recombination frequency (r) threshold less than 0.4 using JoinMap and 40 markers (27 DArTs and 13 SSRs) remained unlinked. The order of markers in each linkage group was finalized using RECORD software, which is faster and more suitable for construction of high-density linkage maps than JoinMap. The map built with JoinMap was inflated by 47.0% when compared with that built using RECORD.

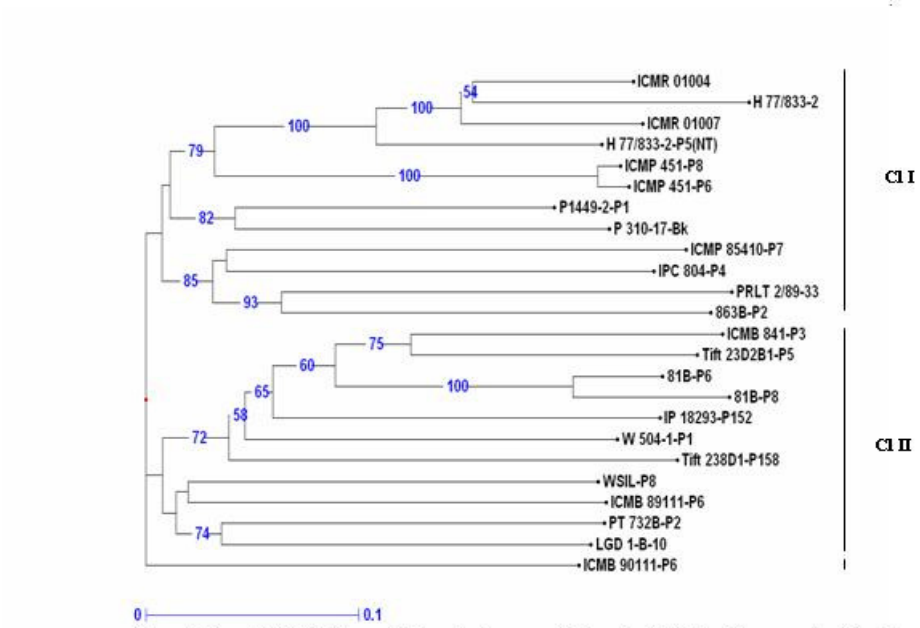

Figure 9. Unweighted Neighbour-Joining dendrogram of 24 pearl millet inbred lines constructed using a Jaccard dissimilarity matrix based on presence/absence of 574 DArT markers. Numbers at branching points indicate per cent bootstrap support of individual nodes; only values (≥50% are reported (Bootstrap no. = 1,000)

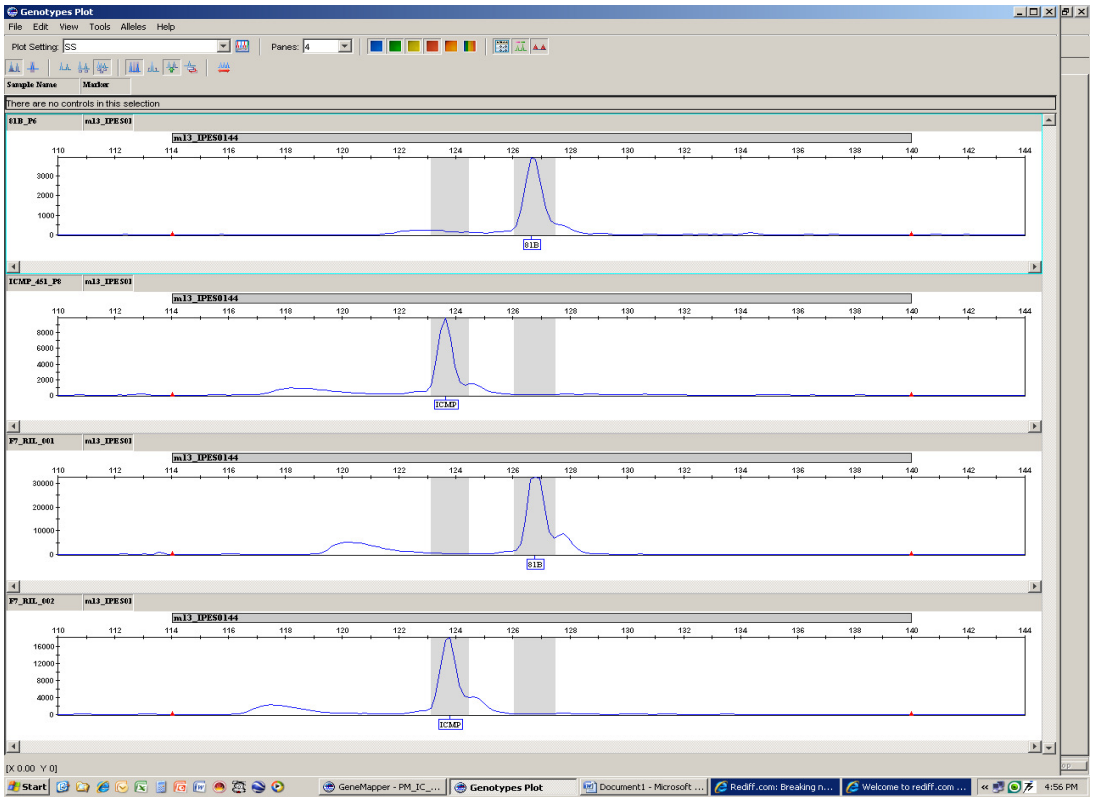

Figure 10 (A). GeneMapper profile for an amplified SSR marker showing polymorphism

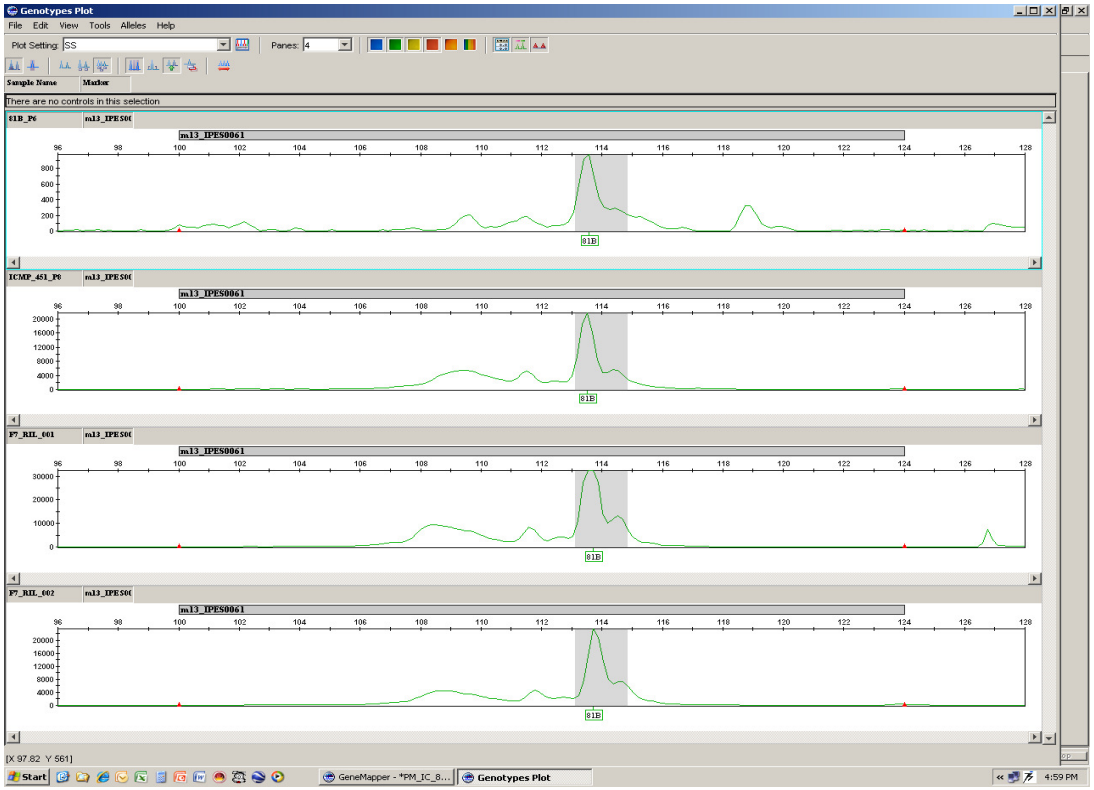

Figure 10 (B). GeneMapper profile for an amplified SSR marker showing monomorphism

Table 5. Distribution of DArT and SSR markers on two genetic linkage maps.

RIL Population	Total no. of markers	DArTs	SSRs			
			Xipes	*Xctm*	*Xpsmp*	*Xicmp*
81B-P6 x ICMP 451-P8	286	229	38	2	15	2
H 77/833-2 x PRLT 2/89-33	321	258	37	4	13	9
Common across the two RIL populations	78	56	15	2	3	2

The final genetic map (Figs. 11 A, B) spanned a total length of 740.3 cM (Haldane) with an average adjacent-marker distance of 2.7 cM, and an average density of 0.39 markers/cM. The total number of mapped loci per linkage group (LG) ranged from 23 on LG 6 to 59 on LG 2, and the average was 40.9 loci/LG. The longest individual linkage group map was for LG 7 (153.5 cM), the shortest was for LG 6 (45.0 cM), and the average LG length was 105.8 cM. The density of markers on the individual linkage group maps ranged from 0.29 markers/cM on LG 5 to 0.51 markers/cM on LG 6. Map distances between 2 consecutive markers varied from 0 to 21 cM, and 263 of the 279 intervals (94.3%) were less than 10 cM. There were only 16 intervals (5.7%) larger than 10 cM, and the largest gap between markers was observed on LG 7 (21.0 cM). Many DArT markers were present as clusters in telomeric regions (*e.g.*, the top of LG 1) (Fig. 11 A). The details of each linkage group are described below and in Table 6.

Table 6. Linkage group details of DArT- and SSR- based genetic map for pearl millet RIL population based on cross (81B-P6 x ICMP 451-P8)

Linkage group	DArT marker loci	SSR marker loci	Total marker loci	Length (cM)	Adjacent-marker interval (cM)	Density (markers/cM)
LG 1	40	14	54	128.0	2.42	0.42
LG 2	49	10	59	118.5	2.04	0.50
LG 3	28	7	35	69.6	2.05	0.50
LG 4	36	6	42	133.6	3.26	0.31
LG 5	21	6	27	92.1	3.54	0.29
LG 6	15	8	23	45.0	2.05	0.51
LG 7	40	6	46	153.5	3.41	0.30
Total	229	57	286	740.3	2.65	0.39

Linkage Group 1

Total map length of LG 1 was 128.0 cM and it consisted of 54 markers. Map distances between 2 consecutive markers varied from 0 to 18.0 cM, with an average adjacent-marker interval of 2.42 cM and average marker density of 0.42 markers/cM.

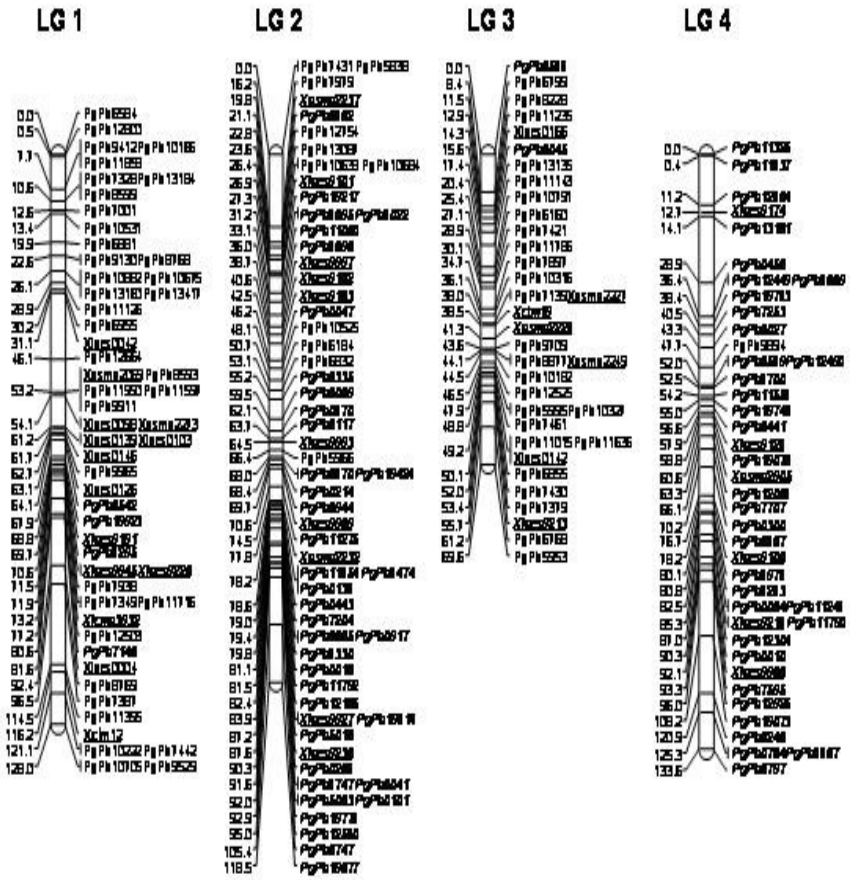

Figure 11 (A). Linkage groups LG 1 thru LG 4 of the genetic linkage map for the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population. Map distances (Haldane cM) and marker names are shown on the left and right side of each linkage group, respectively. SSR markers are underlined and DArT marker names begin with the prefix PgPb. Markers that showed distorted segregation are shown in italics.

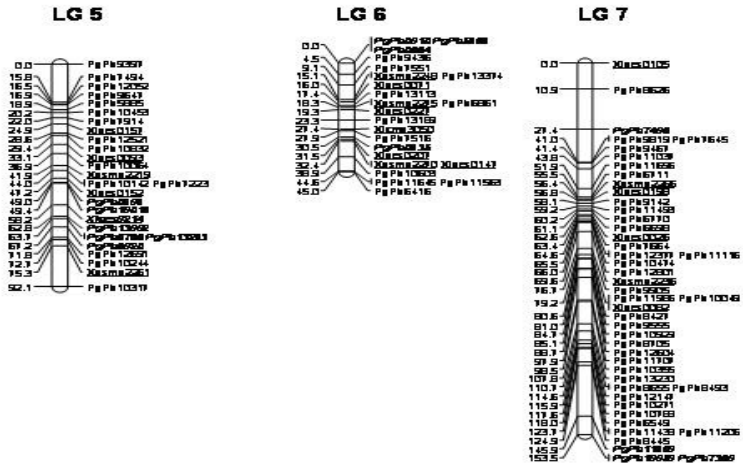

Figure 11 (B). Linkage groups LG 5 thru LG 7 of the genetic linkage map for the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population. Map distances (Haldane cM) and marker names are shown on the left and right side of each linkage group, respectively. SSR markers are underlined and DArT marker names begin with the prefix PgPb. Markers that showed distorted segregation are shown in italics.

Linkage Group 2

LG 2 had the highest number of markers mapped *i.e.* 59, with segregation of 49 of these distorted in favor of alleles from female parent 81B-P6. The total map length was 118.5 cM and map distances between 2 consecutive markers varied from 0 to 16.2 cM, with an average adjacent-marker interval of 2.04 cM, which was smallest among all 7 linkage groups for this RIL population. The average density of markers in this group was 0.50 markers/cM.

Linkage Group 3

Thirty-five markers were placed in LG 3, which had a total length of 69.6 cM. The adjacent-marker intervals ranged from 0 to 8.4 cM, with an average of 2.05 cM and an average density of 0.50 markers/cM.

Linkage Group 4

LG 4 is the second longest group with a map length of 133.6 cM. 42 markers (41 showing distorted segregation favoring alleles from male parent ICMP 451-P8) were placed in this group. Adjacent-marker intervals ranged from 0 to 14.8 cM with an average of 3.26 cM and an average density of 0.31 markers/cM.

Linkage Group 5

The total length of LG 5 (Fig. 11 B) was 92.1 cM with 27 markers. Map distances between 2 consecutive markers varied from 0 to 16.8 cM, with the largest average adjacent-marker interval of 3.54 cM

Linkage Group 6

LG 6 had the lowest number of markers mapped, *i.e.* 23. This was the shortest group with a total map length of only 45.0 cM. Map distances between 2 consecutive markers varied from 0 to 6.5 cM, with an average adjacent-marker interval of 2.05 cM, and average marker density of 0.51 markers/cM, which is highest among all 7 linkage groups for this RIL population.

Linkage Group 7

Forty-six markers were placed in LG 7, which had a longest map length of 153.5 cM. The adjacent-marker intervals observed in this group ranged from 0 to 21.0 cM, with an average of 3.41 cM, and an average marker density of 0.30 markers/cM.

4.3.3.2 Segregation distortion of markers

Significant segregation distortion from the expected 1:1 Mendelian ratio was found for 124 (38.0%) out of 326 markers genotyped across these 146 RILs. Sixty markers (18.4%) showed distortion in favor of the 81B-P6 allele (49 mapped to LG 2) whereas 64 (19.6%) showed distortion in favor of the ICMP 451-P8 allele (41 mapped to LG 4), with missing data (genotypes scored either missing or heterozygote) of 10.7%. Out of the 286 markers mapped, 118 (41.2%) showed distorted segregation with 57 markers (19.9%) showing distortion in favor of the 81B-P6 allele and 61 (21.3%) in favor of the ICMP 451-P8 allele, with 10.8% missing data. Distorted markers (Figs. 11 A, B) favoring 81B-P6 were found on LG 2 (49 out of 59 markers mapping to

this group), LG 3 and LG 6 while those favoring ICMP 451-P8 were mapped on LG 1, LG 3, LG 4 (41 out of 42 markers mapping to this group), LG 5 and LG 7. LG 3 showed skewed markers favoring alleles from either parent. The details of distorted markers are given in Table 7.

Table 7. Segregation distortion of mapped markers in (81B-P6 x ICMP 451-P8)-and (H 77/833-2 x PRLT 2/89-33)-based RIL populations of pearl millet

81B-P6 x ICMP 451-P8			H 77/833-2 x PRLT 2/89-33		
Linkage group	**No. of markers favoring allele 81B-P6**	**No. of markers favoring allele ICMP 451-P8**	**Linkage group**	**No. of markers favoring allele H 77/833-2**	**No. of markers favoring allele PRLT 2/89-33**
LG 1	-	8	LG 1	-	32
LG 2	49	-	LG 2	29	1
LG 3	4	1	LG 3	-	7
LG 4	-	41	LG 4	1	1
LG 5	-	7	LG 5	-	9
LG 6	4	-	LG 6	-	25
LG 7	-	4	LG 7	-	7
Total	57	61	Total	30	82

4.3.4 Genotyping of pearl millet RIL mapping population based on H 77/833-2 x PRLT 2/89-33 using DArT array

High quality genomic DNA samples extracted from 140 F_7 RILs from the cross H 77/833-2 x PRLT 2/89-33 were used for preparation of genomic representations using *Pst*I/*Ban*II-based complexity reduction and homogenous smears were obtained. After screening of 6912 random genomic pearl millet clones with this population, 310 polymorphic DArT markers (4.5%) were identified. The call rate ranged from 79.3 to 98.9% with an average of 87.6%, and the scoring reproducibility range was from 97.2 to 100% with an average of 99.5%. DArT markers displayed PIC values ranging from 0.24 to 0.50 with an average of 0.47. The P and Q values ranged from 60.8 to 94.5% (average 79.5%) and 60.6 to 94.0% (average 79.1%), respectively (Table 4).

4.3.5 Genetic linkage map construction of mapping RIL population based on H 77/833-2 x PRLT 2/89-33

4.3.5.1 Genetic linkage map

Of the 310 available DArT markers, 309 DArT and 80 previously genotyped polymorphic SSR markers (data provided by ICRISAT), *i.e.* a total of 389 markers, were used for assembling the linkage map using data from 137 RILs. Out of this, 318 loci were distributed across 7 linkage

groups using GMendel at a LOD threshold value of 4.0 and a recombination frequency threshold less than 0.12. The unlinked markers were tried using "Try" command of Mapmaker. Markers violating map stability were removed and linkage groups were reanalyzed to construct a stabilized map. Thus, a total of 321 loci (258 DArTs and 63 SSRs) (Table 5) were integrated into the genetic map (Figs. 12 A, B). The order of markers in each linkage group was finalized using RECORD software. The resultant map had a total length of 1148 cM (Haldane), with an average density of 0.28 markers/cM, and an average adjacent-marker interval length of 3.66 cM. The length of individual linkage groups ranged between 77.8 cM for LG 3 and 370.3 cM for LG 2 and the average linkage group length was 164 cM. The total number of mapped loci per linkage group ranged from 28 (LG 5) to 80 (LG 2), with an average of 45.8 loci. The average adjacent-marker interval lengths ranged from 2.15 cM (LG 6) to 4.69 cM (LG 2), with corresponding map densities ranging from 0.22 to 0.48 markers/cM for LG 2 and LG 6, respectively. Map distance between adjacent markers varied from 0 to 35.3 cM and 91.7% of the intervals (288 out of 314 intervals) were <10 cM. There were 26 map regions (8.3%) with adjacent-marker distances >10 cM and the largest distance between adjacent markers was observed on LG 2 (35.3 cM). Many DArT markers were present as clusters in telomeric regions, *e.g.* the top portions of LG 1, LG 2 and LG 4 (Fig. 12 A). The details of each linkage group are described below and in Table 8.

Table 8. Linkage group details of DArT- and SSR-based genetic map for pearl millet RIL population based on cross (H 77/833-2 x PRLT 2/89-33)

Linkage group	DArT marker loci	SSR marker loci	Total marker loci	Length (cM)	Adjacent-marker interval (cM)	Density (markers/cM)
LG 1	44	17	61	215.9	3.60	0.28
LG 2	69	11	80	370.3	4.69	0.22
LG 3	23	7	30	77.8	2.68	0.39
LG 4	42	5	47	156.1	3.39	0.30
LG 5	21	7	28	111.8	4.14	0.25
LG 6	33	6	39	81.7	2.15	0.48
LG 7	26	10	36	134.4	3.84	0.27
Total	258	63	321	1148.0	3.66	0.28

Linkage Group 1

The map length of LG 1 was 215.9 cM. It was the second longest group for this RIL population and had 61 markers. Map distances between 2 consecutive markers varied from 0 to 14.9 cM, with an average adjacent-marker interval length of 3.60 cM and average marker density of 0.28 markers/cM.

Linkage Group 2

LG 2 was the lengthiest group having a map length of 370.3 cM along with the highest number of markers, *i.e.* 80 markers. Map distances between 2 consecutive markers ranged from 0 to 35.3 cM, with the highest average adjacent-marker interval length of 4.69 cM and lowest average marker density of 0.22 markers/cM among all seven groups for this RIL population.

Linkage Group 3

LG 3 was the shortest group with a map length of 77.8 cM accommodating 30 markers. Map distances between 2 consecutive markers varied from 0 to 9.5 cM, with an average adjacent-marker interval length of 2.68 cM, and an average map density of 0.39 markers/cM.

Linkage Group 4

Forty-seven markers occupied LG 4, spanning 156.1 cM. Map distances between 2 consecutive markers ranged from 0 to 12.1 cM, with an average adjacent-marker interval of 3.39 cM, and an average map density of 0.30 markers/cM.

Linkage Group 5

LG 5 had a map length of 111.8 cM (Fig. 12 B), along with smallest number of markers, *i.e.* 28. Map distances between 2 consecutive markers varied from 0 to 15.8 cM, with an average adjacent-marker interval of 4.14 cM, and an average map density of 0.25 markers/cM.

Linkage Group 6

Thirty-nine markers were placed in LG 6, which had a map length of 81.7 cM. The adjacent-marker distance ranged from 0 to 9.9 cM, with the lowest average adjacent-marker interval of 2.15 cM and highest average map density of 0.48 markers/cM among all seven groups for this RIL population.

Linkage Group 7

LG 7 accommodated 36 markers within its map length of 134.4 cM. The adjacent-marker distance ranged from 0 to 23.4 cM, with an average adjacent-marker interval of 3.84 cM, and an average map density of 0.27 markers/cM.

4.3.5.2 Segregation distortion of markers

Segregation analysis data indicated that distortion was found in 136 (34.9%) out of 389 markers analyzed for these 137 RILs. Thirty-seven marker loci (9.5%) showed distortion in favor of the H 77/833-2 allele, whereas 99 (25.4%) showed distortion in favour of the PRLT 2/89-33 allele, with missing data of 12.2%. Out of the 321 mapped markers, 112 (34.9%) showed significant segregation distortion from the expected 1:1 Mendelian ratio. The number of mapped markers showing segregation distortion (Figs. 12 A, B) in favour of the PRLT 2/89-33 allele were more numerous *i.e* 82 (25.5%) than the 30 markers (9.3%) showing distortion in favor of the H 77/833-2 allele, with 12% missing data. The 30 markers that showed distortion in favor of the H 77/833-2 allele were distributed on LG 2 (29 of 80 mapped loci) and LG 4 (1 out of 47 mapped loci). The 82 markers that showed distortion in favor of the PRLT2/89 - 33 allele were distributed across all

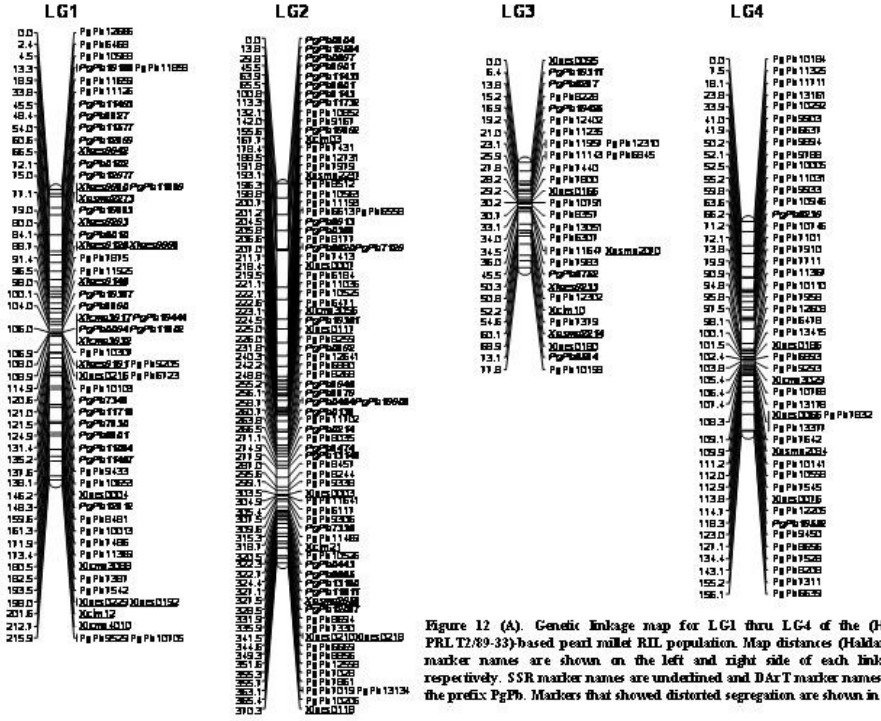

Figure 12 (A). Genetic linkage map for LG1 thru LG4 of the (H77/833-2 x PRLT2/89-33)-based pearl millet RIL population. Map distances (Haldane cM) and marker names are shown on the left and right side of each linkage group, respectively. SSR marker names are underlined and DArT marker names begin with the prefix PgPb. Markers that showed distorted segregation are shown in italics.

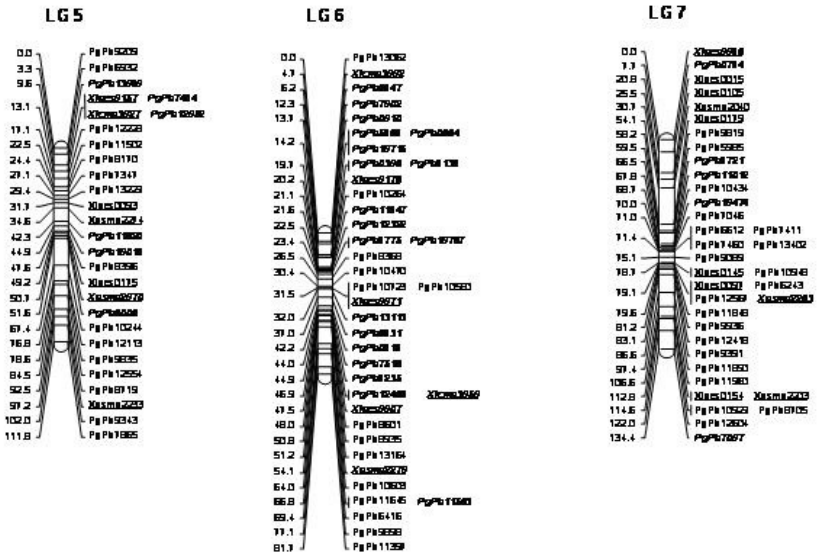

Figure 12 (B). Genetic linkage map for LG5 thru LG7 of the (H 77/833-2 x PRLT 2/89-33)-based pearl millet RIL population. Map distances (Haldane cM) and marker names are shown on the left and right side of each linkage group, respectively. SSR marker names are underlined and DArT marker names begin with the prefix PgPb. Markers that showed distorted segregation are shown in italics.

seven linkage groups, but were concentrated on LG 1 (32 of 61 mapped loci) and LG 6 (25 of 39 mapped loci). LG 2 and LG 4 showed regions with distortion favouring alleles of either parent. The details of distorted markers are given in Table 7.

4.3.6 Comparison of genetic linkage maps of two populations

Both the linkage maps had relatively high marker densities, with the highest numbers of markers mapped on LG 2 in both populations while it was lowest on LG 5 for the (H 77/833-2 x PRLT 2/89-33)-based map and on LG 6 in case of that for (81B-P6 x ICMP 451-P8) (Tables 6, 8). More markers (321), distributed over a larger portion of the genome (1148 cM) with a larger average adjacent-marker interval of 3.66 cM and lower density (0.28 markers/cM), were mapped for the (H 77/833-2 x PRLT 2/89-33)-based RIL population; while there were 286 markers spanning a total length of just 740.3 cM (average adjacent-marker interval of 2.65 cM and map density of 0.39 markers/cM) mapped using the (81B-P6 x ICMP 451-P8)-based RIL population (Figs. 11 A, B and 12 A, B). There were 78 common markers (56 DArTs and 22 SSRs) (Table 5) across the two RIL populations and the number of common markers was 20, 16, 8, 8, 6, 13, and 7 for LG 1 to LG 7, respectively (Table 9). The markers were in the same order in two populations except LG 1 and LG 2 where some order swapping of (mostly) closely linked markers was observed within some blocks, which was not unexpected given the moderate sizes of the two mapping populations.

4.4 QTL analysis for rust resistance

4.4.1 Analysis of variance (ANOVA)

Artificial inoculation of pot-grown seedlings was performed to assess rust reaction on 167 RILs derived from the cross (81B-P6 x ICMP 451-P8). Rust severity (%) was recorded 12 days after inoculation based on percentage of infected leaf area within each pot and it ranged from 0 to 95%. Highly significant differences were detected by ANOVA between individual RIL progenies. Mean of rust severity (%) was calculated for each RIL using the data from four replications and it ranged from 0.25 to 89.38% (grand mean 35.0%) (Fig. 13) with an operational heritability of 99%, SEm of 2.5%, and CV of 7.1%. Parental line ICMP 451-P8 was resistant and exhibited some symptoms of infection against rust in a few replications, with a mean rust severity of 4.6%, while parental line 81B-P6 was completely susceptible and recorded 77.8% disease incidence. Among various control entries, ICML 11 was moderately resistant (10.6% rust severity), ICMB 89111 (55.4%) was susceptible and ICMB 06222 (83.5%) was highly susceptible. Of the 167 RILs, 32 were resistant, 18 moderately resistant, 73 moderately susceptible, 40 susceptible and remaining 4 lines were highly susceptible to rust (Table 10).

4.4.2 QTL mapping

For QTL mapping, the linkage map constructed with marker data from 146 F_7 RILs derived from the cross (81B-P6 x ICMP 451-P8) was used. The primary data of rust severity percentage was converted later into resistance percentage and used for QTL analysis which was performed by

Table 9. List of common markers across linkage maps of pearl millet RIL populations based on crosses (81B-P6 x ICMP 451-P8) and (H 77/833-2 x PRLT 2/89-33)

S. No.	Marker	Linkage group	S. No.	Marker	Linkage group
1	*PgPb10166*	LG 1	40	*PgPb6845*	LG 3
2	*PgPb11858*	LG 1	41	*PgPb11143*	LG 3
3	*PgPb11126*	LG 1	42	*PgPb10791*	LG 3
4	*Xipes0042*	LG 1	43	*Xctm10*	LG 3
5	*PgPb11990*	LG 1	44	*PgPb7379*	LG 3
6	*Xipes0098*	LG 1	45	*PgPb11325*	LG 4
7	*Xpsmp2273*	LG 1	46	*PgPb13161*	LG 4
8	*Xipes0146*	LG 1	47	*PgPb9894*	LG 4
9	*Xipes0126*	LG 1	48	*PgPb9788*	LG 4
10	*Xipes0101*	LG 1	49	*PgPb10746*	LG 4
11	*PgPb9205*	LG 1	50	*Xipes0186*	LG 4
12	*PgPb7938*	LG 1	51	*PgPb9293*	LG 4
13	*PgPb7349*	LG 1	52	*Xipes0066*	LG 4
14	*PgPb11716*	LG 1	53	*PgPb7494*	LG 5
15	*Xicmp3032*	LG 1	54	*PgPb12052*	LG 5
16	*Xipes0004*	LG 1	55	*Xipes0157*	LG 5
17	*PgPb7387*	LG 1	56	*Xipes0093*	LG 5
18	*Xctm12*	LG 1	57	*PgPb10816*	LG 5
19	*PgPb10705*	LG 1	58	*PgPb10244*	LG 5
20	*PgPb9529*	LG 1	59	*PgPb8018*	LG 6
21	*PgPb7431*	LG 2	60	*PgPb5969*	LG 6
22	*PgPb7979*	LG 2	61	*PgPb8664*	LG 6
23	*Xpsmp2237*	LG 2	62	*Xipes0071*	LG 6
24	*Xipes0007*	LG 2	63	*PgPb13113*	LG 6
25	*PgPb10525*	LG 2	64	*Xicmp3050*	LG 6
26	*PgPb6184*	LG 2	65	*PgPb7516*	LG 6
27	*PgPb6117*	LG 2	66	*PgPb8935*	LG 6
28	*Xipes0003*	LG 2	67	*Xpsmp2270*	LG 6
29	*PgPb8214*	LG 2	68	*PgPb10603*	LG 6
30	*PgPb9474*	LG 2	69	*PgPb11645*	LG 6
31	*PgPb8139*	LG 2	70	*PgPb11563*	LG 6
32	*PgPb8443*	LG 2	71	*PgPb6416*	LG 6
33	*PgPb6665*	LG 2	72	*Xipes0105*	LG 7
34	*PgPb9338*	LG 2	73	*PgPb9819*	LG 7
35	*PgPb11702*	LG 2	74	*PgPb10474*	LG 7
36	*PgPb12598*	LG 2	75	*PgPb10929*	LG 7
37	*PgPb8228*	LG 3	76	*PgPb8705*	LG 7
38	*PgPb11235*	LG 3	77	*PgPb12604*	LG 7
39	*Xipes0166*	LG 3	78	*PgPb11960*	LG 7

Table 10. Performance of pearl millet RILs derived from cross (81B-P6 x ICMP 451-P8) for pearl millet rust resistance under glasshouse conditions with artificial inoculation

Percentage severity	Reaction	No. of RILs
0-10	Resistant	32
10-20	Moderately resistant	18
20-50	Moderately susceptible	73
50-75	Susceptible	40
75-90	Highly susceptible	4

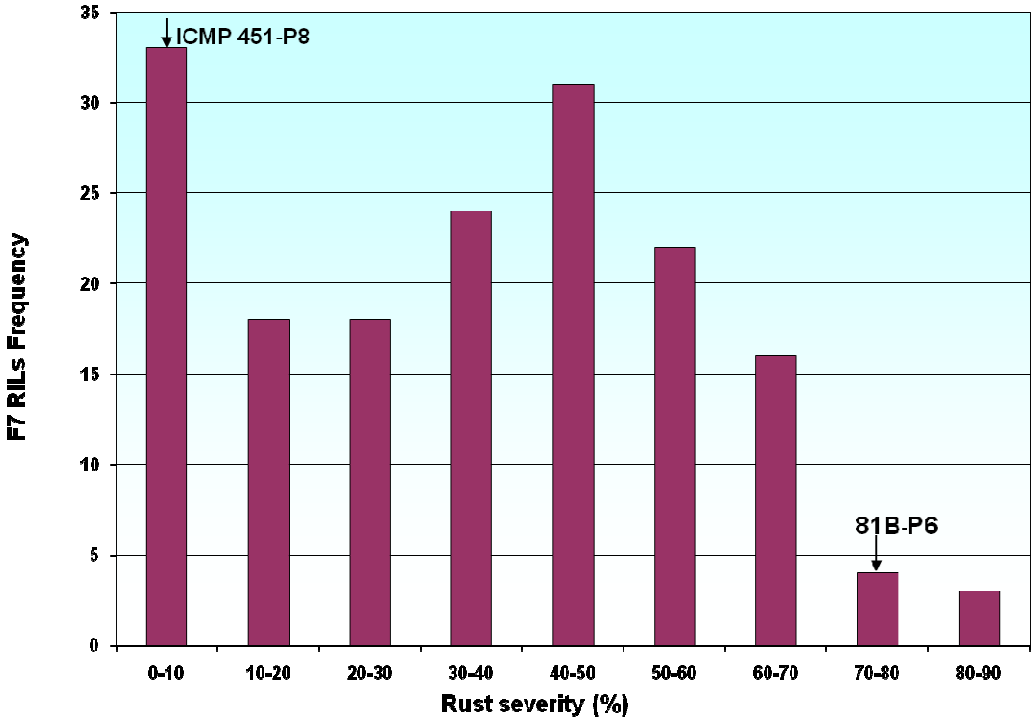

Figure 13. Frequency distribution of rust severity (%) among F_7 RIL progenies from the pearl millet crosses (81B-P6 x ICMP 451-P8)

Composite Interval Mapping with PlabQTL using a LOD of 2.5 as the threshold value for QTL significance. A major QTL with a LOD value of 27 was mapped near the top of the LG 1 (Fig. 14), explaining 58% of the observed phenotypic variation in rust reaction of the RIL progenies. At this locus, the allele of resistant parent ICMP 451-P8 conferred resistance.

In addition to this major QTL, two modifiers were also detected - one each on LG 4 and LG 7, explaining 9.0% and 8.3% of the observed phenotypic variation, respectively.The favorable allele for the LG 4 modifier was inherited from susceptible parent 81B-P6 whereas that for the LG 7 modifier was inherited from ICMP 451-P8. The details of QTLs detected are shown in Table 11 and the graphical representation of LOD values for all seven linkage groups are shown in Figures 15 A and B.

Table 11. Summry of QTLs for pearl millet rust resistance detected using PlabQTL and data from RILs from cross (81B-P6 x ICMP 451-P8)

Linkage group	Flanking Markers	Position (cM)	LOD	Variance explained	Additive effect	Inheritance
LG 1	*PgPb9412-PgPb7328*	8.0	27.30	57.8%	16.9	Major QTL; ICMP 451-P8
LG 4	*PgPb12440-PgPb10793*	38.0	2.97	9.0%	-1.1	QTL modifier; 81B-P6
LG 7	*PgPb12801-Xpsmp2236*	68.0	2.73	8.3%	2.6	QTL modifier; ICMP 451-P8

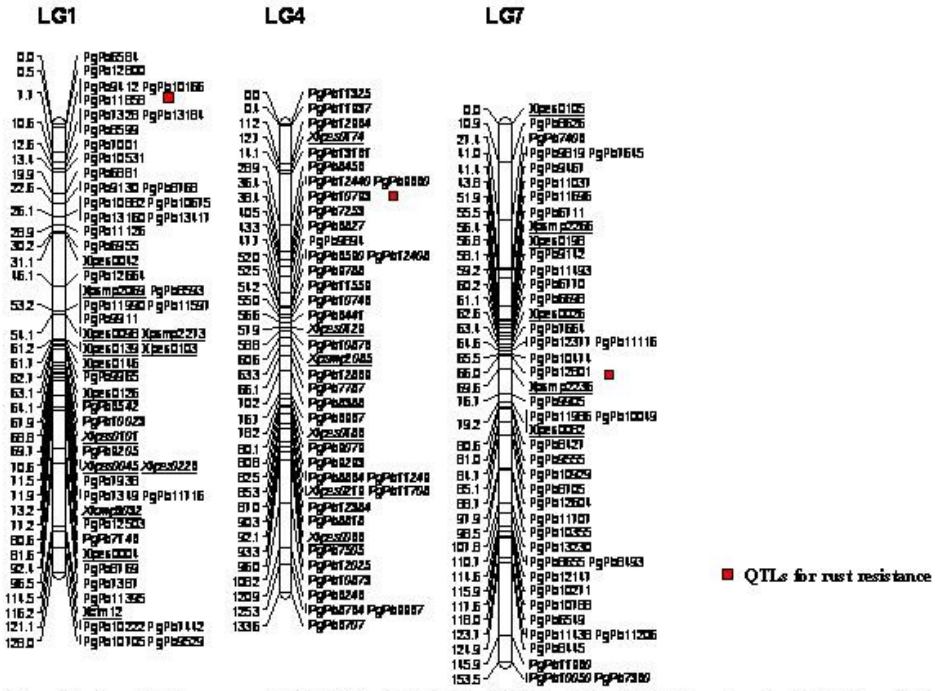

Figure 14. Genetic linkage map of LG 1, LG 4 and LG 7 of the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population showing QTL positions for rust resistance.

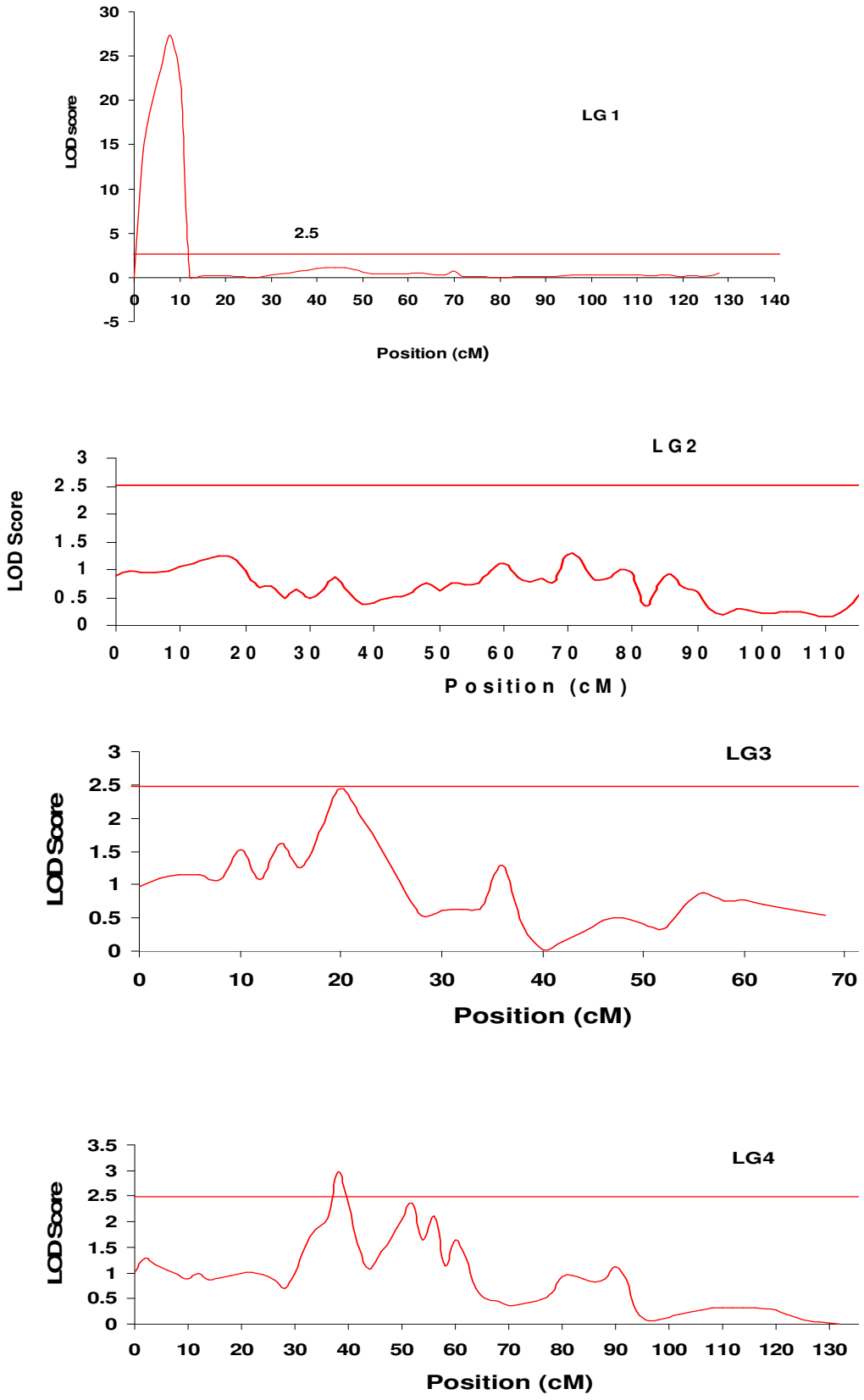

Figure 15(A). Logarithm of odds (LOD) profiles for LG 1 thru LG 4 for rust resistance QTLs segregating in the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population. The horizontal line across each graph indicates the threshold level used for QTL identification

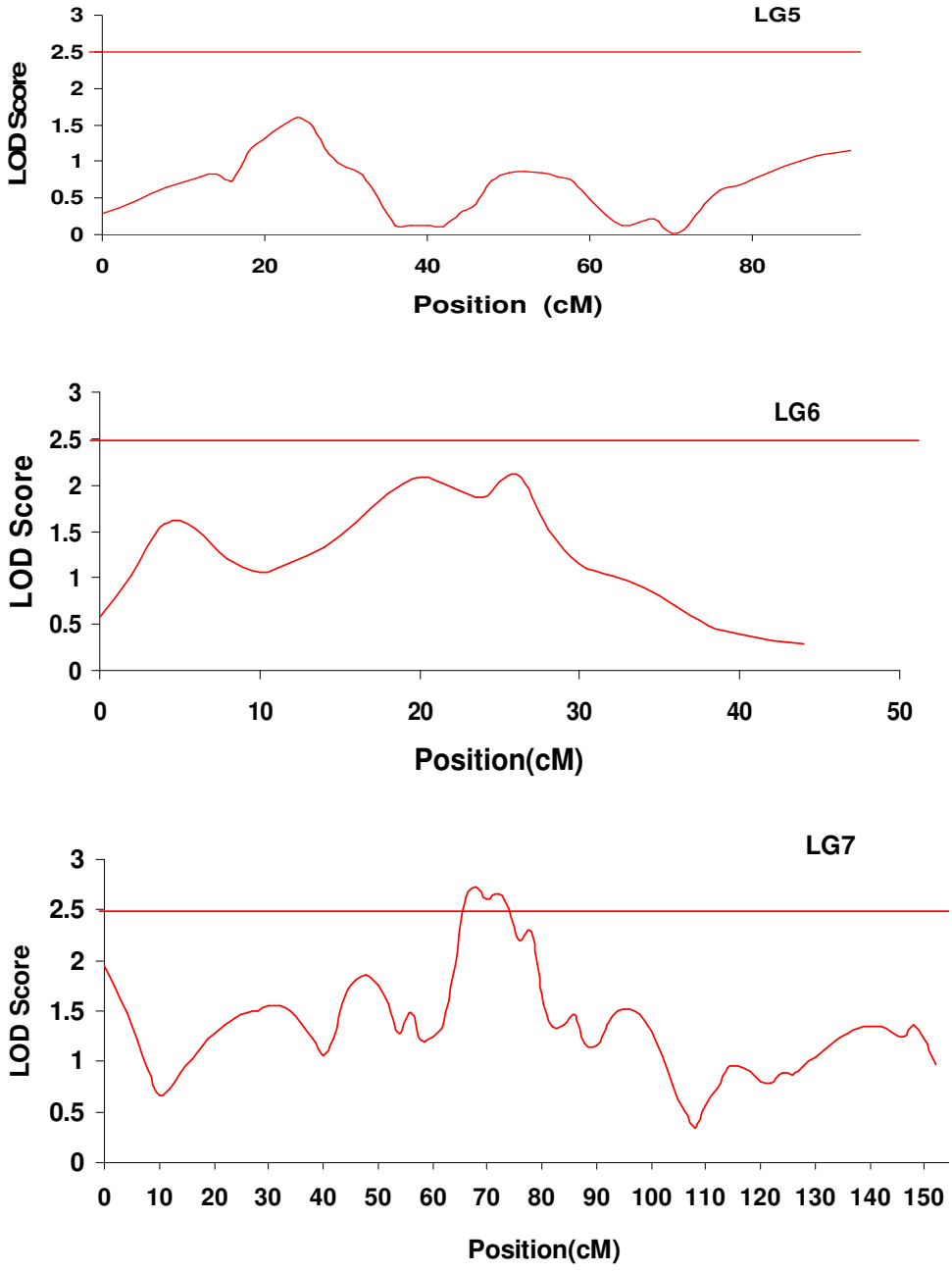

Figure 15(B). Logarithm of odds (LOD) profiles for LG 5 thru LG 7 for rust resistance QTLs segregating in the (81B-P6 x ICMP 451-P8)-based pearl millet RIL population. The horizontal line across each graph indicates the threshold level used for QTL identification

CHAPTER – V

DISCUSSION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important grain, forage and stover crop, grown on more than 26 million ha in arid and semi-arid regions of Asia and Africa, where its grain is often used as basic staple food by the poorest people. However, this crop has received relatively little attention of researchers. So, there is a need to better understand the diversity and genetic basis of this crop. Molecular markers are efficient tools to speed up crop improvement (Langridge, 2005; Varshney and Tuberosa, 2007) and for the construction of molecular linkage maps, the first step in the genetic dissection of target traits. During the past decades, various types of DNA-based molecular markers (e.g., RFLPs, AFLPs, RAPDs, and SSRs) have been developed and applied in crop genetic diversity analysis, gene and QTL mapping, and molecular marker-aided selection. However, all of these techniques share two common features: reliance on gel electrophoresis and low throughput. Consequently, a low cost, high-throughput and electrophoresis-independent technique was required to improve upon these tedious and time-consuming methodologies, especially for those crops with less-developed molecular markers like pearl millet, barley, maize, potato and tobacco.

Diversity arrays technology (DArT), for which proof of concept was first reported by Jaccoud *et al.* (2001), is becoming increasingly adopted in many species. The technology combines a complexity reduction method (Wenzl *et al.,* 2004) with hybridization-based polymorphism detection using high-throughput, solid state platforms and has the potential to generate hundreds of high-quality genomic dominant markers with a cost- and time-competitive trade-off (Kilian *et al.,* 2005). Thus, the present investigation is an attempt to develop and use DArT technology in pearl millet for genetic diversity assessment and QTL mapping.

5.1 Complexity reduction and development of DArT array

The first step in the DArT procedure involves reduction of the number of fragments present in a genomic representation to reveal large amount of genetic polymorphism. This process is called complexity reduction. If the number of unique genomic fragments (complexity) in the targets increases, possibilities for cross-hybridization and obtaining non-specific or low signal intensities increase. It is therefore essential to present subsets of the genome to the arrays to derive meaningful and specific signals. As DArT is a hybridization-based method, the genomic representation can be much more complex compared to those used in gel-based systems such as RAPDs and AFLPs. However, to discover polymorphisms based on SNPs, InDels or epigenetic differences and to obtain a sufficient labeling of all fragments present in the representation, complexity reduction is needed. A good complexity reduction method exhibits a high degree of reproducibility in sampling genomic fragments. Although a nearly unlimited number of methods

of sampling polymorphic sites in the genome could be envisioned, the methods used usually rely on restriction enzyme (RE) digestion, adapter ligation and amplification of adapter-ligated fragments. The initial proof-of-concept paper used AFLP protocols to create representations for DArT (Jaccoud *et al.,* 2001). However, later the AFLP technique was abandoned for DArT for two reasons. First was that genomic representations produced exclusively by RE digestion were more polymorphic than those produced by RE digestion in combination with selective primers. Second, the proprietary status of AFLP in some countries imposes some restriction on the availability and delivery of DArT methods using this type of complexity reduction. Most of the current methods are based on *Pst*I, the RE that was most frequently used to create genomic libraries of low-copy RFLP probes. *Pst*I is sensitive to CXG methylation and does not cut the highly methylated, predominantly repetitive fraction of plant genomes (Rabinowicz *et al.,* 1999). For most plant species, the number of fragments generated by *Pst*I digestion, adapter ligation and amplification of *Pst*I fragments is too large for efficient DArT assays. Therefore, *Pst*I is used in combination with one or more frequently cutting enzymes, while only ligating adapters to the ends created by *Pst*I and amplification of adapter-ligated fragments and this is now one of the methods of choice for plant genomes (Kilian *et al.,* 2005). Restriction and ligation are performed simultaneously to minimize fragment-to-fragment ligation.

The percentage of polymorphism that can be obtained with a certain complexity reduction method can be estimated by either *in silico* simulations or small-scale testing of different enzyme combinations. Computer simulations that calculate the number of fragments when using different enzyme combinations can be of high value if sequence information is present. These *in silico* calculations have been used for optimizing AFLP (Peters *et al.,* 2001) and DArT (Wenzl *et al.,* 2004; Wittenberg *et al.*, 2005) complexity reduction methods. If there is no sequence information available, then testing a number of restriction enzyme combinations to obtain homogenous smear of fragments is a good alternative. These approaches have been used successfully in a number of DArT applications (Wenzl *et al.,* 2004; Xia *et al.,* 2005; Akbari *et al.,* 2006; Yang *et al.,* 2006). Such practical experiments and computer simulations have shown that a complexity of between 10,000 and 20,000 fragments is optimal for DArT. With higher complexities it becomes difficult to obtain a sufficient labelling of all fragments present in the genomic representation and with lower complexities the redundancy (same restriction fragment identified more than once as a marker) in the DArT markers will increase. Based on this concept, different restriction enzyme combinations were tested for pearl millet and all representations showing one or more strong bands were excluded. The *Pst*I/*Ban*II representation was free from observable bands (Fig.3), and so was used to construct the library. Nearly all DArT complexity reduction methods reported so far have used *Pst*I RE to generate adaptor-compatible overhangs, either alone (Wenzl *et al.,* 2004; Xia *et al.,* 2005) or in combination with *Eco*RI (Wittenberg *et al.,* 2005). Yang *et al.* (2006) used a new enzyme combination, *Nde*I + *Bsp*1286I, to produce the

required adaptor-compatible overhangs. Bonin *et al.* (2008) described a new genome complexity reduction method taking advantage of the abundance of miniature inverted repeat transposable elements (MITEs) in the genome of *A. aegypti.* For *Dendrobium* species, a Chinese research group successfully enriched for the presence of polymorphic sequences by applying a subtraction method prior to library construction (Li *et al.*, 2006). In addition, selective amplification (Jaccoud *et al.,* 2001; Yamamoto and Yamamoto, 2004) and degenerated oligonucleotide primer (DOP)-PCR (Jordan *et al.,* 2002) methods for complexity reduction have successfully been used for genetic and epigenetic analysis on microarrays.

The selection of unique and low-copy sequences is an inherent feature of the hybridization-based assay format of DArT and distinguishes DArT from mobility-based assay technologies such as AFLP. Another important feature of genomic representations is sequence complexity, which is determined by the number of unique fragments and their average length. Although DArT can theoretically work with representations of very high complexity, the platform currently implemented comprising glass microarrays, fluorescently labeled targets and a confocal laser scanner, supports assays with representations containing 5,000-20,000 unique fragments *i.e.* a number low enough to ensure the reproducibility of the PCR reaction, but high enough to yield a reasonable number of polymorphic markers. The pearl millet array generated for the current study consisted of 6912 clones, which is large enough to meet the requirements. The length of the fragments is determined by the restriction enzyme combination and the type of adapters used to amplify the representation. We obtained fragments in the size range of 300-1000 bp (Fig. 6). Representations with an average fragment size between 300 to 700 bp perform well on the microarray platform (Jaccoud *et al.,* 2001; Wenzl *et al.,* 2004; Xia *et al.,* 2005).

To prepare a library from the genomic representation, the adenylated PCR fragments have to be ligated into a cloning vector and transformed into electro-competent *Escherichia coli* cells. To achieve high transformation efficiencies it is important that all PCR products are 3'-end adenylated. An efficient adenylation can be obtained by increasing the final extension time at 72°C after the last PCR cycle or by modifications of the primer used for amplification (Brownstein *et al.,* 1996).

The TOPO TA Cloning Kit having pCR2.1-TOPO vector was used for transformation as it provides a highly efficient, 5-minute, one-step cloning strategy for the direct insertion of *Taq* polymerase-amplified PCR products into a plasmid vector and no ligase, post-PCR procedures, or PCR primers containing specific sequences are required. The linearized vector supplied in this kit has a single, overhanging 3' deoxythymidine (T) residue, which allows PCR inserts to ligate efficiently with the vector. Each restriction fragment was flanked by adapter sequences and small portions of the TOPO vector, which was labeled with carboxy-fluorecein 6-FAM and used as a reference signal for all spots. The signal intensity of the reference is used by DArTSoft to determine for each clone the amount of DNA spotted on the array.

DArTSoft enabled the selection of a high-quality marker dataset, by means of applying thresholds for a set of quality parameters. The results demonstrate that DArT markers are of good quality, as measured by their high PIC values, call rates and scoring reproducibility. The average call rate ranged from 87.6 to 91.6% for different experiments using the pearl millet array. The average scoring reproducibility ranged from 99.5 to 100%. DArT markers displayed high polymorphism information content (PIC), the average PIC values ranging from 0.30 to 0.47; with 0.50 being the highest PIC value expected for a bi-allelic marker system. The average P and Q values ranged from 76.1 to 81.3% and 75.2 to 80.9%, respectively (Table 4), which is comparable to those reported by Risterucci *et al*. (2009), Yang *et al*. (2006) and Wenzl *et al*. (2004).

5.2 Genetic relationships among pearl millet inbred lines

Pearl millet cultivars are generated from a relatively narrow gene pool and current breeding programs make only limited use of landrace germplasm accessions and do not make use of wild pearl millets. Genetic diversity studies of *Pennisetum* germplasm generate information that facilitates efficient selection of such accessions for their use in improving pearl millet hybrids, hybrid parental lines and open-pollinated varieties. In the present study, the usefulness of DArT markers was demonstrated for the first time to effectively describe the genetic relationships among a set of pearl millet inbred lines. Such genetic differences in pearl millet have been studied previously by morphological and isozyme analysis (Tostain *et al.,* 1987; Tostain and Marchais, 1989; Tostain, 1992). A disadvantage of isozyme markers is that they are affected by environmental conditions and different stages of development (Falkenhagen, 1985). In addition, the number of isozyme loci that can be analyzed is limited and discrimination between different genotypes is not always possible (Tobolski and Kempery, 1992). Subsequently, RAPDs (Chowdari *et al*., 1998), RFLPs (Bhattacharjee *et al*., 2002), and SSRs (Budak *et al*., 2003; Kapila *et al*., 2008) have been used to estimate pearl millet genetic diversity. Microsatellites have proven informative to study genetic relationships among closely related plant species as well as among subpopulations of a single species (Bowcock *et al*., 1994) because of their exceptionally high level of polymorphism and these recognise multiple alleles and are distributed throughout the genome (Jeffreys *et al*., 1985; Tautz *et al*., 1986). In addition, microsatellites exhibit codominant inheritance and their detection can be readily automated (Hernandez *et al.,* 2002). These features are essential for effective discrimination between closely related lines (Akkaya *et al*., 1992).

The power of the DArT fingerprinting method lies in its ability to compare different genomes at a large number of loci in a single assay. The large numbers of markers that are simultaneously assayed by DArT provide a high level of resolution in genetic-diversity studies. Genetic-distance estimates derived using DArT are more likely to be accurate because the 'random' nature of DArT markers should reduce the ascertainment bias when compared to technologies relying on targeted marker development. DArT markers allow the identification of

genomic regions shared between related genotypes; they effectively complement other molecular marker technologies for genetic diversity studies, genomics and breeding (Risterucci *et al*., 2009). DArT markers revealed genetic relationships among the pearl millet inbred lines that were consistent with those provided by other marker technologies, but at a significantly higher resolution and speed, and at reduced cost. The DArT-based cluster analysis discriminated well between 24 inbred lines of pearl millet (Fig. 9). Clusters generated using DArT were in complete agreement with the available pedigree data. Inbreds derived from the *Iniadi* landrace formed a perfect subcluster within cluster I. In cluster II, five inbreds (ICMB 841-P3, Tift $23D_2B_1$-P5, 81B-P6 and 81B-P8, and IP 18293-P152) were grouped together, four of which are d_2 dwarf lines. Out of these five inbreds, genetically tall ICMB 841-P3 and *d*2 dwarf Tift $23D_2B_1$-P5, are expected to cluster together as they share genetically tall Tift $23B_1$ as a common ancestor and the two sub-selections of 81B, namely 81B-P6 and 81B-P8, were also clustered with these as 81B is a product of an induced mutation breeding program based on Tift $23D_2B_1$. The known pedigree relationships of these five lines are better captured by this DArT-based diversity analysis than by the SSR- and SNP-based analysis recently reported by Thudi *et al*. (2010).

5.3 Genetic linkage map based on DArT and SSR markers

DArTSoft analysis identified 256 and 310 polymorphic markers segregating in the (81B-P6 x ICMP 451-P8)- and (H 77/833-2 x PRLT 2/89-33)-derived random inbred line populations, respectively, with high reproducibility and good P, Q, PIC values and call rates (Table 4). This good performance is partly due to the fact that SNP detection in DArT is mediated by the higher fidelity of restriction enzymes compared to primer annealing. The 'methylation filtration' effect arising from the use of *Pst*I (a methylation-sensitive restriction enzyme) produces genomic representations enriched in the hypo-methylated 'gene space' of a genome. The actively expressed, low-copy regions of a genome, 'gene space' tends to be located in distal regions of the chromosome arms, a pattern that is clearly reflected in DArT-marker density along chromosome arms. Because the size of the 'gene space' varies much less than total genome size, DArT is fairly insensitive with respect to variation in genome size.

The high number of available DArT markers, their cost-effectiveness and relatively high polymorphism content are ideal characteristics for their application in extensive genome-wide screening for QTL discovery, recurrent parent background recovery in marker-assisted backcrossing, isolation of genes via map-based cloning, comparative mapping, and genome organization studies (Varshney *et al*., 2007). Marker-assisted breeding is generally more efficient when molecular maps are well saturated, due to an increased chance of finding polymorphic markers in any genetic background in any genomic region of interest. This is the second report of the use of DArT technology in pearl millet after Kilian *et al*. (2009) and it is the first report where DArT markers were mapped in this species. The two genetic maps span 740.3 cM and 1148.0 cM, corresponding to an average of 2.6 and 3.6 cM per marker, respectively. The linkage maps

constructed in this study are more highly saturated, include more markers and have smaller marker intervals than any previously constructed with RFLPs and/or SSRs. The genome coverage achieved makes the present maps particularly useful to select markers for use in whole genome breeding strategies and to saturate genomic regions of interest in other mapping populations. The maps showed a high level of genome coverage and distribution of markers was reasonably uniform including the distal regions of all chromosome arms (Figs. 11 A, B and 12 A, B). This resulted largely from the inclusion of DArT and EST-SSRs (Table 5). These markers typically show improved genome coverage compared to anonymous (non-coding) SSRs or AFLPs, which are characteristically clustered around the centromeric regions (Ramsay *et al.,* 2000). This difference in genome coverage is thought to reflect the processes used to develop each type of marker. Anonymous SSRs are usually developed from random genomic libraries, in which microsatellites located in the heterochromatic regions are over-represented (Roder *et al.,* 1998) and the development of EST-SSRs from genic regions reduces the representation of regions that are rich in repetitive DNA (Parida *et al.,* 2006). Improved SSR density on the current maps was also facilitated by fluorescence-based marker detection and capillary electrophoresis (Hayden *et al.*, 2005). This assay platform provides higher resolution for small allele size differences and multi-allelic markers compared to nondenaturing PAGE, which has been typically used in previous mapping studies (Karakousis *et al*., 2003).

Genetic linkage maps built with the RECORD software package were superior to those constructed with JoinMap or GMendel, and showed 47% expansion compared to those made using JoinMap. The RECORD map, therefore, was selected for this study. Various problems were encountered with JoinMap and GMendel. Inspection of the locus order given by GMendel revealed a considerable number of misplaced loci and inversions of complete blocks of loci, which led to artifactual crossovers and resulted in inflated maps. In addition, the program needed a very long time (up to several hours) for the calculation of the order of the markers in the linkage groups. The main cause for these problems was the high number of markers with similar segregation patterns. JoinMap 3.0 is one of the most commonly used programs for constructing linkage maps for plant populations, but this program generated erroneous results with the high-density datasets used in the current study. JoinMap has a function to remove all markers with an identical segregation pattern. However, if two marker scores are very similar, but not identical due to missing values, JoinMap keeps both markers in the dataset. JoinMap tries to place these markers at the same position, which in many cases causes tension in the map. Problems with using JoinMap to analyze high-density datasets have been encountered by others as well (Isidore *et al*., 2003; van Os *et al*., 2005).

To overcome these problems a new software package (RECORD) for ordering loci on genetic linkage maps was developed (van Os *et al.,* 2005). RECORD finds the best possible marker order by minimization of the number of recombination events in a dataset of marker

segregation data. In contrast to JoinMap or MapMaker, the algorithm does not make use of many pairwise distance estimates, but it uses the much simpler raw segregation data and therefore is much faster. In addition, the RECORD algorithm does not have a problem with markers with similar, but not identical, segregation patterns. The value of RECORD for the construction of genetic linkage maps from high-density segregation data in potato (Isidore *et al*., 2003; van Os *et al*., 2006), flax (Vromans, 2006) and for building high-density consensus maps of multiple populations in barley (Wenzl *et al*., 2006) has recently been shown. For the markers that showed an identical scoring pattern, the reason could not be ascertained whether the underlying clones had identical sequences or co-segregated because of close linkage.

SSR marker orders from the present study were compared with those from maps based on SSRs only (recently developed unpublished maps of 81B-P6 x ICMP 451-P8 and H 77/833-2 x PRLT 2/89-33 provided by ICRISAT) and were almost identical except for swapping of some marker orders within some blocks on a few linkage groups. Such differences in marker order among genetic maps is not unexpected, as genetic mapping only gives an indication of the relative positions and genetic distances of the markers to each other (Sourdille *et al.,* 2004). Moreover, inconsistency in map position of these few SSRs could be explained by the presence of closely linked DArT loci. The order of loci that were common between the two maps was also very similar with limited order swapping. 78 markers representing all 7 linkage groups of pearl millet were mapped in both populations, which will permit the development of a well-saturated pearl millet consensus linkage map combining DArT and SSR markers.

In the current study, a high proportion of DArT markers showed clustering in distal regions of several of the 14 chromosome arms (Figs. 11 A, B and 12 A, B) and such clustering of DArT markers was more frequent than that of SSRs. This is not surprising, keeping in mind that DArT markers were over four times more abundant than the SSRs in the two data sets (and the SSRs included both genic and genomic SSRs) (Table 5). It seems that DArT markers may have a stronger tendency than genomic SSR and AFLP markers in particular, to map to such gene-rich regions (Vuylsteke *et al.,* 1999), although in the present study this may well have been due to use of the methylation-sensitive restriction enzyme *Pst*I in complexity reduction of the initial library used in creating the DArT array, and subsequent preparation of DNA samples for hybridization to the array. The occurrence of DArT marker clusters in distal regions of chromosome arms was observed in previous DArT mapping studies on wheat (Akbari *et al.,* 2006; Semagn *et al.,* 2006b) and barley (Wenzl *et al.,* 2004). Similar clustering in distal regions was also found in tetraploid wheat using *Pst*I-based AFLP markers (Peng *et al.,* 2000). The higher density of such clusters in distal regions could well be related to the trend of *Pst*I-based markers to map in gene-rich, hypomethylated areas regions of the genome (Langridge and Chalmers, 1998; Moore, 2000). Nevertheless, it is worth noting that the high number of DArT clusters could also be a consequence of the presence of redundant clones on the arrayed genomic representation (Semagn

et al., 2006 b). Clustering around centromeres is a well-known phenomenon with all types of markers, resulting from centromeric suppression of recombination (Tanksley *et al.,* 1992; Korol *et al.,* 1994). The high proportion of clustering of DArT markers away from the centromeres may, therefore, be indicative of gene-rich regions. If this is indeed the case, DArT markers may prove particularly helpful for fine mapping of genes/QTLs residing in gene-rich regions, thereby facilitating positional cloning.

The relatively large population sizes (146 RILs from 81B-P6 x ICMP 451-P8 and 137 RILs from H 77/833-2 x PRLT 2/89-33) used for construction of the genetic linkage maps presented here as compared with other studies (62-120; Blanco *et al.,* 1998, 2004; Röder *et al*., 1998; Nachit *et al.,* 2001; Elouafi and Nachit, 2004; Quarrie *et al*., 2005; Akbari *et al.,* 2006) is highly advantageous for further exploitation of these maps. These larger population sizes improve the estimation of marker orders, which in turn improves the resolution of QTL mapping of agronomic traits. They also enable a greater resolution in the positioning of QTLs on the genetic map, while distribution of markers across the full length of the genome is required to detect all contributing loci (Chalmers *et al.,* 2001). Furthermore, these maps are the most highly saturated linkage maps for pearl millet in terms of total numbers of markers mapped. However, it will be useful to use larger population sizes with such large numbers of markers in order to get more accuracy in marker order of these maps.

5.4 Segregation distortion of markers

Segregation distortion is defined as the deviation of observed genetic ratios from the expected Mendelian ratios in a given phenotypic or genotypic class within a segregating population. Various causes can lead to segregation distortion: chromosomal rearrangement (Faure *et al*., 1993), alleles inducing gametic or zygotic selection (Xu *et al.*, 1997; Lu *et al.,* 2002), parental reproductive differences (Foolad *et al.,* 1995), presence of lethal genes (Blanco *et al*., 1998), meiotic drive and competition between gametes for preferential fertilization (Lyttle, 1991). In general, normal Mendelian segregation can be viewed as a product of evolutionary co-adaptation, and of adjustment of genomic components within a species rather than an automatic outcome of the eukaryote meiotic mechanics (Korol *et al*., 1994). Indirect evidence for this is provided by the fact that segregation distortions frequently occur in the progeny of interspecific hybrids and are similar in manifestation to meiotic drive systems. Numerous examples of segregation distortion have been reported in many crop species including barley (Graner *et al*., 1991; Devaux *et al*., 1995), rice (Causse *et al.,* 1994; Xu *et al.,* 1997), maize (Wendel *et al.,* 1987; Lu *et al.,* 2002) and wheat (Blanco *et al.,* 2004; Peng *et al.*, 2000; Quarrie *et al.,* 2005). Segregation distortion is most commonly observed in interspecific crosses; however, previous studies showed distortion phenomenon also occurs in intraspecific pearl millet crosses (Liu *et al*., 1994 b; Busso *et al*., 1995). While segregation distortion is a common phenomenon in different types of mapping populations, be it F_2, RILs or double haploid (DH), RIL populations have the highest potential for

such distortions due to repeated generations of selection forces (Singh *et al.,* 2007), which can be accentuated by loss of vigour with enforced inbreeding in outcrossing species such as pearl millet. The phenomenon of segregation distortion can be one of the limitations in map construction as it may affect both the establishment of linkage groups and estimation of recombination frequencies. Calculations of linkage distance usually assume no segregation distortion, which could cause over-estimation of recombination frequency between linked markers (Paran *et al*., 1995). But in the present study, the two F_7 RIL populations, 81B-P6 x ICMP 451-P8 and H 77/833-2 x PRLT 2/89-33 showed 38.0% and 34.9% segregation distortion (Table 7) respectively, which did not appear to have much effect on map construction. In most previous studies, segregation distortion in favor of the female parent alleles was observed (Singh *et al*., 2007). In contrast, the present data showed distortion in favor of the male parent alleles in some genomic regions and female parent alleles in others, with the genomic regions exhibiting distorted segregation varying in the two RIL populations. This result should not be considered as a surprise if we take into account the variety of mechanisms that could contribute to the observed distortions such as meiotic drive, preferential abortion of gametes, effects of unusual gametophyte factors, non-random fertilization, and viability selection at post-syngamic stages. Clearly, these factors may work simultaneously and in opposite directions, favoring the alleles of the two parents in different genomic regions. Segregation distortion favoring alleles from a male parent has previously been reported in pearl millet by Liu *et al.* (1994 b), Azhaguvel (2001) and Kolesnikova (2001). It has been suggested that such segregation distortion is highly likely in pearl millet because of its protogynous nature (Liu *et al.,* 1994 b) and sensitivity to inbreeding depression.

5.5 QTL mapping for rust resistance

The high density linkage map for the RIL population based on cross 81B-P6 x ICMP 451-P8 has been successfully used to identify QTLs for rust resistance. This study is a novel report on QTL mapping for rust resistance in pearl millet. The only previous report of rust resistance mapping (Morgan *et al*., 1998), reported QTLs for resistance to pathogen populations present in the southeastern USA, and these mapped to LG 3 and LG 4. In contrast, in the present study a major QTL effective in India was detected on LG 1, along with two QTL modifiers (one each on LG 4 and LG 7), explaining 58% of observed phenotypic variation in rust reaction among the RIL progenies (Table 11). Highly significant differences detected by ANOVA between individual progenies, and high operational heritability of 0.99 demonstrated that resistance was segregating in the population and that much of the observed variation in rust reaction phenotype was attributable to genetic variation. Host rust reaction was continuously distributed in the population, (Fig. 13). However, this does not necessarily imply that the inheritance of rust reaction is complex and that many genes are segregating. In fact, as the frequency distribution of the RILs showed two peaks, it was anticipated that a large portion of the variation would prove to be

attributable to a single genomic region of large effect, and this was indeed the outcome of the QTL analysis. Andrews *et al.* (1985), and Hanna *et al.* (1985) previously reported that pearl millet rust resistance is conferred by single dominant genes (Rpp_1 and Rr_1, respectively) and susceptibility by their recessive alleles. The major rust resistance gene mapped in the present study is also likely to be genetically dominant although this was not tested. Further, unlike the Rr_1 gene reported by Hanna *et al.* (1985), it has proven durable, as it is still effective >20 years after its initial large-scale deployment in India in 1986 in dual-purpose pearl millet hybrid ICMH 451 (MH 179) = 81A×ICMP 451. This study will help to assess the role of this rust resistance locus in providing a framework for marker-assisted selection and cloning of resistance genes.

The results obtained from the present study indicate that DArT provides high quality markers that can be used to construct of high-density genetic linkage maps for plants with complex genomes even when no sequence information is available. DArT can effectively detect SNPs and InDels in the restriction sites that are used in complexity reduction during creation of the DArT array. An additional advantage is that DArT clones can readily be sequenced and thus provide information for their conversion into PCR-based markers. This can be advantageous in cases, such as the major rust resistance QTL detected in the present study on LG 1, when there are not yet any inexpensively assayed markers closely flanking a potential target that could be used in foreground selection for the favorable allele. DArT can be fine-tuned to detect polymorphism in species with various genome sizes. This fine-tuning can be achieved by using the appropriate complexity reduction method or by making use of enrichment techniques prior to cloning. Integration of DArT maps will be straightforward provided these are developed with the same array. High-density maps for map-based cloning and chromosome-landing approaches (Tanksley *et al*., 1995) could be rapidly built by pyramiding data from a limited number of independent arrays. Therefore, it can be believed that DArT is a good alternative to currently used techniques for whole-genome fingerprinting. By using a properly formatted genotyping array, the generation of a linkage map would typically take only three days. This throughput enables routine use of DArT in plant breeding programs; e.g., for exhaustive fingerprinting of germplasm, for quantitative trait locus identification, for genome background screening, for simultaneous marker-assisted selection of several loci, or for accelerated introgression of selected genomic regions. Thus, DArT opens significant opportunities for plant breeding to benefit from whole-genome profiling, particularly in the context of improving traits with complex inheritance.

CHAPTER – VI

SUMMARY AND CONCLUSION

In the present investigation, attempts have been made to analyze genetic diversity and identify QTLs for rust resistance in pearl millet using DArT. The study was broadly divided into three groups of activities:

1. Development of a pearl millet DArT array and diversity analysis of diverse pearl millet inbred lines using this array.
2. Genotyping of two mapping populations using DArT and SSR markers and linkage map construction.
3. QTL identification for rust resistance.

The results obtained in the present study are summarized below:

1. *Pst*I/*Ban*II-based complexity reduction was used to develop a pearl millet DArT array with a DNA representation from 95 diverse genotypes.
2. The amplified inserts obtained after transformation ranged from 300-1000 bp in size.
3. The DArT array constructed consisted of 7680 clones with overall 10% missing fragments. Thus, overall the array has approximately 6912 clones.
4. A set of 24 diverse pearl millet inbred lines were genotyped using the DArT array for diversity analysis. A total of 574 polymorphic DArT markers were identified from the total of 6912 clones on the array (8.3% of polymorphic clones), with 91.6% average call rate, 100% scoring reproducibility, 0.30 average PIC value, 76.1% average P value and 75.2% average Q value.
5. The DArT-based cluster analysis discriminated well between the 24 inbred lines of pearl millet and these were grouped into two main clusters. Cluster I was comprised of 12 inbred lines including restorer lines, downy mildew resistance sources and *Iniadi* landrace-derived mapping population parental lines. Cluster II consisted of 11 inbreds, which were mainly seed parents; whereas ICMB 90111-P6, an inbred derived from the ICRISAT Early Composite, was entirely separated from the two main clusters. Relationships detected by this cluster analysis between different subsets of the 24 inbreds were in agreement with their known pedigree relationships.
6. 168 F_7 RILs from the cross 81B-P6 x ICMP 451-P8 were genotyped using the DArT array and 256 segregating DArT markers (3.7% of polymorphic clones) were identified. The average call rate was 89.5% with 100% scoring reproducibility, 0.46 average PIC value, 81.3% average P value and 80.9% average Q value.
7. Scorable amplification products were detected by capillary electrophoresis for 25 (83.3%) of 30 SSR markers used for genotyping of the (81B-P6 x ICMP 451-P8)-based

RIL mapping population. This data was merged with that for 49 SSRs previously genotyped at ICRISAT for this RIL population.

8. Out of the total 326 polymorphic markers (256 DArT and 70 SSRs) used for assembling the linkage map using 146 RILs data for the cross 81B-P6 x ICMP 451-P8, 286 loci (229 DArT markers and 57 SSRs) were mapped across the expected 7 linkage groups. The total map length was 740.3 cM (Haldane) with an average adjacent-marker distance of 2.6 cM.
9. After genotyping the RIL mapping population based on cross H 77/833-2 x PRLT 2/89-33 with the DArT array, 310 polymorphic DArT markers (4.5%) were scored. The average call rate was 87.6% with 99.5% scoring reproducibility, 0.47 average PIC value, 79.5% average P value and 79.1% average Q value.
10. Along with 309 DArT markers, 80 previously genotyped polymorphic SSR markers were integrated into the data set. Out of these 389 markers, 321 loci (258 DArTs and 63 SSRs) were mapped across the expected 7 linkage groups. The (H 77/833-2 x PRLT 2/89-33)-based genetic linkage map spanned 1148 cM (Haldane), with an average adjacent-marker distance of 3.6 cM.
11. Out of 326 markers analyzed on 146 RILs of 81B-P6 x ICMP 451-P8, 124 (38.03%) showed segregation distortion from the expected 1:1 ratio. Sixty markers (18.4%) showed distortion in favor of the 81B-P6 allele whereas 64 (19.6%) showed distortion in favor of the ICMP 451-P8 allele.
12. Segregation distortion was found in 136 markers (34.9%) out of 389 markers analyzed on 137 RILs of H 77/833-2 x PRLT 2/89-33. Thirty-seven (9.5%) showed distortion in favour of the H 77/833-2 allele whereas 99 (25.4%) showed distortion in favour of the PRLT 2/89-33 allele.
13. Seventy-eight markers (56 DArT and 22 SSRs) were common between the two RIL populations, permitting the development of a well-saturated pearl millet consensus linkage map of DArT and SSR markers.
14. Rust reaction on 167 RILs of the cross 81B-P6 x ICMP 451-P8 was assessed using artificial inoculation of pot-grown seedlings and rust severity ranged from 0 to 95% with an operational heritability of 99%.
15. Out of the 167 RILs, 32 lines were resistant, 18 moderately resistant, 73 moderately susceptible, 40 susceptible and the remaining 4 lines were highly susceptible to rust. Among the parents, 81B-P6 was highly susceptible and ICMP 451-P8 was resistant to rust.
16. A major QTL (LOD 27) was detected near the top of linkage group 1 (LG 1) explaining 58% of the observed phenotypic variation in rust reaction, along with two modifiers (one each on LG 4 and LG 7) explaining 9% and 8% of the phenotypic variation, respectively.

Favourable alleles for the QTLs on LG 1 and LG 7 were from resistant parent ICMP 451-P8.

It is concluded that the present study is the second report of the use of DArT technology in pearl millet and DArT markers were mapped for the first time in this species. The study has been proved to be useful for diversity assessment of inbred lines and for rapid development of reasonably-well saturated genetic linkage maps of RIL populations that can be used for precise and fine QTL mapping. It is anticipated that this DArT array will also prove useful for background genotyping in marker-assisted backcrossing programs to speed up recovery of elite recurrent parent genetic background on genomic regions outside that targeted for introgression of donor parent alleles. The rust resistance locus identified on LG 1 is a novel report and will be useful for providing a framework for marker-assisted selection and cloning of resistance genes.

BIBLIOGRAPHY

Akbari, M., Wenzl, P., Caig, V., Carling, J., Xia, L., Yang, S., Uszynski, G., Mohler, A., Lehmensiek, A., Kuchel, H., Hayden, M.J., Howes, N., Sharp, P., Vaughan, P., Rathnell, B., Huttner, E. and Kilian, A. 2006. Diversity arrays technology (DArT) for high throughput profiling of the hexaploid wheat genome. *Theor. Appl. Genet.* **113**: 1409-1420.

Akkaya, M.S., Bhagwat, A.A. and Cregan, P.B. 1992. Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics* **132**: 1131-1139.

Allouis, S., Qi, X., Lindup, S., Gale, M.D. and Devos, K.M. 2001. Construction of a BAC library of pearl millet, *Pennisetum glaucum*. *Theor. Appl. Genet*. **102**: 1200-1205.

Alsop, B.P., Kilian, A., Carling, J., Pickering, R.A. and Steffenson, B. J. 2007. DArT marker-based linkage analysis and inheritance of multiple disease resistance in a wild x cultivated barley population. In: *Plant and Animal Genomes XV Conference.* San Diego, CA, Jan.13-17, P333.

Andrews, D. J., Rai, K. N. and Singh, S. D. 1985. A single dominant gene for rust resistance in pearl millet. Crop Sci. 25: 565-566.

Athwal, D.S. and Gupta, V.P. 1966. Genetic evaluation of grain and fodder quality of Pennisetum. Annual Research Report 1965-66. Punjab Agricultural University, Ludhiana, Punjab, India

Azhaguvel, P. 2001. Linkage map construction and identification of QTLs for downy mildew (*Sclerospora graminicola*) resistance in pearl millet (*Pennisetum glaucum* (L.) R. Br.). PhD thesis, Department of Agricultural Botany, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India. 204 pp.

Azhaguvel, P., Hash, C.T., Rangasamy, P. and Sharma, A. 2003. Mapping the d_1 and d_2 dwarfing genes and purple foliage color locus *P* in pearl millet. *The Journal of Heredity* **94**: 155-159.

Bar-Hen, A., Charcosset, A., Bourgoin, M. and Cuiard, J. 1995. Relationships between genetic markers and morphological traits in a maize inbred lines collection. *Euphytica* **84**: 145-154.

Barrett, B.A. and Kidwell, K.K. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop Sci*. **38**: 1261–1271.

Basten, C.J., Weir, B.S. and Zeng, Z.B. 1994. QTL cartographer. Dep. of Statistics, North Carolina State Univ., Raleigh, NC.

Bernardo, R. 1993. Estimation of coefficient of co ancestry using molecular markers in maize. *Theor. Appl. Genet*. **85**: 1055–1062.

Bertin, I., Zhu, J.H. and Gale, M.D. 2005. SSCP-SNP in pearl millet-a new marker system for comparative genetics. *Theor. Appl. Genet.* **110**: 1467-1472.

Bhattacharjee, R., Bramel, P.J., Hash, C.T., Kolesnikova-Allen M. A. and Khairwal, I. S. 2002. Assessment of genetic diversity within and between pearl millet landraces. *Theor. Appl. Genet.* **105**: 666-673.

Bidinger, F.R., Nepolean, T., Hash, C.T., Yadav, R.S. and Howarth, C. J. 2007. Quantitative trait loci for grain yield in pearl millet under variable post flowering moisture conditions. *Crop Sci*. **47**: 969-980.

Blanco, A., Bellomo, M.P., Cenci, A., De Giovanni, C., D'Ovidio, R., Iacono, E., Laddomada, B., Pagnotta, M.A., Porceddu, E., Sciancalepore, A., Simeone, R. and Tanzarella, O.A .1998. A genetic linkage map of durum wheat. *Theor. Appl. Genet*. **97**: 721–728.

Blanco, A., Simeone, R., Cenci, A., Gadaleta, A., Tanzarella, O.A., Porceddu, E., Salvi, S., Tuberosa, R., Figliuolo, G., Spagnoletti, P., Röder, M.S. and Korzun, V. 2004. Extension of the "Messapia x *dicoccoides*" linkage map of *Triticum turgidum* (L.) *Thell. Cell Mol Biol Lett*. **9**: 529-541.

Bolibok-Brągoszewska, H., Heller-Uszyńska, K., Wenzl, P., Uszyński, G., Kilian, A. and Rakoczy-Trojanowska, M. 2009. DArT markers for the rye genome-genetic diversity and mapping. *BMC Genomics* **10**: 578.

Bonin, A., Paris, M., Després, L., Tetreau, G., David, J.P. and Kilian, A. 2008. A MITE-based genotyping method to reveal hundreds of DNA polymorphisms in an animal genome after a few generations of artificial selection. *BMC Genomics* **9**: 459-470.

Borevitz, J.O., Liang, D., Plouffe, D., Chang, H.S, Zhu, T., Weigel, D., Berry, C.C., Winzeler, E. and Chory, J. 2003. Large-scale identification of single-feature polymorphisms in complex genomes. *Genome Res*. **13**: 513-523.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet*. **32**: 314-331.

Bouchet, S., Billot, C., Deu, M., Rami, J.F., Xia, L., Kilian, A. and Glaszmann, J.C. 2007. Whole genome scan and linkage disequilibrium evaluation on a sorghum core collection. In: Plant and Animal Genomes XV Conference. San Diego, CA, P365.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R. and Cavalli-Sforza, L.L. 1994. High resolution human evolutionary trees with polymorphic microsatellites. *Nature* (London) **368**: 455-457.

Breese, W.A., Hash, C.T., Devos, K.M. and Howarth, C.J. 2002. Pearl millet genomics-an overview with respect to breeding for resistance to downy mildew. Pages 243–246 *in* Sorghum and Millets Pathology 2000 (Leslie JF, ed.). Ames, Iowa, USA: Iowa State Press.

Brownstein, M. J., Carpten, J. D. and Smith, J. R. 1996. Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques* **20:** 1004-6, 1008-10.

Brunken, J.N. 1977. A systematic study of *Pennisetum* Sect. *Pennisetum* (Graminae). *Am. J. Bot*. **64**: 161-176.

Buckler, E. 2007. TASSEL: Trait Analysis by aSSociation, Evolution, and Linkage. User manual http://www.maizegenetics. net/tassel; verified 10 July, 2008.

Budak, H., Pedraza, F., Cregan, P.B., Baenziger, P.S. and Dweikat, I. 2003. Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. *Crop Sci*. **43**: 2284-2290.

Burton, G.W. and Wilson, J.P. 1995. Identification and transfer of heterotic chromosome blocks for forage yield in short-day exotic pearl millet landraces. *Crop Sci*. **35:** 1184-1187.

Busso, C.S., Liu, C.J., Hash, C.T., Witcombe, J.R., Devos, K.M., de Wet, J.M.J. and Gale, M.D. 1995. Analysis of recombination rate in female and male gametogenesis in pearl millet (*Pennisetum glaucum)* using RFLP markers. *Theor. Appl. Genet*. **90**: 242-246.

Causse, M.A., Fulton, T.M., Cho, Y.G., , S.N., Chunwongse, J., Wu, K.S., Xiao, J.H., Yu, Z.H., Ronald, P.C., Harrington, S.E., Second, G., Mc-Couch, S.R. and Tanksley, S.D. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **138**: 1251-1274.

Chakauya, E. and Tongoona, P. 2008. Analysis of genetic relationships of pearl millet (*Pennisetum glaucum* L.) landraces from Zimbabwe, using microsatellites. Int. J. Plant Breeding and Genetics, **2**: 1-7.

Chalmers, K.J., Campbell, A.W., Kretschmer, J., Karakousis, A., Henschke, P.H., Pierens, S., Harker, N., Pallotta, M., Cornish, G.B. ShariXou, M.R., Rampling, L.R., McLauchlan, A., Daggard, G., Sharp, P.J., Holton, T.A., Sutherland, M.W., Appels, R. and Langridge, P. 2001. Construction of three linkage maps in bread wheat, *Triticum aestivum*. *Aust. J. Agric. Res.* **52**: 1089-1119.

Chandra-Shekara, A.C., Prasanna, B.M., Bhat, S.R. and Singh, B.B. 2007. Genetic diversity analysis of elite pearl millet inbred lines using RAPD and SSR markers. *J. Plant Biochem. and Biotech.* **16**: 23-28.

Chee, M., Yang, R., Hubbell, E., Berno, A., Huang, X.C., Stern, D., Winkler, J., Lockhart, D.J., Morris, M.S. and Fodor, S.P.A. 1996. Accessing genetic information with high-density DNA arrays. *Science* **274**: 610-614.

Choudhary, K., Choudhary, O.P. and Shekhawat, N.S. 2008. Marker Assisted Selection: A novel approach for crop improvement. *American-Eurasian J. Agron.* **1**: 26-30.

Chowdari, K. V., Davierwala, A. P., Gupta, V. S., Ranjekar, P. K. and Govila, O. P. 1998. Genotype identification and assessment of genetic relationships in pearl millet [*Pennisetum glaucum* (L.) R Br] using microsatellites and RAPDs. *Theor. Appl. Genet.* **97**: 154-162.

Collard, B.C.Y. and Mackill, D.J. 2008. Marker assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. Ser. B. Biol. Sci.* **363**: 557-572.

Cooke, R.J. and Reeves, J.C. 1998. Cultivar identification-a review of new methods. In: Kelly AF and George RAT (editors). Encyclopedia of seed production of world crops. London: John Wiley & Sons, pp. 88-102.

Cox, T.S., Murphy, J.P. and Rodgers, D.M. 1986. Changes in genetic diversity in the red winter wheat regions of the United States. *Proc. Natl. Acad. Sci. USA* **83**: 5583-5586.

Crossa, J., Burgueno, J., Dreisigacker, S., Vargas, M., Herrera-Foessel, S.A., Lillemo, M., Singh, R.P., Trethowan, R., Warburton, M., Franco, J., Reynolds, M., Crouch, J.H. and Ortiz, R. 2007. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics* **177**: 1889-1913.

Crossa, J., Vargas, M., Van Eeuwik, F.A., Jiang, C., Edmeades, G.O. and Hoisington, D. 1999. Interpretating genotype x environment interaction in tropical maize using linked molecular markers and environmental covariables. *Theor. Appl. Genet.* **99**: 611-625.

Cutler, D.J., Zwick, M.E., Carrasquillo, M.M., Yohn, C.T., Tobin, K.P., Kashuk, C., Mathews, D.J., Shah, N.A., Eichler, E.E., Warrington, J.A. and Chakravarti, A. 2001. High-throughput variation detection and genotyping using microarrays. *Genome Res.* **11**: 1913-1925.

Devaux, P., Kilian, A. and Kleinhofs, A.1995. Comparative mapping of the barley genome with male and female recombination-derived, doubled haploid populations. *Mol. Gen. Genet.* **249**: 600-608.

Devos, K.M., Pittaway, T.S. Reynolds, A. and Gale, M.D. 2000. Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. *Theor. Appl. Genet.* **100**: 190-198.

Donini, P., Cooke, R.J. and Reeves, J.C. 2000a. Molecular markers in variety and seed testing. In: Arencibia A.D. (ed.) Proceedings of the International Symposium on Plant Genetic Engineering, Havana, Cuba, 1999. Amsterdam: Elsevier B.V., pp. 27-34.

Donini, P., Law, J.R., Koebner, R.M.D., Reeves, J.C. and Cooke, R.J. 2000b. Temporal trends in the diversity of UK wheat. *Theor. Appl. Genet.* **100**: 912-917.

Dudley, J.W. 1993. Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. *Crop Sci.* **33**: 660-668.

Eathington, S.R., Dudley, J.W. and Rufener, G.K.II. 1997. Usefulness of marker-QTL associations in early generation selection. *Crop Sci.* **37**: 1686-1693.

Edwards, M.D., Stuber, C.W. and Wendel J.F. 1987. Molecular-marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution, and types of gene action. *Genetics* **116**: 113 – 125.

Elouafi, I. and Nachit, M.M. 2004. A genetic linkage map of the Durum x *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. *Theor. Appl. Genet.* **108**: 401-413.

Epplen, J.T., Ammer, H., Epplen, C., Kammerbauer, C., Mitreiter, R., Roewer, L., Schwaiger, W., Steimle, V., Zischler, H., Albert, E., Andreas, A., Beyermann, B., Meyer, W., Buitkamp, J., Nanda, I., Schmid, M., Nurnberg, P., Pena, S.D.J., Poche, H., Sprecher, W., Schartl, M., Weising, K. and Yassouridis, A. 1991. Oligonucleotide fingerprinting using simple repeat motifs: a convenient ubiquitously applicable method to detect hypervariability for multiple purposes. In: Burke, T., Dolf, G., Jeffreys, A.J., Wolff, R. (eds) DNA fingerprinting: approaches and applications. *Birkhauser Verlag, Basel*, pp 50-69.

Falkenhagen, E.R. 1985. Isozyme studies in provenance research of forest trees. *Theor. Appl. Genet.* **69**: 335-347.

Faure, S., Noyer, J.L., Horry, J.P., Bakry, F., Lanaud, C. and Gonzalez de Leon, D. 1993. A molecular marker-based linkage map of diploid bananas (*Musa acuminata*). *Theor. Appl. Genet.* **87**: 517–526.

Flavell, A, J., Bolshakov, V.N., Booth, A., Jing, R., Russell, J., Ellis, T.H. and Isaac, P. 2003. A microarray-based high throughput molecular marker genotyping method: the tagged microarray marker (TAM) approach. *Nucleic Acids Res.* **31**: e115.

Foolad, M.R., Arulsekar, S., Becerra, V. and Bliss, F.A. 1995. A genetic map of Prunus based on an interspecific cross between peach and almond. *Theor. Appl. Genet.* **91**: 262-269.

Frova, C., Krajewski, P., Di Fonzo, N., Villa, M. and Sari-Gorla, M. 1999. Genetic analysis of drought tolerance in maize by molecular markers. I: Yield components. *Theor. Appl. Genet.* **99**: 280-288.

Gale, MD., Devos, K.M., Zhu, J.H., Allouis, S., Couchman, M.S., Liu, H., Pittaway, T.S., Qi, X.Q., Kolesnikova-Allen, M. and Hash, C.T. 2001. New molecular marker technologies for pearl millet improvement. *Int. Sorghum Millets Newsl.* **42**: 16-22

Goldstein, D.B. and Schlotterer, C. 1999. Microsatellites, evolution and applications. Oxford University Press, New York.

Gonzalez, J. 1993. Random amplified polymorphic DNA analysis in *Hordeum* species. *Genome* **36**: 1029-1031.

Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G. and Herrmann, R.G. 1991. Construction of an RFLP map of barley. *Theor. Appl. Genet.* **83**: 250-256.

Gulia, S.K. 2004. QTL mapping for improvement of downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] resistance (DMR) in pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrid parental line ICMB 89111. Ph.D. thesis submitted to CCS HAU Hisar, India.

Gulia, S.K., Hash, C.T. Thakur, R.P. Breese, W.A. and Sangwan, R.S. 2007. Mapping new QTLs for downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. p. 373–386. In: D.P. Singh, V.S. Tomar, R.K. Behl, S.D. Upadhyaya, N.S. Bhale, and D. Khare (eds.), Crop production in stress environments:Genetic and management options. Agrobios Publ., Jodhpur, India.

Gupta, P.K., Balyan, H.S., Sharma, P.C. and Ramesh, B. 1996. Microsatellites in plants: a new class of molecular markers. *Current Sci*.**70**: 45-54.

Gupta, P.K., Varshney, R.K., Sharma, P.C. and Ramesh, B. 1999. Molecular markers and their applications in wheat breeding. *Plant Breeding* **118**: 369-390.

Haley, C.S. and S.A. Knott. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**: 315-324.

Hallauer, A.R. and J.B. Miranda. 1988. Quantitative genetics in maize breeding. 2nd edition, Iowa State University Press, Ames, IA.

Hamrick, J.L. and Godt, M.J.W. 1997. Allozyme diversity in cultivated crops. *Crop Sci.* **37**: 26-30.

Hanna, W.W. 1993. Registration of pearl millet parental lines Tift 8677 and A1/B1 Tift 90D2E1. *Crop Sci*. **33**: 1119.

Hanna, W.W. and Wells, H.D. 1993. Registration of parental line Tift 89D2, rust-resistant pearl millet. *Crop Sci*. **33**: 361-362.

Hanna, W.W., Wells, H.D. and Burton, G.W. 1985. Dominant gene for rust resistance in pearl millet. *J. Heredity* **76**: 134.

Hanna, W.W, Wells, H.D. and Burton, G.W. 1987. Registration of pearl millet inbred parental lines, Tift 85D2A1 and Tift 85D2B1. *Crop Sci*. **27**: 1324-1325.

Hanna, W.W., Wells, H.D., Burton, G.W., Hill, G.M. and Monson, W.G. 1988. Registration of 'Tiftleaf 2' pearl millet. *Crop Sci*. **28**: 1023.

Hash, C.T., Bhasker Raj, A.G., Lindup, S., Sharma, A., Beniwal, C.R., Folkertsma, R.T., Mahalakshmi, V., Zerbini, E. and Blümmel, M. 2003. Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum. *Field Crops Research* **84:** 79-88.

Hash, C.T. and Witcombe, J.R. 2001. Pearl millet molecular marker research. *Int. Sorghum Millets Newsl.* **42**: 8-15.

Hayden, M.J., Nguyen, T.M. and Chalmers, K.J. 2005. Multiplex-ready markers. A technique for high-thoughput, low-cost genotyping on an automated DNA fragment analyser. In: Proceedings of the 12th annual barley technical symposium. Hobart, Tasmania.

Hayden, M.J., Nguyen, T.M., Waterman, A., McMichael, G.L. and Chalmers, K.J. 2008. Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. *Mol. Breeding* doi: 10.1007/s11032-007-9127-5.

Hearnden, P.R., Eckermann, P.J., McMichael, G.L., Hayden, M.J., Eglinton, J.K. and Chalmers, K.J. 2007. A genetic map of 1,000 SSR and DArT markers in a wide barley cross. *Theor. Appl. Genet.* **115**: 383-391.

Heller-Uszynska, K., Carling, J., Evers, M., Piperidis, G., Gilmour, R., Aitken, K., Jackson, P., Huttner, E. and Kilian, A. 2007. Diversity Arrays Technology (DArT) for high throughput, whole-genome molecular analysis in sugarcane. In: Plant and Animal Genomes XV Conference. San Diego, CA, W188.

Hernandez, P., Laurie, D.A., Martin, A. and Snape, J.W. 2002. Utility of wheat simple sequence repeat (SSR) markers for genetic analysis of *Hordeum chilense* and tritordeum. *Theor. Appl. Genet.* **104**: 735-739.

Hilbert, P., Lindpainter, K., Beckmann, J. S., Serikawa, T., Soubrier, F., Dubay, C., Cartwright, P., Gouyon, B. De., Julie, C., Takahasi, S., Vincent, M., Ganten, D., Georges, M. and Lathrop, G.M. 1991. Chromosomal mapping of two genetic loci associated with blood presume regulation in hereditary hypertensive rats. *Nature* **353:** 521-529**.**

Hippolyte, I., Bakry, F., Carreel, F., Jenny, C., Arnaud, E., Roux, N., Glaszmann, J.C., Risterucci, A.M., Garsmeur, O., D'Hont, A. and Baurens, F.C. 2007. Progress of *Musa* (banana and plantain) genetic diversity and genome analysis at Agropolis-CIRAD. In: Plant and Animal Genomes XV Conference. San Diego, CA, W35.

Holloway, J.L. and Knapp, S.J. 1993. GMendel 3.0 Users guide.Department of Crop and Soil Science, Oregon State University, Corvallis, Ore.

Hurtado, P., Olsen, K. M., Buitrago, C., Ospina, C., Marin, J., Duque, M., de Vicente, C., Wongtiem, P., Wenzel, P., Kilian, A., Adeleke, M. and Fregene M. 2008. Comparison of simple sequence repeat (SSR) and diversity array technology (DArT) markers for assessing genetic diversity in cassava (*Manihot esculenta* Crantz). *Plant Genetic Resources:Characterization and Utilization*, **6**: 208–214

Huttner, E., Risterucci, A.M., Hippolyte, I., Caig, V., Carling, J., Evers, M., Uszynski, G., Wenzl, P., Glaszmann, J.C. and Kilian, A. 2007. Establishment of diversity arrays technology for whole-genome profiling of banana. In: Plant and Animal Genomes XV Conference, San Diego, CA, W34.

Huttner, E., Wenzl, P., Akbari, M., Caig, V., Carling, J., Cayla, C., Evers, M., Jaccoud, D., Peng, K., Patarapuwadol, S., Uszynski, G., Xia, L., Yang, S. and Kilian, A. 2005. 'Diversity Arrays Technology: A novel tool for harnessing the genetic potential of orphan crops' in Discovery to Delivery: BioVision Alexandria 2004, Proceedings of the 2004 Conference of The World Biological Forum, Serageldin, I. and Persley, G. J. (eds), CABI Publishing, Wallingford, UK.

Huttner, E., Wenzl, P., Carling, J., Xia, L., Yang, S., Jaccoud, D., Caig, V., Evers, M., Uszynski, G.,Howes, N., Sharp, P., Vaughan, P., Rathmell, B., Kilian, A. 2006. Triticarte: whole-genome profiling service for wheat and barley using Diversity Arrays Technology (DArT). Proceeding of the 13th Australasian Plant Breeding Conference, Christchurch, New Zealand, 18-21, April.

Isidore, E., van Os, H., Andrzejewski, S., Bakker, J., Barrena, I., Bryan, G.J., Caromel, B., van Eck, H., Ghareeb, B., de Jong, W., van Koert, P., Lefebvre, V., Milbourne, D., Ritter, E., van der Voort, J.R., Rousselle-Bourgeois, F., van Vliet, J. and Waugh, R. 2003. Toward a marker-dense meiotic map of the potato genome:lessons from linkage group I. *Genetics* **165**: 2107-2116.

Jaccoud, D., Peng, K., Feinstein, D. and Kilian, A. 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Res.* **29**: e25.

Jacob, H.J., Lindpainter, K., Lincoln, S.E., Kusumi, K., Bunker, R.K., Mao, Y.P., Ganten, D., Dzau, V.J. and Lander, E**.**S. 1991. Genetic mapping of a gene causing hypertension in the stroke-prone rat. *Cell* **67:** 213-224.

Jain, A., Bhatia, S., Banga, S. and Lakshmikumaran, M. 1994. Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. *Theor. Appl. Genet.* **88**: 116-122.

Jain, S.M., Brar, D.S., Ahloowalia, B.S. 2002. Molecular techniques in crop improvement. Kluwer Academic Publishers, The Netherlands.

James, K., Schneider, H., Ansell, S., Vogel, J.C., Pedersen, N., Newton, A., Robba, L., Evers, M. and Kilian, A. 2006. Adapting Diversity Arrays Technology (DArT) for inter-and intraspecific molecular research in wild plants. Botany 2006 Conference, California State University, Chico, USA. July 28-August 2.

Jansen, R.C. and Stam, P. 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* **136**: 1447 – 1455.

Jarne, P. and Lagoda, P. 1996. Microsatellites from molecules to populations and back. Trends *Ecol. Evol.* **11**: 424-429.

Jauhar, P.P. 1968. Inter-and intra-genomal chromosome pairing in an interspecific hybrid and its bearing on basic chromosome number in *Pennisetum. Genetica* **39**: 360-370.

Jauhar, P.P. 1981. Cytogenetics and breeding of pearl millet and related species. Progress and topics in cytogenetics, vol 1. Alan R Liss, New York, pp 1-289.

Jauhar, P.P. and Hanna, W.W. 1998. Cytogenetics and genetics of pearl millet. *Adv. Agron.* **64**: 1-26.

Jeffreys, A.J., Wilson, V. and Thein, S.L. 1985. Hypervariable 'minisatellite' regions in human DNA. *Nature* **314**: 67-73.

Ji, M., Hou, P., Li, S., He, N. and Lu, Z. 2004. Microarray-based method for genotyping of functional single nucleotide polymorphisms using dual-color fluorescence hybridization. *Mutat. Res.* **548**: 97-105.

Jiang, C. and Zeng, Z.B. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* **140**: 1111 – 1127.

Jones, E.S., Breese, W.A., Liu, C.J., Singh, S.D., Shaw, D.S. and Witcombe J.R. 2002. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and glasshouse screens detect the same QTL. *Crop Sci.* **42**: 1316-1323.

Jones, E.S., Liu, C.J., Gale, M.D., Hash, C.T. and Witcombe, J.R. 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. *Theor. Appl. Genet.* **91**: 448-456.

Jordan, B., Charest, A., Dowd, J.F., Blumenstiel, J.P., Yeh, R., Osman, A., Housman, D.E. and Landers, J.E. 2002. Genome complexity reduction for SNP genotyping analysis. *Proc. Natl. Acad. Sci. USA* **99:** 2942-2947.

Jordan, D.R., Hammer, G.L., Rodgers, D., Butler, D.G., Hunt, C.H., Collard, B. and Mace, E.S. 2007. Multi-population to mapping to increase genetic diversity and grain yield in sorghum. In: Plant and Animal Genomes XV Conference. San Diego, CA, W398.

Kapila, R.K., Yadav, R.S., Plaha, P., Rai, K.N., Yadav, O.P., Hash, C.T. and Howarth, C.J. 2008. Analysis of genetic diversity in pearl millet inbreds using microsatellite markers. *Plant Breeding* **127**: 33-37.

Karakousis, A., Gustafson, J.P., Chalmers, K.J., Barr, A.R., Langridge, P. 2003. A consensus map of barley integrating SSR, RFLP and AFLP markers. *Aust. J. Agric. Res.* **54**: 1173-1185.

Karp, A. and Edwards, K.J. 1995. Techniques for the analysis, characterization and conservation of plant genetic resources. Molecular techniques in the analysis of the extent and distribution of genetic diversity. In: Ayad, W.G., Hodgkin, T., Jaradat, A., Rao, V.R. (eds.). Molecular genetic techniques for plant genetic resources. Report of the IPGRI Workshop, 9-11 October Rome, Italy, pp.11-22.

Kearsey, M.J. and V. Hyne. 1994. QTL analysis: A simple 'marker regression' approach. *Theor. Appl. Genet.* **89**: 698-702.

Khairwal, I.S., Rai, K.N., Diwakar, B. Sharma, Y.K., Rajpurohit, B.S., Nirwan, B. and Bhattacharjee, R. 2007. Pearl millet: Crop management and seed production manual. Patancheru, Andhra Pradesh, India: ICRISAT. 108 pp.

Kilian, A. 2007. Towards effective deployment of Diversity Arrays Technology (DArT) in banana genomics and sequencing. In: Plant and Animal Genomes XV Conference. San Diego, CA, 13-17 January ,W36.

Kilian, A., Huttner, E., Wenzl, P., Jaccoud, D., Carling, J., Caig, V., Evers, M., Heller-Uszynska, Cayla C., Patarapuwadol S., Xia, L., Yang, S., Thomson, B. 2005. The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In: Tuberosa R, Phillips RL, Gale M (eds). Proceedings of the International Congress "In the Wake of the Double Helix: from the Green Revolution to the Gene Revolution", May 27-31, 2003, Avenue Media, Bologna, Italy. pp 443-461.

Kilian, A., Xia, L., Wenzl, P., Huttner, E., Jordan, D., Mace, E., Bouchet, S., Rami, J.F., Glaszmann, J.C., Varshney, R., Hash, C.T. and Senthilvel, S. 2009. DArT genome profiling and dedicated information technologies for modern breeding of sorghum and pearl millet. W468. Abstract of oral presentation at PAG–XVII, 10-14 Jan 2009, San Diego, CA, USA.

Knapp, S. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. *Crop Sci.* **38**: 1164-1174.

Koebner, R.M.D., Donini, P., Reeves, J.C., Cooke, R.J. and Law, J.R. 2003. Temporal flux in the morphological and molecular diversity of UK barley. *Theor. Appl. Genet.* **106**: 550-558.

Kolesnikova, M.A. 2001. Mapping new quantitative trait loci (QTL) for downy mildew resistance in pearl millet. PhD thesis, Russian National Academy of Sciences, Moscow, Russia. (English and Russian). 266 pp.

Kopecký, D., Bartoš, J., Lukaszewski, A.J., James, H., Baird, J.H., Černoch, V., Kölliker, R., Rognli, O.A., Blois, H., Caig, V., Lübberstedt, T., Studer, B., Shaw, P., Doležel, J. and Kilian, A. 2009. Development and mapping of DArT markers with in the Festuca-Lolium complex. *BMC Genomics* **10**: 473.

Korol, A.B., Preygel, I.A. and Preygel, S.I. 1994. Recombination variability and evolution. Chapman & Hall, London.

Lande, R. and Thompson, R. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* **124**: 743-756.

Lander, E.S. and Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits by using RFLP linkage maps. *Genetics* **121**: 185-199.

Langridge, P. 2005. Molecular breeding of wheat and barley. In: Tuberosa R, Phillips, R.L, Gale, M. (eds) Proceedings of the international congress in the wake of the double helix: from the green revolution to the gene revolution. Avenue Media, Bologna, Italy, 27-31 May 2003, pp 279-286.

Langridge, P. and Chalmers, K. 1998. Techniques for marker development. In: Proceedings of the 9th international wheat genet symposium, vol 1. Saskatchewan, Canada, pp 107-117.

Law, J.R., Donini, P., Koebner, R.M.D., Reeves, J.C. and Cooke, R.J. 1998. DNA profiling and plant variety registration. 3: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. *Euphytica* **102**: 335-342.

Lee, M. 1995. DNA markers and plant breeding programs. *Adv. Agron*. **55**: 265-344.

Lezar, S., Myburg, A.A., Berger, D.K., Wingfield, M.J., Wingfield, B.D. 2004. Development and assessment of microarray-based DNA fingerprinting in *Eucalyptus grandis*. *Theor. Appl. Genet*. **109**: 1329-1336.

Li, T.X., Wang, J.K., Bai, Y.F. and Lu, Z.H. 2006. Diversity suppression-subtractive hybridization array for profiling genomic DNA polymorphisms. *J. Integrative Plant Biology* **48**: 460-467.

Li, T.X., Wang, J. K., Bai, Y.F., Sun, X. and Lu, Z.H. 2004. A novel method for screening species-specific gDNA probes for species identification. *Nucleic Acids Res.* **32**: e45.

Lincoln, S.E., Daly, M.J. and Lander, E.S. 1993. Mapping genes controlling quantitative traits using MAPMAKET/QTL version 1.1: A tutorial and reference manual. Whitehead Inst., Cambridge, MA.

Liu, C.J., Devos, K.M., Witcombe, J.R., Pittaway, T.S. and Gale, M.D.1996. The effect of genome and sex on recombination rates in *Pennisetum* species. *Theor. Appl. Genet.* **93**:902-908.

Liu, C.J., King, I.P., Pittaway, T.S., Abbo, S., Reader, S.M., Miller, T.E. and Gale, M.D. 1997. Physical and genetical mapping of rDNA sites in *Pennisetum* (pearl millet). *Heredity* **78:** 529-531.

Liu, C.J., Witcombe, J.R., Hash, C.T., Busso, C.S., Pittaway, T.S., Nash, M. and Gale, M.D. 1994 a. In J.R. Witcombe and R.R. Duncan (eds.): Use of molecular markers in sorghum and pearl millet breeding for developing countries. Overseas Development Administration: London, U.K. pp. 57-64.

Liu, C.J., Witcombe, J.R., Pittaway, T.S., Nash, M., Hash, C.T., Busso, C.S. and Gale, M.D. 1994 b. An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). *Theor. Appl. Genet.* **89**: 481-487.

Liu, C.J., Witcombe, J.R., Pittaway, T.S., Nash, M., Hash, C.T. and Gale, M.D. 1992. Restriction fragment length polymorphism in pearl millet, *Pennisetum glauc*um. In: Mounolou, J.C. & Sarr, A. (eds.), Complexes d'especes, flux de genes et ressources genetiques des plantes. Colloque international, 8-10 janvier, pp. 233-241, Publications du Bureau des Ressources Genetiques, Paris.

Lörz, H. and Wenzel, G. 2005. Molecular marker systems in plant breeding and crop improvement. Biotechnology in Agriculture and forestry, Springer, Berlin.

Lu, H, Romero-Severson, J. and Bernardo R. 2002. Chromosomal regions associated with segregation distortion in maize. *Theor. Appl. Genet.* **105**: 622-628.

Luikart, G., England, P.R., Tallmon, D., Jordan, S. and Taberlet, P. 2003. The power and promise of population genomics: from genotyping to genome typing. *Nature Genet.* **4**: 981-994.

Luttrell, E.S.1954.Diseases of pearl millet in Georgia. **38**: 507-514.

Lyttle, T.W. 1991.Segregation distorters. *Annu. Rev. Genet.* **25**: 511-557.

Mace, E.S., Xia, L., Jordan, D.R., Halloran, K., Parh, D.K., Huttner, E., Wenzl, P., Kilian, A. 2008. DArT markers: diversity analyses and mapping in sorghum bicolor. *BMC Genomics* **9**: 26.

Mantovani, P., Maccaferri , M., Sanguineti, M.C., Tuberosa , R., Catizone, I., Wenzl, P., Thomson, B., Carling, J., Huttner, E., De Ambrogio, E. and Kilian, A. 2008. An integrated DArT-SSR linkage map of durum wheat. *Mol. Breeding*, doi 10.1007/s11032-008-9205-3.

Mariac, C., Luong, V., Kapran, I., Mamadou, A., Sagnard, M., Deu, M., Chantereau, J., Gerard, B., Ndjeunga, J., Bezançon, G., Pham, J. and Vigouroux, Y. 2006. Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) in Niger assessed by microsatellite markers. *Theor. Appl. Genet*. **114**: 49-58.

Martel, E., De Nay, D., Siljak-Yakovlev, S., Brown, S. and Sarr, A. 1997. Genome size variation and basic chromosome number in pearl millet and fourteen related *Pennisetum* species. *J. Hered.* **88**:139-143.

Melchinger, A.E., Graner, A., Singh, M. and Messmer, M. 1994. Relationship between European barley germplasm: genetic diversity between winter and spring cultivars revealed by RFLPs. *Crop Sci.* **34**: 1191-1199.

Messmer, M.M., Melchinger, A.E. Herrmann, R.G. and Boppenmaier. 1993. Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. *Crop Sci.* 33: 944-950.

Miller, J. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon. Theor. Appl. Genet.* **80:** 437-448.

Mishra, R.N., Reddy, P.S., Suresh, N., Markandeya, G., Reddy, A.R., Sopory, S.K. and Reddy, M.K. 2007. Isolation and characterization of expressed sequence tags (ESTs) from subtracted cDNA libraries of *Pennisetum glaucum* seedlings. *Plant Mol. Biol.* **64**: 713-732.

Mohammadi, S.A. and Prasanna, B.M. 2003. Analysis of genetic diversity in crop plants - salient statistical tools and considerations. *Crop Sci.* **43**: 1235-1248.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G. and Yano, M. 1997. Genome mapping, molecular markers and marker-assisted selection in crop improvement. *Mol. Breeding* **3**: 87-103.

Moore, G. 2000. Cereal chromosome structure, evolution and pairing. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**: 195-222.

Morgan, R.N., Wilson, J.P. Hanna, W.W. and Ozias-Akin, P. 1998. Molecular markers for rust and *Pyricularia* leaf spot disease resistance in pearl millet. *Theor. Appl. Genet.* **96**:413-420.

Myers, N. 1994. Protected areas - protected from a greater what? *Biodiversity and Conservation* **3**. pp. 411-418.

Nachit, M.M., Elouafi, I., Pagnotta, M.A., El Saleh, A., Iacono, E., Labhili, M., Asbati, A., Azrak, M., Hazzam, H., Benscher, D., Khairallah, M., Ribaut, J.M., Tanzarella, O.A., Porceddu, E. and Sorrells, M.E. 2001. Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. *durum*). *Theor. Appl. Genet.* **102**: 177-186.

Nelson, J.C. 1997. QGENE: Software for marker-based genomic analysis and breeding. *Mol. Breeding* **3**: 239-245.

Neumann, K., Kobiljski, B., Dencic, S., Varshney, R.K. and Borner, A. 2010. Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). *Mol. Breeding*, doi 10.1007/s11032-010-9411-7.

Oumar, I., Mariac, C., Pham, J.L. and Vigouroux, Y. 2008. Phylogeny and origin of pearl millet (*Pennisetum glaucum* [L.] R. Br) as revealed by microsatellite loci. *Theor. Appl. Genet*. **117**: 489-497.

Pannu, P.P.S., Sokhi, S.S. and Aulakh, K.S. 1996. Resistance in pearl millet against rust. *Indian Phytopathol.* **49**: 243-246.

Paran, I., Goldman, I., Tanksley, S.D. and Zamir, D. 1995. Recombinant inbred lines for genetic mapping in tomato. *Theor. Appl. Genet.* **90:** 542-548.

Parida, S.K., Kumar, K.A.R., Dalal, V., Singh, N.K. and Mohapatra, T. 2006. Unigene derived microsatellite markers for the cereal genomes. *Theor. Appl. Genet.* **112**: 808-817.

Pastinen, T., Raitio, M., Lindroos, K., Tainola, P., Peltonen, L., Syvanen, A.C. 2000. A system for specific, high throughput genotyping by allele-specific primer extension on microarrays. *Genome Res* **10**: 1031-1042.

Paterson, A.H. 1996 a. Making genetic maps. In: A.H. Paterson (Ed.), Genome mapping in plants, pp. 23-39. R. G. Landes Company, San Diego, California; Academic Press, Austin, Texas.

Paterson, A.H. 1996 b. Mapping genes responsible for differences in phenotype, In: A.H. Paterson (Ed.), Genome Mapping in Plants, pp. 41-54. R. G. Landes Company, San Diego, California; Academic Press; Austin, Texas.

Paterson, A.H., Damon, S., Hewitt, J.D., Zamir, D., Rabinowitch, H.D., Lincoln, S.E., Lander, E.S. and Tanksley, S**.**D. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. *Genetics* **127:** 181-197.

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E. and Tanksley, S.D. 1988. Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. *Nature* **335:** 721-726**.**

Peng, J.H., Fahima, T., Röder, M.S., Li, Y.C., Grama, A., Ronin, Y.I., Korol, A.B., Nevo, E. 2000. Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. *Genome Res.* **10**: 1059-1031.

Peters, J. L., Constandt, H., Neyt, P., Cnops, G., Zethof, J., Zabeau, M. and Gerats, T. 2001. A physical amplified fragment-length polymorphism map of Arabidopsis. *Plant Physiol* **127**: 1579-1589.

Pilat-Andre, J.S., Lamy, F. and Sarr, A. 1992. Etude de la region Adh du mil (*Pennisetum typhoides*) par utilisation des techniques RFLP. In: Mounolou, J.C. & Sarr, A. (eds.), Complexes d'espèces, flux de gènes et ressources génétiques des plantes. Colloque international, 8-10 janvier, 1992, pp. 58–71. Publications du Bureau des Ressources Génétiques, Paris.

Poncet, V., Lamy, F., Devos, K.M., Gale, M.D., Sarr, A. and Robert, T. 2000. Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L. Poaceae). *Theor. Appl. Genet.* **100**: 147-159.

Poncet, V., Lamy, F., Enjalbert, J., Joly, H., Sarr, A. and Robert, T. 1998. Genetic analysis of the domestication syndrome in pearl millet (*Pennisetum glaucum* L., Poaceae): inheritance of the major characters. *Heredity* **81**: 648-658.

Poncet, V., Martel, E., Allious, S., Devos, K.M., Lamy, F., Sarr, A. and Robert, T. 2002. Comparative analysis of QTLs affecting domestication traits between two domesticated × wild pearl millet (*Pennisetum glaucum* L., Poaceae) crosses. *Theor. Appl. Genet.* **104**: 965-975.

Powell, W., Machray, G.C. and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.* **1**: 215-222.

Pozniak, C.J., Knox, R.E., Clarke. F.R. and Clarke, J.M. 2007. Identification of QTL and association of a phytoene synthase gene with endosperm colour in durum wheat. *Theor. Appl. Genet.* **114**: 525-537.

Qi, X., Lindup, S., Pittaway, T.S., Allouis, S., Gale, M.D., Devos, K.M. 2001. Development of simple sequence repeat markers from bacterial artificial chromosomes without subcloning. *Biotechniques*, **31**: 355-362.

Qi, X., Pittaway, T.S., Lindup, S., Liu, H., Wateran, E., Padi, F.K., Hash, C.T., Zhu, J., Gale, M.D. and Devos, K.M. 2004. An integrated genetic map and new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. *Theor. Appl. Genet.* **109**: 1485-1493.

Quarrie, S.A., Steed, A., Calestani, C., Semikhodskii, A., Lebreton, C., Chinoy, C., Steele, N., Pljevljakusi, T. D., Waterman, E., Weyen, E.J., Schondelmaier, J., Habash, D.Z., Farmer, P., Saker, L., Clarkson, D.T., Abugalieva, A., Yessimbekova, M., Turuspekov, Y., Abugalieva, S., Tuberosa, R., Sanguineti, M.C., Hollington, P.A., Aragués, R., Royo, A. and Dodig, D. 2005. A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring x SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor. Appl. Genet.* **110**: 865-880.

Rabinowicz, P.D., Schutz, K., Dedhia, N., Yordan, C., Parnell, L.D., Stein, L., McCombie, W.R. and Martienssen, R.A. 1999. Differential methylation of genes and retrotransposons facilitates shotgun sequencing of the maize genome. *Nature Genet.* **23**: 305-308.

Ramakrishnan, T.S. and Soumini, C.K. 1948. Studies on cereal rusts I. *Puccinia penniseti* Zimm. and its alternate host. *Indian Phytopathol.* **1**: 97-103.

Ramsay, L., Macaulay, M., degli Ivanissevich, S., MacLean, K., Cardle, L., Fuller, J., Edwards, K.J., Tuvesson, S., Morgante, M., Massari, A., Maestri, E., Marmiroli, N., Sjakste, T., Ganal, M., Owell, W. and Waugh, R. 2000. A simple sequence repeat-based linkage map of barley. *Genetics* **156**: 1997-2005.

Rheault, M.E., Dallaire, C., Marchand, S., Zhang, L., Lacroix, M. and Belzile, F. 2007. Using DArT and SSR markers for QTL mapping of Fusarium head blight resistance in six-row barley. In: Plant and Animal Genomes XV Conference. San Diego, CA, P335.

Risterucci, A.M., Hippolyte, I., Perrier, X., Xia, L., Caig, V., Evers, M., Huttner, E., Kilian, A., and Jean-Christophe Glaszmann, J.C. 2009. Development and assessment of Diversity Arrays Technology for high-throughput DNA analyses in Musa. *Theor. Appl. Genet.* **119**: 1093-1103.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P. and Ganal, M.W. 1998. A microsatellite map of wheat. *Genetics* **149**: 2007-2023.

Sax, K. 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris. Genetics* **8**:552–60.

Schlotterer, C. 2004. The evolution of molecular markers - just a matter of fashion? *Nat. Rev. Genet.* **5**: 63-69.

Schneider, K.A., Brothers, M.E. and Kelly, J.D. 1997. Marker-assisted selection to improve drought resistance in common bean. *Crop Sci.* **37**: 51-60.

Semagn, K, Bjørnstad, Å. and Ndjiondjop, M.N. 2006 a. An overview of molecular marker methods for plants. *African J. Biotech.* **5**: 2540-2568.

Semagn, K., Bjornstad, A., Skinnes, H. Maroy, A.G., Tarkegne, Y. and William, M. 2006 b. Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. *Genome* **49**: 545-555.

Senthilvel, S., Jayashree, B., Mahalakshmi, V., Sathish Kumar, P., Nakka, S., Nepolean, T. and Hash, C.T. 2008. Development and mapping of Simple Sequence Repeat markers for pearl millet from data mining of Expressed Sequence Tags. *BMC Plant Biology* **8**: 119.

Senthilvel, S., Mahalakshmi, V., Sathish Kumar, P., Reddy, A.R., Markandeya, G., Reddy, M.K., Misra, R. and Hash, C.T. 2004. New SSR markers for pearl millet from data mining of Expressed Sequence Tags. *In* Proceedings of the 4th International Crop Science Congress held at Brisbane, Australia, 26 Sept.-1 Oct., 2004.

Sharma, A., Namdeo, Ajay G. and Mahadik, K.R. 2008. Molecular markers: new prospects in plant genome analysis. *Pharmacognosy Reviews* **2**: 23-34.

Sharma, H.C., Crouch, J.H., Sharma, K.K., Seetharama, N., Hash, C.T. 2002. Applications of biotechnology for crop improvement: prospects and constraints. *Plant Sci*. **163**: 381-395.

Singh, K., Ghai, M., Garg, M., Chhuneja, P., Kaur, P., Schnurbusch, T., Keller, B. and Dhaliwal, H.S. 2007. An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* x *T. monococcum* RIL population. *Theor. Appl. Genet.* **115**: 301-312.

Singh, S.D. and King, S.B.1991. Pearl millet rust - present status and future research needs. *Int. J. Trop. Plant Dis*. **9**: 35-52.

Singh, S.D., Wilson, J.P., Navi, S.S., Talukdar, B.S, Hess, D.E. and Reddy, K.N. 1997. Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Information Bulletin no. 48. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 104 pp.

Smith, J.S.C., Paszkiewics, S., Smith, O.S. and Schaeffer, J. 1987. Electrophoretic, chromatograhic and genetic techniques for identifying associations and measuring genetic diversity among corn hybrids. p. 187-203. *In* Proc. 42nd Annu. Corn Sorghum Res. Conf., Chicago, IL. Am. Seed Trade Assoc., Washington, DC.

Smith, J.S.C., Smith, O.S., Wright, S., Wall, S.J. and Walton, M. 1992. Diversity of U.S. hybrid maize germplasm as revealed by Restriction Fragment Length Polymorphisms. *Crop Sci.* **32**: 598-604.

Smith, O.S., and Smith, J.S.C. 1992. Measurement of genetic diversity among maize hybrids; A comparison of isozymic, RFLP, pedigree, and heterosis data. *Maydica* **37**: 53-60.

Sokhi, S. S. and Singh, B. B. 1984. Components of slow rusting in pearl millet infected with *Puccinia penniseti*. *Indian J. Mycol. Plant Pathol.* **14**: 190-192.

Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G.L., Gay, G., Qi, L., Gill, B.S., Dufour, P., Murigneux, A. and Bernard, M. 2004. Microsatellite based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Funct. Integr. Genomics* **4**: 12-25.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. *Plant J.* **3**: 739-744.

Storey, J. and Tibshirani, R. 2003. Statistical significance for genome wide studies. *Proc. Nat. Acad. Sci. USA*. **100**: 9440-9945.

Stuber, C. W., Lincoln, S.E., Wolff, D.W., Helentrjaris, T. and Lander, E.S. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* **132:** 823-839.

Syvanen, A.C. 1999. From gels to chips: 'minisequencing' primer extension for analysis of point mutations and single nucleotide polymorphisms. *Hum. Mutat.* **13**: 1-10.

Talbert, L.E., Blake, N.K., Chee, P.W., Blake, T.K. and Magyar, G.M. 1994. Evaluation of sequence-tagged-site-facilitated PCR products as molecular markers in wheat. *Theor. Appl. Genet.* **87**: 789-794.

Tanksley, S.D., Ganal, M.W. and Martin, G.B. 1995. Chromosome Landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends in Genetics* **11**: 63-68.

Tanksley, S.D., Ganal, M.W., Prince, J.P., de Vicente, M.C. 1992. High density molecular linkage maps of the tomato and potato genomes: biological inferences and practical applications. Genetics **132**: 1141-1160.

Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: new tools for an old science. *Biotechnology* **7**: 257-264.

Tapsoba, H. and Wilson, J.P. 1996. Pathogenic variation in *Puccinia substriata* var. *indica* in the southeastern United States and screening for resistance in pearl millet germplasm. *Plant Disease* **80**: 395-397.

Tapsoba, H. and Wilson, J. P. 1999. Increasing complexity of resistance in host populations through intermating to manage rust of pearl millet. *Phytopathology* **89**: 450-455.

Tautz, D., Trick, M. and Dover, G.A. 1986. Cryptic simplicity in DNA is a major source of genetic variation. *Nature* **322**: 652-656.

Thoday, J.M. 1961. Location of polygenes. *Nature* **191**: 368-370.

Thompson, J.A., Nelson, R.L. and Vodkin. L.O. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Sci.* **38**: 1348-1355.

Thudi, M., Senthilvel, S., Bottley, A., Hash, C.T., Reddy, A.R., Feltus, A.F., Paterson, A.H., Hoisington, D.A. and Varshney, R.K. 2010. A comparative assessment of the utility of PCR-based marker systems in pearl millet. *Euphytica* doi 0.1007/s10681-010-0148-5.

Tinker, N.A., Kilian, A., Wight, C.P., Heller-Uszynska, K., Wenzl, P., Rines, H.W., Bjørnstad, A., Howarth, C.J., Jannink, J.L., Anderson, J.M., Rossnage, B.G., Stuthman, D.D., Sorrells, M.E., Jackson, E.W., Tuvesson, S., Kolb, F.L., Olsson, O., Federizzi, L.C., Carson, M.L., Ohm, H.W., Molnar, S.J., Scoles, G.J., Eckstein, P.E., Bonman, J.M., Ceplitis, A. and Langdon, T. 2009. New DArT markers for oat provide enhanced map coverage and global germplasm characterization. *BMC Genomics* **10**: 39.

Tobolski, J.J. and Kemery, R.D.C. 1992. Identification of red maple cultivars by isozyme analysis. *Hort. Science* **27**: 169-171.

Tostain, S. 1992. Enzyme diversity in pearl millet (*Pennisetum glaucum* L.) 3. Wild millet. *Theor. Appl. Genet.* **83**: 733-742.

Tostain, S. 1994. Isoenzymatic classification of pearl millet (*Pennisetum glaucum*, Poaceae) landraces from Niger (West Africa). *Pl. Syst. Evol.* **193**: 81-93.

Tostain, S. and Marchais, L. 1989. Enzyme diversity in pearl millet (*Pennisetum glaucum*). 2. Africa and India. *Theor. Appl. Genet.* **77**: 634-640.

Tostain, S., Riandley, M.F. and Marchais, L. 1987. Enzyme diversity in pearl millet (*Pennisetum glaucum*). 1. West Africa. *Theor. Appl. Genet.* **74**: 188-193.

Utz, H.F. and Melchinger, A.E. 1994. Comparison of different approaches to interval mapping of quantitative trait loci. pp. 195-204 In: van Ooijen , J.W. and Jansen, J. (eds.) Biometrics in Plant Breeding: Applications of Molecular Markers. Proceedings of the Ninth Meeting of the EUCARPIA Section Biometrics in Plant Breeding, 6 – 8 July 1994, Wageningen. CPRO-DLO, Wageningen, The Netherlands.

Utz, H.F. and Melchinger, A.E. 1996. PLABQTL: A program for composite interval mapping of QTL. Inst. Plant Breed., Seed Sci., Pop. Genet., Univ. of Hohenheim, Stuttgart, Germany.

van Hintum, Th.J.L., and Haalman, D. 1994. Pedigree analysis for composing a core collection of modern cultivars, with examples from barley (*Hordeum vulgare*). *Theor. Appl. Genet*. **88**: 70-74.

van Os, H., Andrzejewski, S., Bakker, E., Barrena, I., Bryan, G.J., Caromel, B., Ghareeb, B., Isidore, E., de Jong, W., van Koert, P., Lefebvre, V., Milbourne, D., Ritter, E., Rouppe van der Voort, J.N.A.M., Rousselle-Bourgeois, F., van Vliet, J., Waugh, R., Visser, R.G.F., Bakker, J., van Eck, H.J. 2006. Construction of a 10,000-marker ultra dense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genome wide physical map. *Genetics* **173**: 1075-1087.

van Os, H., Stam, P., Visser, R.G.F. and van Eck, H.J. 2005. RECORD: a novel method for ordering loci on a genetic linkage map. *Theor. Appl. Genet.* **112**: 30-40.

Varshney, R.K., Graner, A. and Sorrells, M.E. 2005. Genic microsatellite markers in plants: features and applications. *Trends Biotechnol.* **23**: 48-55.

Varshney, R.K., Hoisington, D.A. and Tyagi, A.K. 2006. Advances in cereal genomics and applications in crop breeding. *Trends Biotechnol.* **24**: 490-499.

Varshney, R.K., Langridge, P., Graner, A. 2007. Application of genomics to molecular breeding of wheat and barley. *Adv. Genet.* **58**: 121-155.

Varshney, R.K. and Tuberosa, R. 2007. Genomics-assisted crop improvement: an overview. In: Varshney, R.K., Tuberosa, R. (eds) Genomics-assisted crop improvement, vol **1**: genomics approaches and platforms. Springer, Dordrecht, The Netherlands, pp 1-12.

vom Brocke, K., Christinck, A., Weltzien, E.R., Presterl, T. and Geiger, H.H. 2003. Farmers' seed systems and management practices determine pearl millet genetic diversity patterns in semi-arid regions of India. *Crop Sci*. **43**: 1680-1689.

Voorrips, R.E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered*. **93**: 77-78.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* **23:** 4407-4414.

Vromans, J. 2006. Molecular genetic studies in flax (*Linum usitatissimum* L.). Laboratory of Plant Breeding, Wageningen University, The Netherlands.

Vuylsteke, M., Mank, R., Antonise, R., Bastiaans, E., Senior, M.L., Stuber, C.W., Melchinger, A.E., Lübberstedt, T., Xia, X.C., Stam, P., Zabeau, M. and Kuiper, M. 1999. Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. *Theor. Appl. Genet.* **99**: 921-935.

Wang, J. F., Graham, E. B., Kilian, A., Balatero, C. H., Hautea, D. M., Cameille, A., Besse, P., Hidayati, N., Jaunet, T. X., Dittapongpitch, V., Huang, S. M., Truong, T. H. H., de la Peña, R. C. and Hanson, P. M. 2006. Mapping quantitative resistance loci to bacterial wilt in tomato line Hawaii 7996. Tomato Breeders Roundtable and Tomato Quality Workshop, Tampa, Florida USA.

Weber, J. and May, P. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am.J. Hum. Genet.* **44**: 388-396.

Wells, H.D., Burton, G.W. and Hennen, J.F. 1973. *Puccinia substriata* var. *indica* on pearl millet in the southeast. *Plant Dis. Rep.* **57**: 262.

Wendel, J.F., Edwards, M.D. and Stuber, C.W. 1987. Evidence for multilocus genetic control of preferential fertilization in maize. *Heredity* **58**: 297-302.

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A. and Kilian, A. 2004. Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proc. Natl. Acad. Sci. USA* **101**: 9915-9920.

Wenzl, P., Catizone, I., Huttner, E., Mantovani, P., Maccaferri, M., DeAmbrogio, E., Corneti, S., Sanguineti, M.C., Tuberosa, R. and Kilian, A. 2007. A DArT platform for high-throughput profiling in durum wheat. Plant & Animal Genome XV Conference. San Diego, CA, P263.

Wenzl, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Jaroslava, O., Cakir, M., Poulsen. D., Wang, J., Raman, R., Smith, K.P., Muehlbauer, G.J., Chalmers K.J., Kleinhofs, A., Huttner, E. and Kilian, A. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* **7**: 206-228.

White, J., Law, J.R., Mackay, I., Chalmers, K.J., Smith, J.S.C., Kilian, A. and Powell, W. 2008. The genetic diversity of UK, US and Australian cultivars of *Triticum aestivum* measured by DArT markers and considered by genome. *Theor. Appl. Genet.* **116**: 439-453.

Whittaker, J.C., Thompson, R. and Visscher, P.M. 1996. On the mapping of QTL by regression of phenotypes on markertype. *Heredity* **77**: 23-32.

Williams, J., Kubelik, A., Livak, K., Rafalski, J. and Tingey, S.1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**: 6531-6535.

Wilson, J.P. 1991. Detection of pathogenic races of *Puccinia substriata* var. *indica* in the U.S. *Phytopathology* **81**: 815.

Wilson, J.P. 1993a. Two new dominant genes for resistance to pearl millet rust. *Phytopathology* **83:**1395.

Wilson, J. P. 1993b. Identification of virulence in *Puccinia substriata* var. *indica* to *Rr*1 in pearl millet. *Plant Dis.* **77**: 100.

Wilson, J. P. 1994. Field and greenhouse evaluations of pearl millet for partial resistance to *Puccinia substriata* var. *indica*. *Plant Dis.* **78**: 1202-1205.

Wilson, J. P. 1997. Inheritance of partial rust resistance in pearl millet. *Plant Breed.* **116**: 239-243.

Wilson, J.P. 2006. Inheritance of partial rust resistance in pearl millet. *Plant Breeding* **116**(3): 239-243.

Wilson, J.P., Gates, R.N. and Hanna W.W. 1991. Effect of rust on yield and digestibility of pearl millet forage. *Phytopathology* **81**: 233-236.

Wilson, J.P., Hanna, W.W. and Gascho, G.J. 1996. Pearl millet grain yield loss from rust infection. *J. Prod. Agricult.* **80**: 543-545.

Winter, P. and Kahl, G. 1995. Molecular marker technologies for plant improvement. *World Journal Microbiology and Biotechnology*, **11**(4): 438-448.

Winzeler, E.A., Castillo-Davis, C.I., Oshiro, G., Liang, D., Richards, D.R., Zhou, Y. and Hart, D.L. 2003. Genetic diversity in yeast assessed with whole-genome oligonucleotide arrays. *Genetics* **163**: 79-89.

Wittenberg, A.H.J., Van der Lee, T., Cayla, C., Kilian, A., Visser, R.G.F. and Schouten, H.J. 2005. Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana*. *Mol. Gen. Genomics*, **274:** 30-39.

Wright, A.J. and Mowers, R.P. 1994. Multiple regression for molecular-marker, quantitative trait data from large F2 populations. *Theor. Appl. Genet*. **89**: 305-312.

Xia, L., Peng, K., Yang, S., Wenzl, P., Carmen de Vicente, M., Fregene, M., and Kilian, A. 2005. DArT for high-throughput genotyping of Cassava (*Manihot esculenta*) and its wild relatives. *Theor. Appl. Genet*. **110:** 1092-1098.

Xie, Y., McNally, K., Li, C.Y., Leung, H. and Zhu, Y.Y. 2006. A high throughput genomic tool: Diversity Array Technology complementary for rice genotyping. *J. Int. Plant Biol.* **48**: 1069-1076.

Xu, Y., Zhu, L., Xiao, J., Huang, N. and McCouch, S.R. 1997. Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations of rice (*Oryza sativa* L.). *Mol. Gen. Genet.* **253**: 535-545.

Yadav, O.P., Mitchell. S.E., Zamora. A., Fulton, T.M. and Kresovich, S. 2007. Development of new simple sequence repeat markers for pearl millet. *J. SAT. Agr. Res.* **3**(1), [http://www.icrisat.org/journal]

Yadav, R.S., Bidinger, F.R., Hash, C.T., Yadav, Y.P., Yadav, O.P., Bhatnagar, S.K. and Howarth, C.J. 2003. Mapping and characterization of QTL × E interactions for traits determining grain and stover yield in pearl millet. *Theor. Appl. Genet.* **106:** 512-520.

Yadav, R.S., Hash, C.T., Bidinger, F.R, Cavan, G.P. and Howarth, C.J. 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought-stress conditions. *Theor. Appl. Genet.* **104:** 67-83.

Yadav, R.S., Hash, C.T., Bidinger, F.R., Devos, K.M. and Howarth, C.J. 2004. Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and tester background. *Euphytica* **136**: 265-277.

Yadav, R.S., Hash, C.T., Bidinger, F.R. and Howarth, C.J. 1999. QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet. In: Ito O, O'Toole J, Hardy B (eds). Proc Workshop on Genetic Improvement of Rice for Water-limited Environments, 1-3 Dec 1998, Los Banos, The Phillipines, The International Rice Research Institute, pp 211-223.

Yamamoto, F. and Yamamoto, M. 2004. A DNA microarray-based methylation-sensitive (MS)-AFLP hybridization method for genetic and epigenetic analyses. *Mol. Genet. Genomics* **271**: 678-686.

Yang, S., Pang, W., Ash, G., Harper, J., Carling, J., Wenzl, P., Huttner, E., Zong, X. and Kilian, A. 2006. Low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theor. Appl. Genet*. **113**: 585-595.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. *Genetics* **136**: 1457-1468.

Title	:	**Genetic diversity analysis and QTL mapping in pearl millet (*Pennisetum glaucum*) using Diversity Arrays Technology (DArT)**
Name	:	**Supriya**
Institute	:	**CCS Haryana Agricultural University, Hisar-125 004 (Haryana), India**
Supervisor	:	**Dr. R.C. Yadav**
Submitted	:	**16June, 2010**

Abstract

Diversity Arrays Technology (DArT) was used for diversity analysis and QTL mapping in pearl millet. DArT array was developed from 95 diverse genotypes using *Pst*I/*Ban*II complexity reduction method and consisted of 6912 clones. 574 DArT markers were identified after genotyping a diverse set of 24 inbred lines. Genetic relationships revealed by cluster analysis were in agreement with their known pedigree.

Two mapping populations (81B-P6 x ICMP 451-P8 and H 77/833-2 x PRLT 2/89-33) were genotyped with DArT array and SSR markers and high-density genetic linkage maps were constructed. The maps of respective populations comprised of 286 loci with 740.3 cM map length and 321 loci spanning 1148 cM. DArT markers were mapped for the first time in pearl millet. 78 markers were common across the two populations which allow the development of a well-saturated consensus linkage map.

167 RILs derived from cross 81B-P6 x ICMP 451-P8 were assessed for rust reaction and a major QTL (LOD 27) was mapped near the top of LG 1 explaining 58% of the observed phenotypic variation and the allele of resistant parent ICMP 451-P8 conferred resistance. This study is a novel report on QTL mapping for rust resistance in pearl millet on LG 1.

CURRICULUM VITAE

a) Name : Supriya

b) Date of Birth : 04.03.1983

c) Place of Birth : Haryana

d) Mother's Name : Smt. Sumitra Devi

e) Father's Name : Dr. Jagdev Singh

f) Permanent Address : 10/26, Old Campus CCS HAU, Hisar-125004

g) Telephone : +919255584123

h) Mobile : +919255584123

i) E-mail : supriya_bmb@yahoo.co.in

j) Academic Qualifications :

Degree	**University/ Board**	**Year of passing**	**%age of marks**	**Subjects**
Matric	C.B.S.E.	1998	79.0	Eng., Hindi, S.S., Science, Maths, Sanskrit
10+2	C.B.S.E.	2000	81.6	Eng., Biology, Chemistry, Physics, Sanskrit
B. Sc.	KU,Kurukshetra	2004	75.6	Zoology, Botany, Chemistry, Eng., Sanskrit
M. Sc. (Biotech. & Mol. Biology)	CCS HAU, Hisar	2006	82.0	Principles of Biotech., Genet. Engg., PTC, Mol. Biology, Tech. in Biotech., Biochem. Techn., Bioinfo., Principles of Microbio., Principles of Genetics, General Biochem., Biostat. & computers

k) Co-curricular Activities :

- Second position in college level science exhibition.
- Participation in state level science exhibition.
- Certificate of Merit in National Service Scheme.

l) Medals/Honours received :

- Ph.D. Research Scholar in ICRISAT, Patancheru, Hyderabad.
- Topper in M.Sc. entrance examination -2004 conducted by CCS HAU, Hisar.
- Second position in M.Sc. (Biotech. and Molecular Biology) in CCS HAU, Hisar.
- Merit Scholarship during Post-Graduate programme funded by CCSHAU, Hisar.
- Third position in B.Sc. examination in Govt. College, Hisar.
- Third position in district Rewari in 10+2 C.B.S.E. examination.

m) List of Publications :

1. **Supriya,** Senthilvel S, Nepolean T, Hash CT, Rajaram V, Eshwar K, Sharma R, Thakur RP, Pandurangarao V, Yadav RC. 2010. Identification of quantitative trait loci associated with rust resistance in pearl millet using DArT and SSR based linkage map. National Symposium on Genomics and Crop Improvement: Relevance and Reservations, February 25-27, 2010, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad. P170.
2. Senthilvel S, Nepolean T, **Supriya,** Eshwar K, Rajaram V, Kumar S, Hash CT, Kilian A, Yadav RC, Narasu ML, Singh G, 2010. Development of molecular linkage map of pearl millet integrating DArT and SSR markers. Plant & Animal Genomes Conference- XVIII, January 09-13, 2010, Town & Country Convention Center, San Diego, USA. P368. http://www.intl-pag.org/18/abstracts/P05d_PAGXVIII_368.html
3. Rajaram V, Varshney RK, Vadez V, Nepolean T, Senthilvel S, Kholova J , Choudhary S, Kumar S, **Supriya**, Kumar S, Thakur RP, Sharma R, Pandurangarao V, Rai KN, Velu, G, Sahrawat KL , Bhasker Raj AG, Blümmel M, Narasu ML, Kocova M, Kavi Kishor PB, Yadav RC, Singh G, Hash CT. 2010. Development of EST resources in pearl millet and their use in development and mapping of EST-SSRs in four RIL populations. Plant & Animal Genomes Conference-XVIII, January 09-13, 2010, Town & Country Convention Center, San Diego, USA. P373. http://www.intl-pag.org/18/abstracts/P05d_PAGXVIII_373.html
4. Rajaram V, Varshney RK, Vadez V, Nepolean T, Senthilvel S, **Supriya**, Kumar S, Narasu ML, Yadav RC, Singh G, Hash CT. 2010. Mapping EST-SSRs, developed from ESTs of stressed root and leaf tissues, in four pearl millet RIL mapping populations. National Symposium on Genomics and Crop Improvement: Relevance and Reservations, February 25-27, 2010, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad. P166.

Citation (Biological Abstracts): All papers listed above.

(Supriya)