Studies on comparative mapping in cereals

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Biotechnology

By

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This is to certify that the dissertation entitled "**Studies on comparative mapping in cereals**" submitted in partial fulfillment for the award of the degree of Master of Technology in Biotechnology to the Jawaharlal Nehru Technological University, is a bontafide work carried out by Ms. B. Manjula Rani, under my guidance and supervision.

The results embodied in this dissertation have not been submitted to any other university or institution for the award of any degree or diploma.

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Dedicated to
my parents

ABSTRACT

The availability of molecular marker systems and their applications in genome mapping have lead to direct comparisons of the relative order of homologous sequences along the chromosomes of closely and distantly related species and the development of the concept of genome synteny. Comparative genome mapping provides insights into genome evolution and genetic information among divergent species. Therefore the primary objective of this study was to use RFLP markers to compare the presence of the same putative disease resistance genes in the sorghum and rice. During this term of my project work, I have learnt molecular marker techniques like isolation of high quality DNA, restriction fragment length polymorphism (RFLP), and other related techniques like handling clones, transformation by electroporation, and isolation of the enzyme *Taq DNA polymerase.* In addition, various computer applications like *MS-Office*, information search through *Internet* has also been undertaken during the course.

Most applications of molecular markers in plants have so far utilized RFLPs. Several high density genetic maps have been developed for many important crop species. In addition, RFLP's have been used for obtaining markers linked to important agronomic traits and to study plant phylogenetics and evolution. Molecular analysis of the sorghum genotypes and rice genotype was accomplished by using RFLP markers. DNA was extracted from 15 sorghum genotypes and rice, RFLP analysis was carried out using 3 different sorghum probes. These probes are putative disease resistance gene clones obtained from Dr. S. Sivaramakrishnan, ICRISAT.

Five restriction enzymes, *EcoRI, EcoRV, BamHI, HindIII* and *XbaI*, were used to identify the probes which produced RFLPs between the parental lines of the mapping populations of sorghum and rice genotype. All the three of the probes tested hybridized to genomic blots of sorghum DNA under the hybridization conditions, but only two probes hybridized to rice genomic DNA. The genomic blots, digested with *EcoRI* and *EcoRV* were probed with S-2-2. The enzyme-probe combination of *EcoRI* and S-2-2 detected polymorphism in genotypes E 36-1 and 296 B, whereas *EcoRV* when probed with S-2-2 detected polymorphism in genotypes IS 9830 and 296 B. The enzyme probe combinations of *XbaI* + S-30-5 and *XbaI* + S-27-2 showed polymorphism between E 36-1 and 296 B. The enzyme *BamHI* when probed with S-30-5 detected polymorphism in only one genotype, 296 B. *HindIII* and S-27-2 combination showed no polymorphism in sorghum genotypes. In the given probes, only S-30-5 and S-27-2 hybridized with rice, when digested with enzymes *XbaI* and *HindIII* respectively and detected polymorphism. Similarly, it is necessary to find polymorphism between parents of rice mapping populations for comparative mapping. To make comparisons of genomes of sorghum and rice, one would need maps of both crops with common set of probes.

Studies on cereal synteny have revealed a suprising level of conservation between cereal genomes inspite of large differences of genome size. Molecular marker detection of syntenic relationships provides researchers with the potential for collating the genetics of many different plant species, such that studies of any one member can be considerably enhanced by examination of the results of the group as a whole. As Bennetzen and Freeling have proposed, perhaps the time has come to take

combined advantage of the attributes of the individual members of the Graminae and to consider them as a single genetic system for study based upon the synteny for their genomes as determined through molecular marker analysis. Genes defined as important in one species could be both studied and isolated in related Graminae species, and the results can be extrapolated across many other species based upon both genomic and genic homology. In this aspect, the current study is an attempt to compare the RFLP loci for the genes of agronomic importance in both the species of Graminae i.e., sorghum and rice.

CONTENTS

List of legends for the figures

List of Tables

List of Abbreviations

1. INTRODUCTION 1

1.1 GENETIC MAPPING IN GRASSES 1

1.2 MOLECULAR MARKERS 2

1.3 GENETIC LINKAGE MAP OF SORGHUM AND RICE 4

1.4 CEREAL SYNTENY 5

2. REVIEW OF LITERATURE 8

2.1 TYPES OF DNA MARKERS 9

2.1.1 *Restriction Fragment Length Polymorphism (RFLP)* 9

2.1.2 *Random amplified polymorphic DNA (RAPD)* 12

2.1.3 *Simple sequence repeats (SSRs)* 13

2.1.4 *Amplified Fragment length polymorphisms (AFLPs)* 15

2.2 GENETIC MAP CONSTRUCTION 16

2.2.1 *Applications of genetic maps* 17

2.2.2 *Locating markers in defined chromosomal segments* 17

2.3 COMPARATIVE MAPPING 19

2.3.1 *Techniques for comparative mapping* 19

2.3.2 *Comparative mapping in various plants* 20

2.3.3 *Comparative mapping in grasses* 21

2.3.4 *Advantages of comparative mapping* 22

2.4 STATUS OF COMPARATIVE MOLECULAR MAPPING IN SORGHUM RESEARCH 23

2.4.1 *RFLP mapping in sorghum* 23

2.4.2 *Future prospects in comparative mapping of cereals* 25

3. MATERIALS AND METHODS 27

3.1 PLANT MATERIAL 27

3.2 ISOLATION OF GENOMIC DNA OF SORGHUM AND RICE 29

3.2.1 *Extraction of DNA* 29

3.2.2 *Purification of DNA* 29

3.3 QUANTIFICATION OF DNA 30

3.3.1 *Spectrophotometric method* *30*
3.3.2 *Ethidium bromide staining* *30*
3.4 RFLP ANALYSIS 31
3.4.1 *Restriction Digestion* *31*
3.4.2 *Preparation of Master mix for DNA restriction reactions* *32*
3.4.3 *Separation of DNA fragments* *32*
3.4.4 *Southern blotting* *33*
3.4.5 *Labelling of probe* *33*
3.4.6 *Prehybridization & Hybridization* *34*
3.4.7 *Processing after hybridization* *34*
3.4.8 *Scoring of autoradiographs* *34*

4. RESULTS AND DISCUSSION **35**

4.1 COMPARISON OF HYBRIDIZATION WITH LABELLED PROBES 35
4.2 CONCLUSIONS 45

5. REFERENCES 46

LEGENDS FOR THE FIGURES

Figure 1 Genomic DNA of sorghum lines and rice digested with restriction enzymes, *EcoRI* and *EcoRV*.....38

Figure 2 Genomic DNA of sorghum lines and rice digested with restriction enzymes, *HindIII* and *BamHI*.....39

Figure 3 Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *EcoRI* and hybridized with sorghum RFLP probe S-2-2.....40

Figure 4 Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *EcoRV* and hybridized with sorghum RFLP probe S-2-2.....41

Figure 5 Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *HindIII* and hybridized with sorghum RFLP probe S-27-2.....42

Figure 6 Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *XbaI* and hybridized with sorghum RFLP probe S-27-2.....43

Figure 7 Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *XbaI* and hybridized with sorghum RFLP probe S-30-5.....44

LIST OF TABLES

Table 1. Description of the sorghum genotypes used in this Study.....27

Table 2. Spectrophotometric readings of the DNA samples.....28

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AP-PCR	Arbitrary Primed-Polymerase Chain Reaction
DAF	DNA Amplification fingerprinting
DNA	Deoxyribonucleic acid
dNTP	deoxy Nucleotide-tri Phophate
dATP	deoxy Adenine tri Phosphate
MAS	Marker assisted Selection
PCR	Polymerase Chain Reaction
QTL	Quantitative trait loci
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeats
STMS	Sequence Tagged Microsatellites

1. INTRODUCTION

Plant breeding is both an art and a science dealing with a wide range of both quantitative and qualitative traits. Having as much genetic information as possible about major and minor traits and their interactions improves the efficiency and probability of success in achieving an end product with the desired attributes. Construction of a detailed genetic map for the crop of interest will make available a precise but vast amount of information that plant breeders can use to identify, manipulate, and complement traits to their maximum advantage. Integration of linkage information derived from various types of markers has significantly improved the resolution of crop genome architecture and created opportunities for improved interpretation of the genetic basis of crop improvement.

1.1 Genetic mapping in grasses

The current and ever-expanding power of genetic mapping with DNA markers has allowed identification of genomic regions that make even minor contribution to the within-species variability in a particular physiological or biochemical process. These quantitative trait loci (QTL), like major or qualitative genes, are both identified and positionally localized when large populations are mapped with a reasonable number of well-distributed markers (Bennetzen, 1996). Mapping of qualitative and quantitative traits on comparable grass maps will uncover similar map positions for many related traits in the different species.

The identification of novel genes would be a particularly powerful and exciting outcome of parallel grass genome mapping. These genes would be identified

as novel by their unique map position relative to the position of similar genes in other species that affects a similar process. It is likely that novel genes found in one grass species is usually present in other grass species but might have been missed due to limitations in genetic analysis (Bennetzen, 1996).

Gene orders are not perfectly identical across the grasses; inversions and translocations involving large portions of chromosome arms often differentiate the genetic maps of the cereals. However, within any given arm of chromosome or small part of it, the lower gene orders are found to be highly conserved. This conservation of gene content and map position indicates that individual grass genomes can be viewed as manifestations of a single grass genetic system (Bennetzen and Freeling, 1993). Further the recombination distances between such stretches of genes are also conserved (Bennetzen, 1996).

1.2 Molecular markers

Molecular markers are DNA sequence polymorphisms that can be readily assayed without obtaining the explicit DNA sequence and that show Mendelian inheritance. For genome mapping, the ideal genetic marker is codominant, multiallelic, and hyper variable (i.e., segregates in almost every family) (O'Malley, 1996). Use of molecular genetic markers allows the entire genome to be scanned for differences linked to a gene of interest (Bates et al, 1996).

Several categories of molecular markers have emerged and include those polymorphisms detected by hybridization assays, such as restriction fragment length polymorphisms (RFLPs) and those detected using the polymerase chain reaction

(PCR). PCR-based markers includes those involving arbitrary or random priming such as arbitrarily-primed PCR (AP-PCR), randomly amplified polymorphic DNA (RAPD), or DNA amplified fingerprinting (DAPs). A more specific PCR marker technique involves detection of polymorphic regions of di-, tri-, or tetranucleotide repeats, commonly called as short tandem repeats (STRs), which exhibit simple sequence repeat polymorphisms (SSRs). Amplified fragment length polymorphisms (AFLP) use restriction endonucleases to fragment the DNA but selective PCR amplification for detection. All these methods attempt to exploit differences in DNA sequences for the purpose of defining individual differences and constructing linkage maps (Bates et al., 1996).

In this study for screening polymophism and comparative mapping of sorghum and rice genotypes RFLP marker technology has been applied. RFLP analysis is used extensively in the construction of genetic maps and has been successfully applied to genetic diversity assessments, particularly in cultivated plants (Castagna et al., 1994; Deu et al., 1994; Jack et al., 1995) and also in populations and wild accessions (Besse et al., 1994; Laurent et al., 1994; Bark and Harvey 1995). As a technique for diversity studies, there are three important advantages, which should be considered. The first is that RFLPs are highly reproducible between laboratories and the diversity profiles generated can be readily transferred. The second is that RFLPs are codominant markers, enabling heterozygotes to be distinguished from homozygotes. The third advantage is that no sequence-specific information is required and, provided suitable probes are available, the approach can be applied immediately for diversity screening in any system.

1.3 Genetic linkage map of Sorghum and Rice

The present study is based on comparative mapping of sorghum and rice genotypes for the presence of disease resistance genes. Sorghum is one of world's most important cereal crops. It belongs to the family *Graminae*, tribe Andropogoneae. It is a diploid species with 2n (2x) = 10 chromosomes and a genome size of 0.8 pg (where 1 pg = 965 million base pairs) (Shields, 1993). It is well adapted to semi-arid conditions, and both grain and crop residues have numerous valuable uses.

Grain yield and quality of sorghum are constrained by many factors including disease and insects. However, this species is poorly characterized genetically, when compared to other cereals like maize and rice. Despite the relatively small amounts of attention that molecular biologists have paid to sorghum, the last few years have seen tremendous advances in mapping (Hulbert et al., 1990; Binelli et al.,1992; Melake-Berhan et al., 1993; Chittenden et al., 1994; Pereira et al., 1994), and diversity analyses (Tao et al., 1993; Vierling et al., 1994).

DNA markers can be utilized to characterize sorghum lines for their suitability as parents in a breeding programme. For instance, lines containing DNA polymorphisms associated with particular desirable characteristics can be identified in a collection once enough correlations and cosegregations of these traits and markers have been established. The vast number of potential DNA markers, and high levels of DNA polymorphism, essentially guarantee that any two sorghum lines can be differentiated.

Rice is one of the most important food crops in the world and is a staple food for much of the world's population. It is a diploid species with 12 chromosomes and

has the smallest genome of any monocotyledon crop known, with a genome size of 0.45 pg (Shields, 1993). It belongs to the tribe *oryzeae* under the subfamily *Pooideae* in the grass family *Gramineae (Poaceae).* High-density RFLP maps of rice have been constructed (Causse et al., 1994; Kurata et al., 1994), and some quantitative traits have also been studied by using molecular markers (Ahn et al., 1993; Wang et al., 1994; Xiaco et al., 1994; Li et al., 1995, Lu et al., 1997).

Recent studies have shown that the grasses, covering the whole range of crops like sorghum, maize, rice, wheat, barley and others have very similar gene content and regions of conserved gene order (Hulbert et al., 1990; Ahn et al., 1993; Devos et al., 1993; Melake- Berhan et al., 1993; Hont et al., 1994; Kurata et al., 1994; Periera et al., 1994). Because they contain nearly the same gene content by homology criteria, the different agronomic properties of these species must be due to allelic variation in these genes.

The mapping of agronomically significant genes from multiple grass species will provide the basic information that can be used to plan enhanced breeding strategies using DNA markers. Thus the whole range of grass species can be used to identify superior alleles of specific genes, and these "superior" genes will often function effectively when transferred into other grass species (Bennetzen and Freeling, 1993).

1.4 Cereal Synteny

A recent and important observation in the use of plant molecular markers is the finding that many distantly related species have colinear maps for positions of genomes: a similarity in the constitution of the chromosomes, termed synteny. In the

race to build dense genetic maps, the early reports of the genomes were interesting but not remarkable. Later the cross-genome comparisons became more compelling. These comparisons all employed hybridization based mapping procedures, which with variable stringency conditions, allowed the detection of similar but imperfectly matched DNA sequences. Large numbers of characterized DNA probes were still not available, and researchers of the day, therefore, used RFLP probes available in one species to create genetic maps in related genomes.

In this approach, in order to obtain comparative maps between the cereal genomes, anchor set of probes have been produced at the Cornell University. Different probes like the anchor set of rice probes, provided by the Cornell University are being tested. The DNA obtained from the Cornell University is transformed into the competent cells of the DH5α strain of *E coli* bacteria by electoporation. The competent cells are prepared and transformed at high voltage. These cells are grown on selection medium (antibiotic medium) and are screened for the presence of transformed cells. The plasmid DNA is isolated from the transformed cells and digested with the restriction enzyme, *PstI* to obtain the desired fragment of DNA (probe) which can be separated by electrophoresis. The probe can also be obtained by amplification by PCR using M13 primers. The probes thus obtained are used to compare genomes of different grass species.

The obvious result is that, once careful interspecific comparisons have been made, predictions for gene locations mapped in one species can be made in the second. In the short run, regions that are sparsely mapped in one grass species might be filled by markers from the molecular map of the second grass species.

My object of work is to familiarize myself with certain techniques of molecular analysis like RFLP and PCR-based markers, besides basic techniques of molecular analysis. Therefore, a set of sorghum genotypes (mapping parents) and a rice variety (*Taipei 309*) are screened for DNA polymorphism using RFLP markers. These lines of sorghum carry traits of economic importance, so this information on DNA polymorphism facilitates marker-assisted breeding. By comparing the marker position for disease resistance genes in both the species one can suggest that these genes may be allelic and the genetic engineer can choose whichever allele preferred for crop modification process.

2. REVIEW OF LITERATURE

The advent of molecular technologies for the mapping of complex genomes now permits wholly new approaches to crop improvement. Molecular genetic markers which describe differences at the level of DNA sequence provide the investigator with powerful tools for the analysis of the inheritance of quantitative traits (Beckmann and Soller 1988; Paterson et al., 1988, 1991; Ottaviano et al 1991) and evolutionary phenomenon (Debener et al.,1990; Miller and Tanksley 1990), and for varietal and species identification (Soller and Beckmann 1983). Molecular markers could be used to provide a better description of the level of diversity of the resistance sources as a tool to ensure diversity among the parent lines developed.

Coverage of the entire genome with molecular markers offers geneticists a historically unique view of the genome. Geneticists now have the ability to not only determine the genotype of the individual and thereby correlate markers with traits, but they can evaluate the genotypic composition of entire populations. Segments of chromosomes or individual markers can be followed through initial breeding programs to cultivar release, or through generations of cultivar development. By evaluating the genotypic compositions of cultivars, we can begin to determine the allele combinations breeders have consistently selected for, or against. A major constraint to developing resistance is to identify molecular markers that are closely associated with resistance and to use these markers to select indirectly to resistance.

2.1 Types of DNA markers

The most informative DNA markers are characterized by a high polymorphism information coefficient (PIC) number, indicating relatively large number of alleles with similar frequencies in each locus (Bostein et al., 1980). The more polymorphic DNA markers available, the better is the chance that useful markers can be used for both genotype identification and breeding purposes.

Molecular markers could be used to provide a better description of the level of diversity of the resistance sources and as a tool to ensure diversity among the parent lines developed for breeding, especially the hybrids.

Avise (1994) lists the advantages of molecular genetic markers

1. Molecular data are genetic.
2. Molecular methods open the entire biological world for genetic scrutiny.
3. Molecular methods access a nearly unlimited pool of genetic variability.
4. Molecular data can distinguish homology from analogy.
5. Molecular data provide a common measure for assessing divergence.
6. Molecular approaches facilitate mechanistic appraisals of evolution and finally,
7. Molecular approaches are challenging and exciting.

A wide variety of techniques to visualize DNA sequence polymorohism are available which include:

2.1.1 *Restriction Fragment Length Polymorphism (RFLP)*

The ability of a class of enzymes known as restriction endonucleases to cleave DNA at specific nucleotide sequence or recognition sites has greatly enhanced our ability to examine structure and variation of the primary genetic material. The loss or gain of a

restriction site between two related taxa is generally assumed to be caused by a single nucleotide mutation at the recognition site and allows for the estimation of percent nucleotide difference between the two taxa. Other forms of mutation including indels, inversions, and translocations can disrupt a recognition site, but the pattern then produced are generally quite distinctive (Hahn and Grifo, 1996). The resultant restriction fragment polymorphisms (RFLPs) can provide broad coverage of the genome under consideration when a sufficiently large array of probes or primer pairs are used to generate and visualize the fragments. Increased character sampling can then be accomplished by using more enzymes or selecting more frequently cutting enzymes (Helentjaris et al., 1985; Holsinger and Jansen., 1993; Olmstead and Palmer., 1994).

The use of RFLP markers in plant breeding is based on the tight linkages found between these markers and genes of interest. Such linkage infers the presence of a desirable gene by assaying for the RFLP marker. One example of this is to transfer disease resistance genes into susceptible cultivars. Traditionally, progeny are screened for the presence of disease resistance genes by inoculation with the pathogen. Screening the plant with several different pathogens simultaneously or even sequentially is difficult. However, detecting disease resistance genes by their linkage to RFLP probes makes it possible to screen for many different disease resistance genes simultaneously without the need to inoculate the pathogens. Similar tight linkages between RFLP markers and other major genes are currently being sought by scientists working with sorghum.

Summary of characteristics of RFLPs as genetic markers:

A. Genetic

1. Ubiquitous
2. Numerous
3. Almost entire genome accessible
4. Stably inherited
5. Mendelian inheritance for nuclear genome
6. Maternal inheritance for organelle genomes
7. Multiple alleles
8. Codominant
9. Lack of pleiotropic effects
10. Uneffected by environmental or developmental patterns
11. Detects variation within hybridizing region and flanking sequences
12. Probes for hypervariable regions available
13. Informative about the nature of mutations

B. Methodological

1. Can be tested in all tissues and at all ages
2. Long shelf-life of DNA samples
3. Virtually unlimited number of probe x enzyme combinations available
4. Heterologous genes can be used as probes
5. Multiple loci can be tested with a single probe, e.g. multigene families, hypervariable regions.

6. Any probe can serve as a base point to facilitate isolation of closely linked markers by chromosome walking, generating haplotypes
7. Probes for specific chromosomal regions can be obtained by way of chromosome dissection or isolation
8. Additional random or site-specific polymorphisms can be formed *de nova* by insertions

C. Limitations

1. Costly in materials
2. Labour intensive
3. Satellite and highly reiterated DNA sequences unaccessible with naturally occurring probes.
4. RFLP cannot be automated

As RFLP is the oldest and most reliable, but cumbersome technique therefore, other types of markers have overtaken it.

2.1.2 *Random amplified polymorphic DNA (RAPD)*

Randomly amplified polymorphic DNA (RAPD) is a popular technique that uses the polymerase chain reaction (PCR) but with short nonspecific primers under conditions of modest stringency (Williams et al., 1990). Multiple uncharacterized annealing sites are used as PCR primer targets in the strategies variously termed random amplified polymorphic DNA (RAPDs) analysis (Williams et al.,1990), arbitrary primed PCR (AP-PCR) (Welsh and McClelland, 1990), and DNA amplification fingerprinting (DAF) (Caetano-Anolles et al, 1992). These techniques often result in several

polymorphic sites per PCR reaction, and the PCR primers are available commercially so that the initial screening of a genome with primers for those that show polymorphic products is relatively inexpensive (Bates et al., 1996).

The RAPD technology finds its greatest application in detecting polymorphisms in closely related organisms (low divergence) such as those that compose a species complex, different populations of a single species or individuals within a population. RAPD markers are extensively used in gene mapping research, in individual and strain identification and in those issues in ecology and population biology requiring genetic analysis of relatedness or identity. This method is simple, fast and permits analysis of a large number of individuals at reasonable cost.

This technique has certain limitations. Because of the random nature of the amplifications, one cannot be certain that all comigrating bands seen are homologous (related by evolutionary descent) in all samples analyzed. This technique is limited to closely related organisms where total sequence divergence is low. Another limitation of the method is that a good number of the RAPD markers show dominance/recessive inheritance in diploid organisms whereby a particular fragment is amplified from some individuals but not from others, making it impossible to distinguish heterozygotes from homozygotes for the dominant allele. Concern has also been raised on use of the method in parentage analysis because of the occasional appearance of nonparental bands in offspring of known parentage.

2.1.3 *Simple sequence repeats (SSRs)*

SSRs are microsatellites, which reveals polymorphisms for numbers of simple sequence repeat units at mappable sites through the genome (Smith and Beavis,

1996). To develop SSRs as genetic markers the surrounding sequence must be obtained. PCR primers are then designed for regions directly outside the repeat.The primers must amplify uniquely the desired region, which must also be polymorphic in the segregating population. If SSR loci are cloned and sequenced, primers to the flanking regions can be designed and used to amplify only that single region containing the SSR, which is then referred to as sequence-tagged microsatellite (STMS or a sequence tagged SSR) (Morgante and Olivieri 1993).

There are several important advantages of sequence-tagged SSRs. They are usually single loci which, because of their high mutation rate, are often multi-allelic (Saghai- Maroof et al., 1994). They are codominant markers and they can be detected by a PCR (non-hybridization based) assay. They are very robust tools that can be exchanged between laboratories and their data is highly informative. The assay is relatively quick, but throughput can be increased by selecting a small number of different SSRs with alleles that have different non-overlapping size ranges, amplifying and running them together in the gel (multiflexing) (Karp and Edwards, 1995).

There are, nonetheless, some negative aspects of using sequence tagged SSRs. Although they are codominant markers, their mode of evolution is different from normal coding loci. It is difficult to use them to estimate relatedness beyond a few generations (Karp and Edwards, 1995). Because of the large number of nonpolymorphic repeats, success rates for finding polymorphic SSRs is not high.

2.1.4 *Amplified Fragment length polymorphisms (AFLPs)*

Amplified Fragment Length Polymorphisms (AFLP) are recently developed PCR-based fingerprinting technique (Zabeau and Voss, 1992). The DNA is cut into defined fragments using restriction endonucleases and adaptor oligonucleotides are ligated to the overhanging ends. These adaptors serve as recognition sequences for PCR primers. The complexity of the mixture of fragments is decreased using selective PCR amplification, by adding additional nucleotides to the 3' termini of the PCR primers. The number of additional bases is adjusted such that 50-150 DNA fragments are amplified during the PCR. This can produce 10-30 polymorphisms per PCR reaction, depending upon the genomes being assayed, making AFLPs a very cost effective marker system (Bates et al, 1996)

The primary reason for the superiority of AFLP technique is that it detects very large number of DNA bands enabling simultaneous identification of many polymorphic markers (Becker et al., 1995). The AFLP does not necessarily offer higher rates of polymorphism but is more efficient than other approaches of detecting polymorphic DNA. AFLPs detect more point mutations, enable detection of very large number of polymorphic DNA, and are simpler than microsatellites as no prior sequence information is needed.

Although AFLP approach is highly informative, a few limitations of this technique are, the use of multiple procedures, expensive, cumbersome, and laborious protocol. AFLP technology has an added template preparation step relative to other PCR-based assays. Slightly more genomic DNA is required, and it must be of

sufficient quality to allow restriction endonuclease digestion and ligation of adaptor oligonucleotides (Bates et al, 1996).

2.2 Genetic map construction

A major breakthrough occurred when it was realized that genetic maps could be constructed by using pieces of chromosomal DNA as direct markers for the segregation pattern of chromosome segments (Bostein et al., 1980). The genetic maps of most crop species are based on polymorphisms of low- or single-copy sequences. Most reasonably detailed maps are based on analysis of restriction fragment length polymorphism (RFLP), which is detected by the hybridization of the cloned DNA segments to genomic DNA digested with restriction enzymes. In future, however, most maps will probably be based on amplified fragment length polymorphisms (AFLP) (Vos et al., 1995), simple sequence repeats (SSRs or microsatellites) and, to a lesser extent, random amplified polymorphic DNAs (RAPD) (Williams et al., 1990).

Genetic maps are usually constructed to locate genes of biological or economic interest in relation to molecular markers. For single genes of large effect this is straightforward since a chromosome location can easily be established by segregational analysis. However, many traits of interest to plant breeders and researchers are controlled by several to many genes acting in combination. The availability of high-resolution genetic maps has greatly increased the ability to identify the number and chromosome locations of such quantitative trait loci (QTL) (Lander and Bostein 1989; Jansen, 1994; Kearsey and Hyne, 1994).

2.2.1 *Applications of genetic maps*

There are two main applications for ganetic maps. The first is the use of DNA markers linked to the gene or genes of interest to select individuals in breeding programmes without the need to assess the phenotype at each stage of development (Gebhardt and Salami, 1992). Such marker-assisted selection (MAS) is already used in breeding programmes. It is particularly useful for the manipulation of quantitative traits, which are the most difficult to evaluate phenotypically in conventional breeding programmes (Laurie et al., 1997).

The second application of genetic maps is the isolation of genes by a map-based cloning approach such as chromosome walking (Tanksley et al., 1995). This approach has been used, for example, in the isolation of genes conferring disease resistance from tomato (Martin et al., 1993).

2.2.2 *Locating markers in defined chromosomal segments*

In the application of molecular maps to plant breeding applications it is often desirable to 'tag' an agronomically valuable gene with closely linked molecular markers. One way to accomplish this is to map the trait relative to a set of molecular markers in a segregating population. If there is no prior knowledge from conventional mapping about the location of the gene, molecular markers have to be rescored systematically in the new population until one is found which is linked (Kochert, 1994). Using isogenic lines or a bulked segregant approach, one can identify molecular markers that are linked to agronomically important, but as yet, unmapped traits. The chromosomal location of these traits can then be established by mapping

the linked marker(s) in existing mapping population of that crop, provided the markers exhibit variation.

More efficient ways of obtaining linked markers utilize near-isogenic lines (NILs). NILs have been produced for a variety of traits in several crop plants by repeated cycles of back crossing and selection. Ideally they consist of a single chromosome segment containing the trait of interest. If there is sufficient polymorphism for molecular markers between the parent lines used to make the NIL, molecular markers can be rapidly screened to locate those which are in the introgressed segment and thus polymorphic between the recurrent parent and NIL. No mapping or use of segregating populations is theoretically necessary to locate linked markers. However, NILs so far investigated have been found to contain segments of introgressed DNA other than the one selected in the breeding program, and it is necessary to check the putative positive clones by segregation analysis (Young et al., 1988).

Pooling of DNA samples can also be used to locate molecular markers in defined chromosomal segments or those, which are closely linked to genes of interest. This is, in effect, a way of creating isogenic lines *in vitro*. This approach was used by Arnheim et al. (1985) to locate RFLP markers closely linked to human disease genes. Michelmore et al.(1991) called the technique 'bulked segregant analysis'. F_2 plants from a lettuce population segregating for resistance to downy mildew were separated into two groups: those which were homozygous resistant and those which were homozygous for susceptibility (heterozygtes previously identified by progeny tests were excluded). DNA was then combined from several plants from each group to

form two DNA pools and differences in banding pattern were deteced to subsequently identify candidates for mapping markers linked to the trait of interest (Kochert, 1994).

2.3 Comparative mapping

The cloned DNA fragments used to detect RFLP often hybridize well to DNA of related species, giving a series of common markers that enable respective genetic maps to be aligned. This is termed comparative mapping (Laurie et al., 1997). It has recently been used to demonstrate that a suprisingly small number of chromosomal rearrangements (inversions and/or translocations) distinguish many major crops. Moreover, comparative maps provide a conduit for communication- permitting information gathered during study of one species to be quickly and efficiently applied to related species.

Vivilov's "law of homologous series in variation" (Vivilov 1922) was perhaps the earliest recognition of fundamental similarity between different cultivated species. Most plant breeders now recognize that similarities between their target species and related taxa transcend diversity in breeding objectives.

2.3.1 *Techniques for comparative mapping*

As individual DNA sequences evolve relatively slowly, and because many techniques for studying DNA are generally tolerant of relatively small differences, but intolerant of large differences in DNA sequence, experiments can readily be designed to establish orthology across different genera.

Two main techniques are used in comparative mapping. One is the "classical" restriction fragment length polymorphism (RFLP) technique, by which a visualizable label is incorporated into a specific cloned DNA element. Most comparative maps, and indeed most molecular mapping of plants to date, have relied on RFLP analysis. So far, the "polymerase chain reaction" (PCR), has provided limited viable alternatives to RFLP for comparative mapping. A variety of PCR-based techniques that use "arbitrary primers," while well suited to some applications, are poorly suited to comparative mapping. Such techniques include random amplified polymorphic DNA (RAPD) (Williams et al., 1990), arbitrary- primer PCR (AP-PCR) (Welsh and McClelland 1990) and AFLP (Zabeau 1995). Difficulty in using these techniques for comparative mapping stems from the fact that each simultaneously reveals many genetic loci that are independent of each other, except for sharing limited DNA sequence homology.

2.3.2 *Comparative mapping in various plants*

Comparative mapping studies in plants have only recently begun and have concentrated on comparative maps of agronomically important species. Comparative mapping in the *Solanaceae* showed that RFLP maps of potato (*Solanum tuberosum)* and tomato (*Lycopersion esculentum*) were similar. When compared, the two genomes were identical in gene content and order; only four paracentric inversions differ between them (Bonierbale et al 1998). However, tomato and pepper chromosomes have been extensively rearranged relative to one another. Large

conserved linkage blocks are still present, however (Tanksley et al., 1989; Prince et al., 1993).

Comparative mapping has also been reported extensively in legumes, where several studies show conserved linkage groups between species such as mungbean (*Vigna radiata*) and cowpea (*V. unguiculata*) (Menancio-Hautea et al., 1993) and pea (*Pisum sativum*) and lentil (*Lens culinaris*) (Weeden et al., 1992; Muehlbauer et al., 1995).

Comparison of *Brassica* species shows that there are a greater number of chromosome rearrangements, but there are still large segments with conserved marker orders (Ferreira et al., 1994; Teutonico and Osborn, 1994; Parkin et al., 1995). *Brassica* species and the model plant species *Arabidopsis thaliana* are both in the *Cruciferae* family and are sufficiently closely related for *Arabidopsis* clones to be used directly for mapping and gene isolation in *Brassica* (Laurie et al., 1997)

2.3.3 *Comparative mapping in grasses*

Comparative mapping studies have revealed a suprising level of conservation between cereal genomes (Dunford et al., 1995; Moore et al., 1995; Van Deynze et al., 1995) in spite of large differences in genome size. Comparative maps of maize and sorghum are well established (Hulbert et al., 1990; Melake Berhan et al., 1993; Pereira et al., 1994; Pereira and Lee, 1995) and comparative maps of Oryza species are being developed. Remarkably, comparison of the genetic maps of wheat and rice (Kurata et al., 1994), rice and maize (Ahn and Tanksley, 1993) and wheat and maize

(Devos et al., 1994) also reveals large linkage segments that can be aligned (Moore et al., 1993; Bennetzen and Freeling, 1993).

Furthermore, equivalent linkage blocks can be recognised in rice, wheat, maize, sorghum, sugar cane and pearl millet. Thus a model of cereal genomes can be constructed using segments of the rice genetic map as the building blocks. This aligns the maps of the various species, revealing the genetic relationships between their chromosomes (Moore et al., 1995).

2.3.4 *Advantages of comparative mapping*

Comparative genetic mapping can be used to provide a more comprehensive picture of the repertoire of genes that potentially influence a trait and directly link individual genes to DNA markers. A 'comparative phenotypic map' finds potential application both in crop improvement and in molecular cloning. In breeding programs, a comparative phenotypic map might predict the locations of new height mutants (for example) in which allelic variation has not yet been found.

The existence of a common basis for related crops should allow drawing information separately on each of these plants on the same complex trait. It will be possible to study several species and take advantage of the respective ranges of allelic variation in order to cumulate the genetic factors tractable through mapping analysis. Thus, comparative mapping opens the way for comparative plant physiology or pathology.

2.4 Status of comparative molecular mapping in sorghum research

Sorghum is a crop with extreme genetic diversity. It ranks fifth in world grain production, serving as the stuff of life for more than 300 million people in the semi-arid tropics, and is an important forage crop in dry land areas worldwide (Doggett, 1988). Despite the relatively small amounts of attention that molecular biologists have paid to sorghum, the last few years have seen tremendous advances in mapping (Hulbert et al., 1990; Binelli et al., 1992; Melake-Berhan et al., 1993; Chittenden et al., 1994; Pereira et al., 1994), and diversity analyses (Tao et al., 1993; Vierling et al., 1994). With this full set of molecular tools in hand, biotechnology can be applied to sorghum once appropriate improvements are identified.

The long-term utility of marker-assisted selection in sorghum improvement is likely to be jointly determined by the identification and mapping of phenotypes with a direct impact on productivity or quality. Design of marker-assisted selection procedures needs to accommodate many of the same environmental parameters as testing of inbreds or cultivars in order to assure that the final product performs as expected in the target environment.

2.4.1 *RFLP mapping in sorghum*

RFLP mapping has provided new information on chromosomal duplication in the *Poaceae* (particularly sorghum). Doggett (1988) summarized genetic linkage of morphological and physiological mutants involving 49 loci. Nine linkage groups have been established but these consist of only 2-10 loci. Several reports have been made of linkage maps for sorghum that are based on restriction fragment length polymorphism (RFLPs). The ability of maize genomic probes to detect RFLP's in

sorghum was tested (Hulbert et al., 1990). Eight linkage groups were established and they observed that RFLP loci linked to maize were usually linked to sorghum. About 35 maize genomic probes were used to identify five linkage groups in sorghum (Binelli et al., 1992). By using 85 maize genomic probes and seven isozyme loci, 13 linkage groups were identified (Whitkus et al., 1992). A genetic map with 96 RFLP loci arranged in 15 linkage groups was generated (Berhan et al., 1993). As completed genetic maps of sorghum were published, it became clear that those duplicate loci which were found showed patterns in the genome which were consistent with ancient duplication (Chittenden et al., 1994; Pereira et al., 1994).

A large number of useful DNA markers have been identified and mapped, and there is little need for more to be acquired from sorghum. However, there are too few fingerprinted lines, mapped traits and mapped populations in sorghum. The populations that have been mapped were often too small to provide precise mapping information. Important single gene and quantitative traits need to be placed on sorghum genetic map (Oh et al., 1994) and the traits need to be screened under a variety of environmental conditions and from a number of parental sources. Such comprehensive studies will be needed to identify regions of the sorghum genome (that enhance productivity and) to identify the lines that have the superior alleles within these regions.

This important gene identification and mapping process can be greatly assisted by use of information and markers from other grass species. Recent studies have shown that the grasses have very similar gene order. This means that genes can be effeciently (and comparatively) mapped in any grass species using an anchor set of

grass DNA markers and that any grass species can be used as a source of genes for sorghum improvement.

2.4.2 *Future prospects in comparative mapping of cereals*

Efforts have been made within the sorghum improvement programme to broaden the genetic background of the working materials in order to develop favourable genotypes. The main objective of diversifying the genetic base is to improve disease resistance while maintaining desirable agronomic characteristics and stability.

The relationships between the chromosomes of sorghum and those of other cultivated Graminae are quickly being established. The chromosomes of *Sorghum* and *Zea* differ primarily by inversions, with few, if any translocations (Whitkus et al., 1992). Several groups are working to collate the sorghum chromosomes with those of sugarcane, rice, wheat, and pearl millet. Most of the labs that made sorghum maps (Hulbert et al., 1990; Binelli et al., 1992; Whitkus et al., 1992; Chittenden et al., 1994; Periera et al., 1994; Xu et al; Ragab et al., 1994), as well as many others, are pursuing mapping of discrete mutations and/or QTLs affecting a wide range of traits (Paterson, A.H., 1994).

Collectively, the molecular maps of sorghum have reached a level of detail paralleling those of most other major crops. Ongoing work in sorghum, as well as comparative mapping of sorghum and related genera, will quickly add further detail to the sorghum map(s), and clarify questions about the evolution of sorghum and other grasses. This extensive collinearity can now be exploited to map agronomically important traits more efficiently, to isolate genes in crops irrespective of their genome size.

From the present review, it is evident that mapping of qualitative and quantitative traits on comparative maps will uncover similar map positions for many related traits in different species. This current investigation includes initiation of comparative mapping of sorghum and rice genotypes for the presence of putative disease resistance genes by using RFLP marker technology.

This dissertation deals with some of the experiments I carried out to learn comparative mapping. The main emphasis was on learning DNA marker technology. The genomic DNA of sorghum lines and rice was isolated and digested with four restriction enzymes viz., *EcoRI*, *EcoRV*, *HindIII*, and *BamHI*. The blots were hybridized with sorghum probes for mapping the genomic sequences between the two crop species.

3. MATERIALS AND METHODS

3.1 Plant material

Table 1. Description of the sorghum genotypes used in this study

S.No	Genotypes	Comments
1.	N13 (IS 18881)	Striga resistant, dual purpose variety
2.	IS 9830	Striga resistant/ drought susceptible
3.	E 36-1 (IS30469)	Striga susceptible,but staygreen
4.	IS 18729	Mold resistant
5.	IS 24756	Mold resistant
6.	R 16	senescent
7.	IS 22380	senescent
8.	PB 15881-3	Borer resistant & midge susceptible
9.	ICSV 745	Midge resistant and borer susceptible
10.	IS 18551	Shootfly resistant
11.	296 B	Standard seed parent in India, widely used
12.	BTX 623	Standard seed parent in the USA; widely used
13.	MS 35-1	Standard rabi parent
14.	B-35	Staygreen B line from US conversion program
15.	CSV5	Drought susceptible rainy season variety

Table 2. Spectrophotometric readings of the DNA:

Genotype	260.0 nm (A1)	280.0 nm (A2)	A1-A2	A1/A2
N-13	0.807	0.403	0.403	1.9988
IS-9830	0.468	0.240	0.228	1.9512
E 36-1	0.590	0.305	0.285	1.9360
IS 18729	0.101	0.057	0.043	1.7692
IS 24756	0.706	0.358	0.347	1.9687
R-16	0.894	0.443	0.450	2.0165
IS-22380	0.653	0.329	0.324	1.9866
PB-15881-3	0.331	0.167	0.163	1.9709
ICSV 745	0.467	0.240	0.227	1.9451
IS 18551	0.195	0.103	0.092	1.9005
296-B	0.371	0.187	0.184	1.9818
BTX-623	0.646	0.330	0.316	1.9586
M 35-1	0.584	0.295	0.289	1.9769
B-35	0.299	0.154	0.145	1.9399
CSV-5	0.348	0.179	0.168	1.9402
Taipei 309	0.149	0.099	0.057	1.6243

3.2 Isolation of genomic DNA of sorghum and rice

3.2.1 *Extraction of DNA*

The tissue samples for extraction of DNA are collected from greenhouse, kept in sealable plastic bags, frozen in liquid nitrogen and stored at -70°C or extracted immediately. The sample is pulverized with liquid nitrogen using a pestle and mortar and transferred to CTAB extraction buffer (1M Tris, 5M NaCl, 0.5M EDTA, β-mercapto-ethanol, CTAB). About 15 ml of warm CTAB extraction buffer was added to frozen ground tissue in a 30 ml falcon tube, mixed well on rotating shaker and incubated for 3 hours at 65°C with occasional mixing. The tubes were removed from water-bath, cooled to room temperature and 10 ml of chloroform:isoamyl alcohol (24:1) is added and mixed gently by inverting several times and centrifuged at 6000 rpm for 20 min at room temperature. The aqueous phase is transferred to new tubes and 10 ml of chloroform:isoamyl alcohol (24:1) is added and mixed gently for several times and centrifuged at 6000 rpm for 20 min at 2°C. The aqueous layer is taken into new tubes and 10 ml of propanol is added to each tube and mixed gently by inversion for several times. The contents are kept at -20°C for 10-15 min and centrifuged at 6000 rpm for 20 min at 2°C and the pellet was saved.

3.2.2 *Purification of DNA*

The pellet was washed with 100% ethanol and dried. Next, 2 ml RNase (10 mg/ml) was added to each tube and kept overnight at room temperature. About 200 µl of 5 M NaCl was added, mixed gently and kept at 4°C for 15-20 min, and centrifuged at

6000 rpm for 20 min at 2°C. The supernatant was transferred to new 5 ml *corex* tubes and 2 ml of phenol was added, mixed well and centrifuged at 2500 rpm for 10 min at 2°C. The contents were washed twice with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 2500 rpm for 10 min at 2°C. To the supernatant, 200 µl 0f 2.5 M sodium acetate (pH 5.2) was added mixed well and incubated at -20°C for 15 min. The precipitated DNA was spooled with a glass hook and placed in 1.5 ml eppendorf tubes. The pellet in the tubes was dissolved by adding 300 µl of T_{10} E_1 buffer (10 mM Tris Cl, 1 mM EDTA, pH 8.0) and stored at 4°C until further use.

3.3 Quantification of DNA

3.3.1 *Spectrophotometric method*

The quantity and purity of DNA samples were determined spectrophotometrically by measuring the absorbance at 260 and 280 nm. DNA was quantified considering that 1.0 OD unit at 260 nm is equivalent to 50 µg of DNA per ml. More precise quantification can be obtained by comparing the absorbance of the samples at 260 and 280 nm. The ratio of OD_{260} AND OD_{280} indicates the purity of the DNA samples. Pure DNA preparations should have an OD_{260}/OD_{280} of 1.8.

3.3.2 *Ethidium bromide staining*

In some cases, such as the amount of plasmid inserts (to be used as probes), DNA is often present in amounts too small to be detected by direct spectroscopy. In this case, the fluorescent dye ethidium bromide can be used to estimate DNA. Ethidium bromide binds to the DNA molecule by intercalating between adjacent base pairs. It

absorbs ultra-violet light at 300 nm and emits light at 590 nm in the red/orange region of the visible spectrum. An aliquot of genomic DNA was run on 0.8% ethidium bromide stained agarose gel. Comparisons with a standard λ *HindIII* digest DNA, gives an estimate of the amount of DNA.

3.4 RFLP analysis

3.4.1 *Restriction Digestion*

The following restriction enzymes are used for digestion of DNA: *EcoRI*, *EcoRV*, *HindIII*, and *XbaI*.

Restriction enzymes recognize specific base sequence in double stranded DNA and break the phosphodiester bonds between two nucleotides within this sequence. Recognition sequences are:

EcoRI Restriction site sequence	5' G ↓ AATTC 3' 3' CTTAA ↑ G 5'
EcoRV Restriction site sequence	5' GATAT ↓ C 3' 3' C ↑ TATAG 5'
HindIII Restriction site sequence	5' A ↓ AGCTT 3' 3' TTCGA ↑ A 5'
XbaI Restriction site sequence	5' T ↓ CTAGA 3' 3' AGATC ↑ T 5'

3.4.2 *Preparation of Master mix for DNA restriction reactions*

Reagents	For 1 reaction	For 16 reactions
10x buffer	4.0 µl	64.0 µl
Restriction enzyme (10units/µl)	10.0 µl	160.0 µl
BSA	1.0 µl	16.0 µl
Reaction volume	15.0 µl	90.0 µl

Concentration of genomic DNA - 15 µg

Reactions for restriction digestion are setup with 15 µg DNA in a final volume of 40 µl (15 µl master mix + DNA + DDW). The tubes were briefly centrifuged and incubated at 37°C overnight for complete digestion of sorghum genomic DNA.

3.4.3 *Separation of DNA fragments*

DNA fragments are most commonly separated by electrophoresis through an agarose gel matrix in the presence of a buffer. The samples were run on 0.8% agarose gel stained by ethidium bromide. TAE (1x) buffer is used, and a voltage of 40V was maintained.

The lane containing molecular weight size standards is useful for determining DNA fragment lengths as well as orienting the blot. A common marker is *lambda* phage DNA that has been digested with *HindIII*. Since the marker lane was to be probed along with single or low copy sequences, it is easiest to use a small amount of marker in order to avoid excessive signal from the marker lane.

3.4.4 *Southern blotting*

Depurination of gel is carried out in 0.25M HCl for 15 min at room temperature with gentle agitation. The gel is then rinsed briefly in distilled water, and DNA is denatured in 0.4N NaOH for 1 hour at room temperature by gentle agitation. The nylon membrane (*Amersham Hybond N*) was cut according to gel size and labeled. The gel was then carefully transferred on to the membrane placed on the vacuum blot apparatus to transfer the DNA fragments to the nylon membrane. The transfer is carried out in 20X SSC solution for one hour at 5 Hg pressure. After the transfer, the DNA was cross-linked to the nylon membrane, in a UV cross linker. The blot was stored at 4°C until further use.

3.4.5 *Labelling of probe*

The probes used for hybridization are sorghum probes, which are putative disease resistance gene clones. About 25 ng of purified insert DNA was used as the probe. It was denatured for 5 min in a boiling water-bath; flash-cooled and final volume was made upto 50 µl as shown below and incubated at 37°C for 3 hours.

Sorghum DNA was labelled with α-^{32}P(dATP) as follows

DNA	-	3µl
dNTP without dATP	-	6µl
dATP (α32P)	-	4µl
Klenow	-	1µl
Random primer	-	5µl
SDW	-	31µl
Total volume	-	**50µl**

3.4.6 *Prehybridization & Hybridization*

Southern blots were washed with DDW and then with 3X SSC. Prehybridization was carried out in bottles containing prehybridization solution (500 mM Na_2HPO_4, 7% SDS, 1% BSA, 20 µg/ml of denatured salmon sperm DNA), at 65°C for 3 hours in hybridization oven. The denatured-labelled probe was added to the prehybridization solution. The hybridization was carried out in hybridization oven for 16 hours at 65°C with continuous agitation.

3.4.7 *Processing after hybridization*

After hybridization the excess probe was removed by washing the blots twice in 150 ml wash solution (3X SSC, 0.5% SDS) for 15 min at 65°C, and again in 400 ml of the same solution for 20 min at 65°C with constant agitation. The blots were wrapped in *saran wrap* and exposed to *KODAK* X-ray film at -80°C for 4 –5 days before developing the film. X-ray films were developed by undergoing the process of developing for 2 min followed by treatment with stopper solution (1% acetic acid solution) for 1 min and fixer solution for 2 min. Then, the films were washed and air-dried.

3.4.8 *Scoring of autoradiographs*

Polymorphism was detected by scoring the presence or absence of bands. Comparison of genotypes is based upon the analyses of the number of 'bands shared' between the two species.

4. RESULTS AND DISCUSSION

4.1 Comparison of hybridization with labelled probes

A total of 15 genotypes of sorghum and *Taipei* variety of rice were assessed for RFLPs using three probes originating from sorghum. The probes used were the disease resistance gene clones namely, S-2-2, S-30-5 and S-27-2. Four restriction enzymes viz. *EcoRI, EcoRV, HindIII,* and *XbaI* were used to detect RFLPs. Nearly all of the sorghum clones tested hybridized to genomic blots of sorghum DNA, but only two probes hybridized to rice genomic DNA.

Genomic DNA was isolated from the sorghum lines and rice by CTAB extraction method from young leaves. The purity of DNA was confirmed by spectrophotometric analysis at 260 and 280 nm. The absorbance ratio at 260 and 280 nm were between 1.6 to 1.9, which suggests that the genomic DNA preparation is reasonably pure. Further the DNA obtained was digested with four restriction enzymes, *EcoRI, EcoRV, HindIII* and *XbaI.* Complete digestion of the DNA was evident from the appearance of the smear of DNA as shown in figure 1 and 2 when analysed on 0.8% agarose gels. The blots were prepared by vaccum blotting method maintaining 5 Hg pressure and hybridization was carried out with the labelled probes of sorghum DNA.

The hybridization of the S-2-2 probe with genomic blot of sorghum lines and rice DNA digested with *EcoRI* detected two bands which is represented in figure 3. One band is more prominent but no polymorphism has been detected, whereas in the second faint band genotype 296 B is found to be polymorphic. Rice has not shown hybridization with this probe.

When the DNA is digested with *EcoRV* and hybridized with the same probe S-2-2, only one locus was detected, which is indicated by the presence of single band. Polymorphism was detected in the genotypes IS 9830 and 296 B, and the probe has not hybridized with rice DNA as seen in figure 4.

The blot of sorghum and rice genomic DNA, when digested with the restriction enzyme *HindIII* and probed with S-27-2 showed no polymorphism in the parental lines of sorghum (figure 5). The probe hybridized to rice DNA and detected numerous bands. Since the band that is present in sorghum lines was not detected in rice, so they are not conserved across species.

The enzyme probe combination of *XbaI* and S-27-2 found hybridization only with sorghum lines and not with rice DNA. Polymorphism was detected in the parental lines of E 36-1 and 296 B genotypes (figure 6).

The genomic blot digested with *XbaI* and hybridized with the probe S-30-5 detected hybridization with both sorghum lines and rice DNA (figure 7). Two bands are found common in both the sorghum lines as well as in rice genomic DNA. Polymorphism is seen in the sorghum E 36-1 and 296 B genotypes. These bands are also found in rice, which depicts conservation of these particular sequences across different genotypes of sorghum and between the sorghum and rice genotypes.

From this particular investigation, it is evident that most of the enzyme-probe combinations revealed polymorphism in the genotypes 296 B and E 36-1. Only two of the probes hybridized to rice genomic DNA, and could detect numerous bands. The enzyme probe combination of *XbaI* and S-30-5 could detect common bands

in sorghum parental lines and in the rice genotype, which shows that, the sequences in the given genotypes are conserved between the two crop species. This observation may be useful for the future research in comparative mapping of different cereal crops.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 M

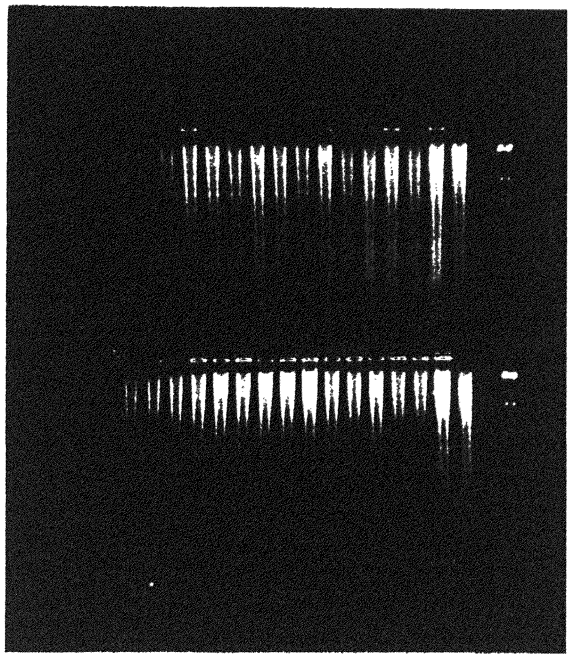

Figure 1. Genomic DNA of sorghum lines and rice digested with restriction enzymes, *EcoRI* and *EcoRV*

Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15. CSV5, 16. Taipei 309 rice.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 M

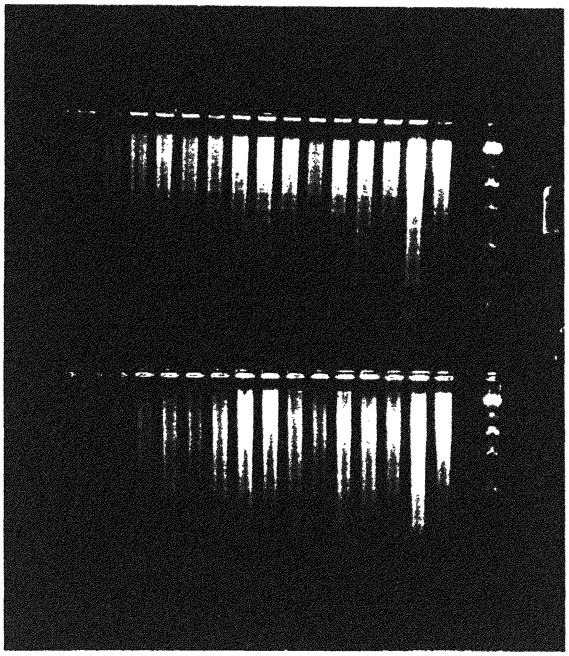

Figure 2. Genomic DNA of sorghum lines and rice digested with restriction enzymes, *HindIII* and *XbaI*

Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15. CSV5, 16. Taipei 309 rice.

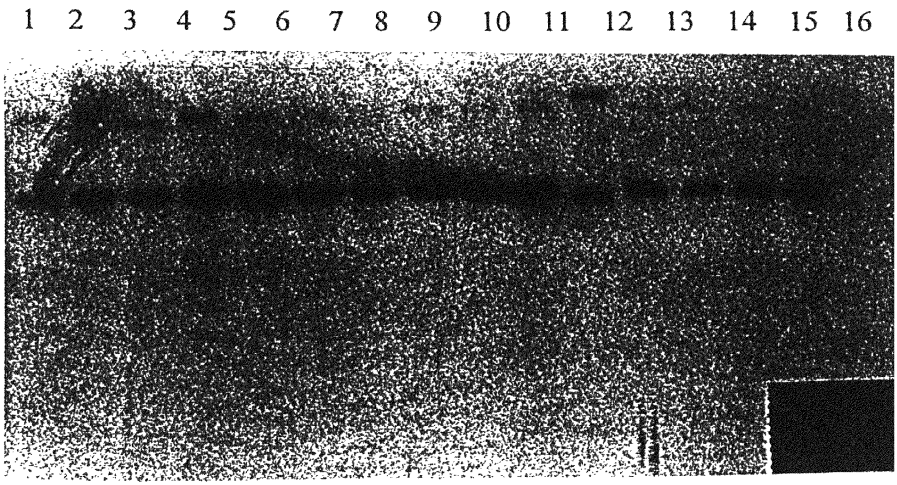

Figure 3. Autoradiograph of genomic blot of sorghum and rice DNA digested with *EcoRI* and hybridized with sorghum RFLP probe, S-2-2. Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15. CSV5, 16. Taipei 309 rice.

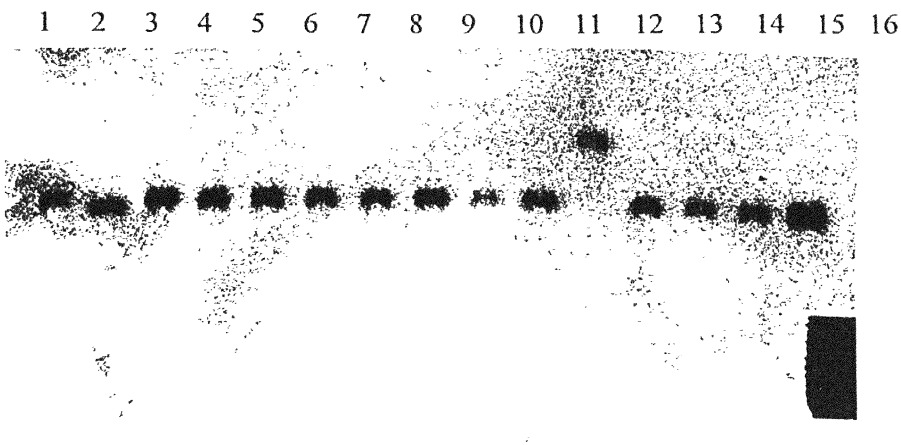

Figure 4. Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *EcoRV* and hybridized with sorghum RFLP probe, S-2-2. Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15. CSV5, 16. Taipei 309 rice.

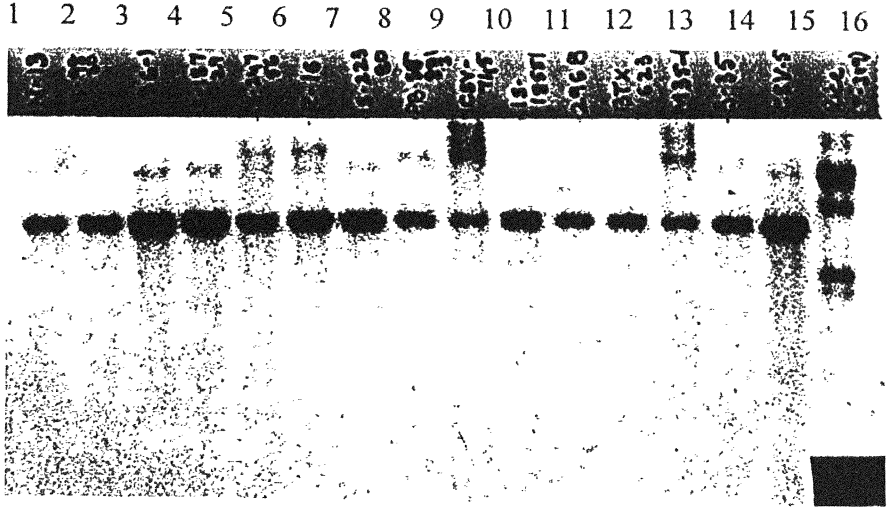

Figure 5. Autoradiograph of genomic blot of sorghum lines and rice DNA digested with *HindIII* and hybridized with sorghum probe, S-27-2.

Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15 CSV5, 16 Taipei 309

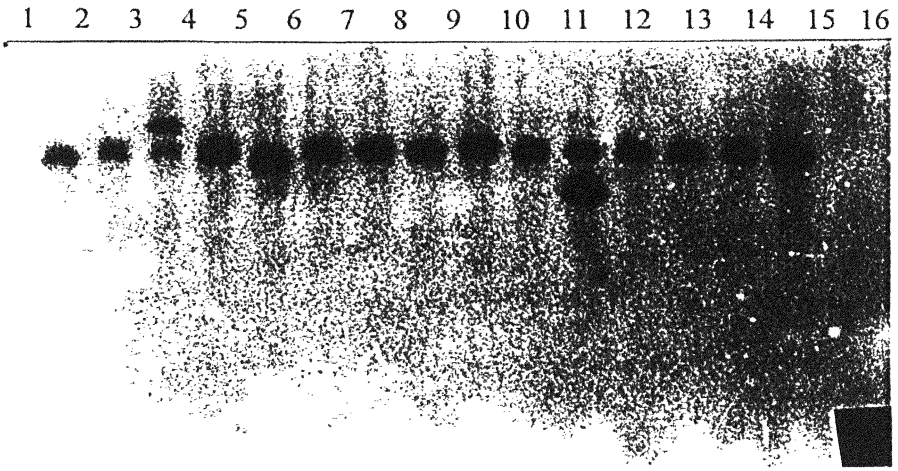

Figure 6. Autoradiograph of genomic blot of sorghum and rice DNA digested with *XbaI* and hybridized with sorghum RFLP probe, S-27-2. Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15. CSV5, 16. Taipei 309 rice.

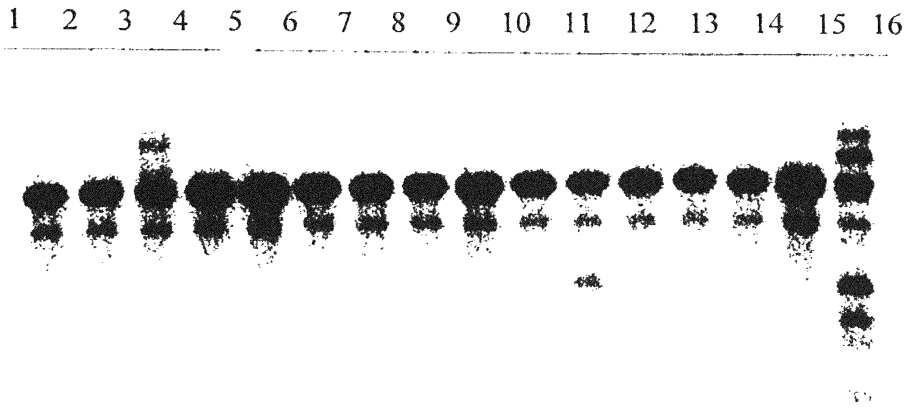

Figure 7. Autoradiograph of genomic blot of sorghum and rice DNA digested with *XbaI* and hybridized with sorghum RFLP probes S-30-5. Lane 1. N13, 2. IS 9830, 3. E 36-1, 4. IS 18729, 5. IS 24756, 6. R 16, 7. IS 22380, 8. PB 15881-3, 9. ICSV 745, 10. IS 18551, 11. 296 B, 12. BTX 623, 13. MS 35-1, 14. B-35, 15. CSV5, 16. Taipei 309 rice.

4.2 Conclusions

From these results, it is obvious that the probe S-30-5, when hybridized to the genomic DNA of sorghum and rice showed conservation of sequences between the two species. So there is a possibility to use common probes to reveal RFLP across several taxa which can lead to the observation of an extensive similarity between the genomes of related species.

In future experiments, DNA from appropriate mapping populations of both sorghum and rice should be hybridized to several probes and maps can be constructed for comparison of map positions in the two crops.

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