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Study of Genetic Diversity in Karnataka Rice (*Oryza Sativa*) Landraces Using Trait Specific Simple Sequence Repeat (SSR) Markers

Ms. KAMAROUTHU DIVYA

M.tech. Dept. Of Biotechnology
REVA INSTITUTE OF SCIENCE AND MANAGEMENT
Bangalore University

I. INTRODUCTION

RICE:

Rice *(2n=24) belonging to the* family *Graminae and subfamily Orazoidea* is staple food of more than 60% of world's population. Rice is the seed of the monocot plants *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). It is a major cereal crop of high agronomic and nutritional importance as global rice production is over 650 million tonnes (http://www.fao.org/). It is highly polymorphic with wide geographical and genetic differentiation (Sarla *et al.*, 2005).It is the grain with the second-highest worldwide production, after maize (corn), according to data for 2010.

Rice is the first food crop for which complete genome sequence is available. It is an ideal model plant for the study of grass genetics and genome organization due to its relatively small genome size of 430 Mb (Causse et al., 1994) compared with other major crops and because of the availability of a high-precision genomic sequence and highly saturated molecular markers.

Having the smallest genome of all cultivated cereals, being diploid and self pollinating, it is the most extensively studied species among cereals. It is grown under diverse cultural conditions and over wide geographical range. Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population.

It is one of the most important crops in the world, growing on over 1.5 billion hectares of land with overall worldwide production of 596 million tons per annum. Now Rice is grown in 117 countries. India alone produces nearly one fourth (22%) of the Rice in the world.

In addition to staple food, rice has extensive protective and curative properties against human ailments like epilepsy, chronic headache, rheumatism, paralysis, skin diseases, diabetes, arthritis, indigestion, blood pressure, colon cancer, internal rejuvenation of tissues and overcoming postnatal weaknesses (Kirtikar and Basu, 1935).

There are four major categories of rice worldwide: Indica, Japonica, Aromatic and glutinous. There is wide genetic variability available in rice among and between landraces leaving a wide scope for future crop improvement. Approximately 11% of the world's arable land is planted annually to rice, and it ranks next to wheat.

Owing to world population growth, there is a tremendous increase in the demand for rice. The price for rice is not homogenous in the international rice markets due to its variability in quality, variety and processing. According to a USDA report, the world's largest exporters of rice in 2012 were India (9.75 million tonnes), Vietnam (7 million tonnes), Thailand (6.5 million tonnes), Pakistan (3.75 million tonnes) and the United States (3.5 million tonnes).

LANDRACES - A source of important genes :

A landrace is a local variety of a domesticated plant species which has developed largely by natural processes, by adaptation to the natural and cultural environment in which it lives. Landraces have particular traits, such as their adaptability to local conditions and constraints, which are not easily found in formal varieties.

Landraces, also known as local populations, traditional cultivars, or farmers varieties (Zeven 1998), provide a valuable resource for plant breeding as well as for the preservation of genetic diversity. Landraces have been shown to be excellent sources of genes for novel alleles. (McCouch et al., 1997; Hoisington et al., 1999; Jackson 1999; Loresto et al., 2000). They are precious genetic resources, because they contain huge genetic variability which can be used to complement and broaden the gene pool of advanced genotypes.

The extent of genetic diversity in a crop population depends on recombination, mutation, selection and random genetic drift. Mutation and recombination bring new variations to a population, whereas selection and genetic drift remove some alleles, often from agronomically important lines. The use of adapted rice landraces, as the primary source of variation into which desired characters present in modern cultivars are introgressed may be an effective strategy for producing cultivars adapted to difficult production environments (Hawtin *et al*., 1997).

The demand for productivity and homogeneity in crops has resulted in a limited number of standard, high-yielding varieties and a loss of heterogeneous traditional local varieties (landraces), a process known as genetic erosion. Landraces and older crop varieties preserve much of this lost diversity and comprise the genetic resources for breeding new crop varieties to cope with environmental and demographic changes (Zhu et al., 2004; Esquinas-Alacazar, 2005).

Rice landraces, maintained through traditional farming practices, possess high genetic diversity and specific traits such as disease resistance, environmental constraint tolerance and nutritional quality which are often used in crop improvement (Camacho-Villa *et al.*, 2005). Furthermore, landraces are adapted to local agro-environmental conditions which contributes to yield stability and hence, they continue playing an important role in traditional and subsistence farming (Camacho-Villa *et al.*, 2005).Thus, landraces of rice play a very important role in the local food security and sustainable development of agriculture (Tang et al.,2002).

GENETIC DIVERSITY:

The use of rice genetic resources available at gene banks is an important strategy for incorporating genetic variability into rice breeding programs, which can potentially generate new cultivars with broadened genetic basis and allows new and useful allelic combinations (McCouch, 2005). Crosses to broaden the genetic basis of rice also can promote the preservation of rare alleles that can be incorporated in elite germplasm. The use of adapted rice landraces, as the primary source of variation into which desired characters present in modern cultivars are introgressed may be an effective strategy for producing cultivars adapted to difficult production environments (Hawtin *et al*., 1997). Speed, reproducibility and the ability to detect genetic variation within and between accessions determine the utility of molecular techniques for germplasm bank management (Gilbert *et al*., 1999).

BLAST DISEASE:

The rice blast disease is caused by the fungus *Pyricularia grisea*, which, in its sexual state, is known as *Magnaporthe grisea* (Hebert) Barr., a filamentous heterothallic ascomycoteous fungus.In all rice growing areas blast disease caused by (Magnaporthe grisea (Herbert) Borr. (anamorph Pyricularia oryza Cav. = P. grisea Cav.) is the most serious fungal disease causing heavy yield losses from 10 to 80%. Blast occurs at different growth stages. The fungus produces spots or lesions on leaves, nodes and different parts of panicles and grains. The neck blast makes more significant yield and quality losses than leaf blast.

Blast disease is the most destructive disease worldwide. Growing resistant varieties has been the most effective and economic way to control this disease but resistance is often lost in a few years after cultivars released because of the high variability of the rice blast fungus. To breed rice varieties with more durable blast resistance, multiple resistance genes utilizing both qualitative and quantitative genes must be incorporated into individual varieties. This necessitates the exploration of more efficient selection and breeding strategies than those currently exist. Recent advancements in DNA marker technology may provide new solutions for selecting and maintaining more durable resistance genotypes in rice. In contrast to the traditional selection based on phenotypic screening molecular markers are refractory to environmental

variation. Upon identification of molecular markers closely linked to desirable traits, marker assisted selection (MAS) can be performed for multiple resistance in early segregating generations and at early stages of plant development.

DROUGHT:

Drought stress is a major constraint to rice (*Oryza sativa* L.) production and yield stability in many rainfed regions of Asia, Africa, and South America. The genetic improvement of adaptation to drought is addressed through the conventional approach by selecting for yield and its stability over locations and years. Recent development of molecular linkage maps of rice and other advances in molecular biology offer new opportunities for drought resistance breeding. Molecular markers linked to root traits and osmotic adjustments are being identified, which should lead to marker-assisted selection.

MOLECULAR MARKERS:

A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily monitored. Over the past decade, morphological characteristics such as height, grain shape, size, etc. were used in rice variety identification. Recently, DNA profiles based on various molecular markers have been widely applied across different fields (Popping, 2002; Terzi et al., 2005; Primrose et al., 2010). Molecular markers are powerful tools for evaluation of genetic diversity.

Ability and potential of biochemical markers (electrophoresis of proteins and isoenzymes) for distinguishing crop varieties have been demonstrated by many workers. Though biochemical markers are less influenced by the environmental conditions, they offer limited polymorphism and often do not allow discrimination between closely related genotypes (Ainsworth and Sharp, 1989; Aldrich *et al*., 1992). DNA marker is a new approach based on DNA polymorphism among tested genotypes, and thus applicable to biological research. It offers many advantages over other categories of markers such as morphological, cytological or biochemical markers. For example, DNA marker can cover the whole genome and, therefore, is much larger in quantity. Many DNA markers are co-dominant and can differentiate between the homozygous and heterozygous genotypes. Furthermore, DNA markers are 'neutral', and they have no effect on phenotype, no epistatic effect, and are not influenced by environmental conditions and developmental stages. Therefore, it has been applied widely in the identification, registration of plant variety, and in monitoring of the seed purity and the authenticity with high accuracy, high reliability and low cost.

Unambiguous, reliable, fast and cost-effective identification, assessment of genetic diversity and relationships within and among crop species and their wild relatives is essential for the effective utilization and protection of plant genetic resources (Paterson et al., 1991; Barcaccia, 2009). Traditionally used morphological markers are not discriminative enough, warranting more precise techniques. Further, these markers are not reliable because many characters of interest have low heritability and genetically complex in nature. Molecular marker technology provides powerful tool for assessment of genetic diversity among cultivars, identification of cultivars and thus add to management and protection of plant genetic resources (Virk et al., 2000). Use of molecular markers is considered best for analysis of genetic diversity and varietal identification since there is no effect of stage of development, environment or management practices.

These markers include restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), random amplified polymorphic DNA (RAPD) (Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), microsatellite or simple sequence repeat (SSR) (Tautz, 1989; Chen et al., 1997; McCouch et al., 2002), and single nucleotide polymorphism (SNP) (Hour et al., 2007; Ganal et al., 2009).

- **RFLP**:

Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. This technique is mainly based on the special class of enzyme i.e. Restriction Endonucleases.

The two main advantages of RFLP markers are co-dominance and high reproducibility. Disadvantages are the requirement of relatively large amounts of pure and intact DNA and the tedious experimental procedure.

- **RAPD:**

RAPD markers are based on the PCR amplification of random DNA segments with single, typically short primers of arbitrary nucleotide sequence (Willams *et al*. 1990). A disadvantage of RAPD markers is the fact that the polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity. Besides being dominantly inherited, RAPDs also show some problems with reproducibility of data. Their major advantages are the technical simplicity and the independence of any prior DNA sequence information.

- **AFLP:**

The AFLP technique combines elements of RFLP and RAPD. It is based on the selective PCR amplification of restriction fragments. Possible reasons for AFLP-Polymorphisms are (i) sequence variations in a restriction site, (ii) insertions or deletions within an amplified fragment and (iii) differences in the nucleotide sequence immediately adjoining the restriction site (not detected with RFLPs). Thus, the usage of AFLP technologies results in the detection of higher levels of polymorphisms compared with RFLPs. AFLPs also have a much higher multiplex ratio (more markers per experiment) and better reproducibility than RAPDs. A drawback can be that most AFLP markers are dominant rather than co-dominant, due to the complex banding patterns.

SNP markers are based on sequence differences at single-base pair positions in genomes. Single nucleotide exchanges in genomes are numerous; therefore SNP markers provide a great marker density. Another important advantage of SNP is that it is not a gel-based technology. For the large-scale genotyping required in marker assisted breeding programs, technologies based on gel electrophoresis are often too labor intensive and time consuming.

Among these markers, SSR markers have several advantages, their co-dominant, stable and highly polymorphic characteristics have been used intensively for rice cultivar identification, genetic diversity evaluation and phylogenetic comparison and marker assisted selection.

- **SSR MARKERS:**

SSR (Simple sequence repeats) markers are class of repetitive DNA sequences usually 2-6 bp that are distributed throughout whole genome and are flanked by highly conserved region. They are also known as microsatellites or short tandem repeats (STRs). It is a type of variable number tandem repeats (VNTR).

Of the wide array of DNA markers available, microsatellite or simple sequence repeat (SSR) markers are considered to be appropriate for assessment of genetic diversity and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Smith et al., 1996; Varshney et al., 2005; Ijaz, 2011). Microsatellite markers have been ideal for identification and purity checking of rice varieties.

Microsatellites are PCR-based markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome (Panaud *et al*., 1996, Temnykh *et al*., 2000). In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity (McCouch *et al.*, 1997). These markers can detect a significantly higher degree of polymorphism in rice (Ni *et al*., 2002, Okoshi *et al.*, 2004) which becomes ideal for studies on genetic diversity and intensive genetic mapping (Cho *et al*., 2000).

In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species (Siwach *et al*., 2004, Brondani *et al*., 2005, Neeraja *et al*., 2005). In rice, more than 2,000 SSR markers are available from SSR-enriched libraries and from the sequenced rice genome (McCouch *et al*., 2002). This high number of markers permits the selection of the most informative and well-distributed SSR loci in the rice genome to be used in molecular analysis.

Advantage of SSR markers are:

- The method is relatively simple and can be automated
- Most of the markers are monolocus and show Mendelian inheritance
- They are highly informative

- A high number of public SSR primer pairs are available
- Effective cost per genotype and primer

II. REVIEW OF LITERATURE

Rice is the staple food for a large segment of the Asian population. The quality of rice seed has distinct effect on the yield potential; hence proper inspection of rice seed quality is very important. Varietal identification has attained critical importance worldwide especially in the context of plant variety protection. Due to the proliferation of varieties in all major crop species, the number of combinations of morphological and physiological descriptors available to establish the uniqueness of a variety has narrowed down (Song *et al*., 1999). Characterization and identification of cultivars are crucial for the varietal improvement, release and seed production programmes.

A collection of terms was used to describe the different types of drought stress responses in rice that allow a plant to produce grains under stress (O'Toole and Chang 1978): 1) escape (e.g., early flowering, or matching crop duration and development to the rainy season length), 2) avoidance (e.g., deep root growth to allow continued water uptake), 3) drought tolerance (e.g., the ability to withstand very negative soil water potentials), and 4) drought resistance, an overall term for the ability to produce grains through any of the above mechanisms . Root morphology and rate of development were thought to be the most important characteristics for drought resistance (Krupp et al. 1972).

The Njavara is the most widely used medicinal rice in the world. This rice can also be prompted as a health food. The promotion of medicinal rice can open up greater awareness, benefit a broader public and result in increased income for the poor farming community of India. Unambiguous, reliable, fast and cost-effective identification, assessment of genetic diversity and relationships within and among crop species and their wild relatives is essential for the effective utilization and protection of plant genetic resources (Paterson et al., 1991; Barcaccia, 2009).

Rice landraces, maintained through traditional farming practices, possess high genetic diversity and specific traits such as disease resistance, environmental constraint tolerance and nutritional quality which are often used in crop improvement (Camacho-Villa *et al.*, 2005).

In tropical Asian countries such as India, few of the traditional native landraces are still under cultivation by resource poor farmers who practice subsistence farming (Ram *et al.*2007).

Although less productive, these landraces have shown excellent adaptation to local conditions and they are known to harbour great genetic potential for rice improvement, particularly for stress tolerance (Hanamaratti *et al.* 2008; Lisa *et al.* 2011) and quality (Huang *et al.* 2010; Pervaiz *et al.* 2011). Therefore, the need to characterize available landraces has become important in modern day crop improvement (Dale *et al.* 1985; Rezai and Frey 1990).

Genetic Diversity:

Agriculture relies heavily on the genetic diversity of crop plants. Ever since the very beginning of agriculture (more than 10,000 years ago), during the process of domestication and cultivation of crop plants, a wealth of genetic diversity has been utilized and partly preserved. It is estimated that not even 15 percent of the potential diversity has been utilized. Thousands of valuable allelic variations of traits of economic significance remain unutilized in nearly all crop plants. These can be discovered and effectively used to meet the existing and emerging challenges that threaten world food security. Sadly, this genetic wealth is being eroded due to neglect and over-exploitation. Developmental activities and exploitive land-use planning are destroying natural habitats, and modern varieties are replacing native species and landraces, resulting in a reduction of varietal diversity. Major crop species (rice, wheat and millet) suffered the most during the green revolution. In order to successfully meet future food requirements, it is necessary to manage the continuing genetic erosion and address the issues of genetic conservation and optimum utilization of what remains of the genetic diversity of important crop plants.

Genetic diversity can be seen as a defence against problems caused by genetic vulnerability. Traditional farmers built this defence into the genetic structure of landraces through selection over many generations and it may be necessary to introgress such defence mechanisms into modern cultivars to make them sustainable (Martin et al., 1991; Chang, 1994; Kannenberg and Falk, 1995).

It is well known that during early domestication and evolutionary selection, cultivated rice differentiated into two major subspecies, *indica* and *japonica* (Chang 1976; Oka 1988; Morishima *et al.* 1992)

58 random primers were used, most of these primers (96.5%) detected polymorphism among the genotypes. Of the 465 amplified bands, 314 were polymorphic (P. Ray Choudhury *et al* 2000*).* The dendrogram based on 58 primers was highly similar to that based on 10 and 15 primers with
matrix correlation (r) of 0.88 and 0.91, respectively. This suggested that a set of 10 primers can be employed for an initial assessment of genetic diversity in a large number of collections.

The characterization and quantification of genetic diversity within closely related crop germplasm has long been a major goal, as it is essential for a rational use of genetic resources. Above and beyond, the analysis of genetic variation among breeding materials is of fundamental interest to plant breeders, as it contributes immensely to se-lection, monitoring of germplasm, and also to pre-diction of potential genetic gains (Chakravarthy and Rambabu 2006).

Some recent studies have reported considerable crop genetic diversity being maintained on farm in the form of traditional crop varieties, as in the increase in the genetic diversity of rice even when the number of varieties decreases in traditional production (Jarvis et al. 2008; Steele et al. 2009).

2.1 APPROACHES TO VARIETAL IDENTIFICATION

Genetic markers have been observed and used for the cultivar identification since the dawn of genetics. Genetic markers fall into one of the three broad classes: those based on visually assessable traits (morphological and agronomic traits), those based on gene product (biochemical markers), and those relying on a DNA assay (molecular markers).

2.1.1 Morphological characterization

The earliest noteworthy attempt at a detailed classification of rice varieties was that of Kikkawa (1912) based on agronomical and physiological characters. Graham (1913) classified the Indian rice varieties based on leaf sheath colour and then on grain dimensions.

Morphological characters in various parts of plants have traditionally been used to distinguish one cultivar from other (Simmonds and Shepherd, 1955).

Vanangamudi *et al.* (1988) grouped 85 rice varieties based on colour of hulled grain, vitreous characters, length, shape, profile value (width), 100 grain weight, presence or absence of pearl spots and shape of pearl spot.

Various grain characters such as number of panicles, grains per panicle, grain yield, grain weight and shape are useful in identification of rice hybrids (Geetha *et al.*, 1994).

2.2 Molecular characterization

Molecular marker technology is the powerful tool for determining genetic variation in rice varieties (Xu and Wang, 1974).

Molecular marker has been widely used to verify the purity of seed. The majority of the work utilizing molecular marker in seed production has the base on genetic mapping using various

The DNA marker system have given substantial impetus to genetic diversity studies and marker assisted selection programmes. Establishing the identity of crop varieties has assumed greater importance for protecting plant breeders and farmers rights particularly in the developing countries. DNA fingerprinting has applications in crop improvement like study of genetic diversity within taxa and for the study of evolutionary and genetic relationships; tagging of economically useful traits; assessment of genetic purity of inbred lines and varieties; selection of recurrent parental genome in back cross and segregating generations thereby decreasing the number of generations required to breed pure lines. The usefulness of DNA fingerprinting technique for cultivar identification analysis was demonstrated first by Dallas (1988).

Markers based on differences in DNA sequence between individuals generally detect more polymorphism than morphological and protein based markers and constitutes a new generation of genetic markers (Bostein *et al.,* 1980; Tanksley *et al.,* 1989).

Traditionally used morphological and biochemical markers are not discriminative enough, warranting more precise techniques. Further, these markers are not reliable because many characters of interest have low heritability and genetically complex in nature. Molecular marker technology provides powerful tool for assessment of genetic diversity among cultivars, identification of cultivars and thus add to management and protection of plant genetic resources (Virk et al., 2000).

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species and several molecular markers *viz*. RFLP, RAPD, SSRs, ISSRs, AFLP and SNPs are presently available to assess the variability and diversity at the molecular level (Joshi *et al*., 2000).

Saker *et al*. (2005) observed the genetic diversity of seven Egyptian rice genotypes using eight RAPD, six SSR and eight AFLP primer combinations and concluded that some varieties originated from closely related ancestors possessing high degree of genetic similarity.

Simple Sequence Repeat (SSR) markers:

Despite the fact that the mechanism of microsatellite evolution and function remains unclear, SSRs were being widely employed in many fields soon after their first description (Litt & Luty1989; Tautz 1989; Weber & May 1989) because of the high variability which makes them very powerful genetic markers. Microsatellites have proven to be an extremely valuable tool for genome mapping in many organisms (Schuler et al., 1996; Knapiket al., 1998) their applications span over different areas ranging from kinship analysis, to population genetics and conservation/management of biological resources (Jarne & Lagoda 1996). microsatellites are powerful for a variety of applications because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage (Liu & Cordes, 2004).

The first report of microsatellites in plants was made by Condit and Hubbel, (1991) and suggested that they are abundant in plant system.

Microsatellites are tandem repeats of 1-6 nucleotides found at high frequency in the nuclear genomes of most taxa (Beckmann and Weber, 1992).

In rice Presence of short tandem repeats of varying length is characteristic of microsatellite loci (Akkaya *et al*.1992)

A microsatellite locus typically varies in length between 5 and 40 repeats, but longer strings of repeats are possible. Dinucleotide, trinucleotide and tetranucleotide repeats are the most common choices for molecular genetic studies. Dinucleotides are the dominant type of microsatellite repeats in most vertebrates characterized so far, although trinucleotide repeats are most abundant in plants (Beckmann & Weber, 1992; Chen et al., 2006; Kantety et al., 2002).

Rice microsatellites have been demonstrated to be polymorphic between (Wu and Tanksley, 1993; Yang *et al.,* 1994; Panaud *et al.,* 1996) and within rice varieties (Olufowote *et al.,* 1997).

SSR markers are considered to be appropriate for assessment of genetic diversity and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Smith et al., 1996; Varshney et al., 2005; Ijaz, 2011)
More than 2,200 SSR markers had been described in rice by McCouch*et al.* (1997, 2002a,b).

Liu and Wu (1998) stated that SSR markers were able to detect a high level of polymorphism among inbred lines. Thus, SSR markers are good molecular markers for hybrid rice research.

A random set of microsatellite markers should facilitate an unbiased assay of genetic diversity and unambiguous molecular description of rice genotypes. The medicinal properties of different rice cultivars are yet to be supported by sound scientific data. No serious efforts have been made at national level for genetic improvement except for reports on collection, characterization and evaluation of the medicinal rice, Njavara (Menon and Potty, 1998; Sreejayan et al., 2003; Leena Kumary, 2004, Thomas et al., 2006; Deepa et al., 2008).

Junjian Ni *et al*. (2002) suggested that a relatively small number of microsatellite markers could be used for the estimation of genetic diversity and the identification of rice cultivars. Molecular genetic techniques can be applied for the evaluation of genetic diversity and to traditional approaches in the conservation and utilization of plant genetic resources (Gauthier *et al.*, 2002).

The number of alleles detected by a single SSR locus varied from 1 to 31 depending upon the fingerprinting techniques and materials used in the studies (Ni et al., 2002; Blair et al., 2002; Lu et al., 2005; Jayamani et al., 2007; Thompson et al., 2009; Kaushik et al., 2011).

To select SSR markers for efficient differentiation of various rice varieties, they conducted a preliminary screening of 120 microsatellite markers (McCouch et al., 2002) by agarose gel analysis using 16 rice accessions from different countries (data not shown). These rice varieties were selected according to the following criteria: (1) where they are currently grown in Taiwan, (2) the higher price available in current rice market, (3) the total trade volume of imported foreign rice and (4) the adulterated rice preferred by customers. Those SSR markers displaying non-specific banding patterns, no polymorphisms or without PCR products were discarded. The identity of either foreign or domestic Taiwan rice varieties was easily determined using RM1387 (Hsue-Yu CHUANG *et al.*, 2011)

SSR markers revealed varying degrees of genetic similarity among the accessions of cultivated and wild species of rice (Ren et al., 2003; Juneja et al., 2006; Jayamani et al., 2007; Joshi et al., 2010).

SSRs presented a higher level of polymorphism and greater information content, as assessed by the expected heterogosity, than AFLPs and RAPDs. (A. Belaj *et al*., 2003).

Microsatellite markers have been ideal for identification and purity checking of rice varieties (Nandakumar et al., 2004; Singh et al., 2004).

SSR markers are more popular in rice because they are highly informative, mostly monolocus,
codominant, easily analyzed and cost effective (Gracia et al., 2004).

Kosakivska *et al.*, 2008) discovery of polymerase (PCR) technique (saiki *et al., 1988*) there has been an exponential increase in the marker systems suitable for genetic analyses. These aspects have been revieved in detail by several authors (Staub *et al*., 1996; Mohan *et al.*, 1997; Karp et al., 1997, 1998; Koebner et al., 2001).

The multivariate nature of SSR markers has the unambiguous advantage of discriminating genotypes more precisely (A . Prabakaran et al., 2010)

SSR loci with di-nucleotide repeats detected greater number of alleles than those with tri-nucleotide repeats (Kaushik et al.,2011). The abundance of information on DNA sequences of plant genomes permits to design sequence-related primers for PCR amplification (Rafalski et al., 2002).

A set of 36 microsatellite markers distributed over 12 chromosomes of rice were used to assess genetic diversity in 33 medicinal rice genotypes. L. Behera *et al*.,2012 reported a total of 166 alleles were polymorhic, of the total 169 amplified . All the rice genotypes showed the presence of multiple alleles. Genetic similarities among genotypes varied from 0.239 to 0.827 with an average of 0.5. All the genotypes included in the study could be uniquely distinguished from each other.

Microsatellite marker (SSR) analysis was used to differentiate between domestic Taiwan rice varieties and foreign rice cultivars for authentication and traceability. A panel of 32 microsatellite DNA markers and 36 rice varieties from different countries were used for comparative polymorphism analysis. A total of 306 alleles were observed in 32 loci. The most useful markers identified for efficient differentiation of domestic Taiwan rice identities were RM21, RM22, RM101, RM333, RM475, RM1387, RM5704, and RM7545. Principle component analysis (PCA) score plot and clustering analysis were sufficient to discriminate between two different groups of rice varieties based on geographic origins. To prevent fraudulent commercial activity, these results provide effective SSR marker sets and workflow for regular genotype verification and premium rice variety purity monitoring in Taiwan (Hsue-Yu CHUANG *et al.*, 2012*).*

Objectives:

- Characterization of the genetic diversity and addressing cultivars relationships based on the genetic similarity are the major goals of the research project.
- Assessment of genetic diversity and relatedness among various landraces for Blast disease and for drought related traits.

III. MATERIALS AND METHODS

The experiments were conducted at the Department of Genetics and Plant breeding, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra (GKVK). A total of 11 trait specific SSR markers were analyzed on 26 local land races of rice collected from various rice fields of Karnataka.

Leaf sample collection:

Leaves were harvested from the field grown plants. Young leaves without necrotic lesions were used. The midrib was removed and the leaves were cut into 10-15cm sections and placed in a plastic bag along with the tag identifying the sample.

Grinding:

The leaf samples were cut into 1-2 cm sections and grind with liquid Nitrogen in a mortar and pestle till fine powder was obtained.

DNA Extraction:-

26 rice genotypes maintained at GKVK field was used for the study. DNA was extracted based on a previously reported cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) with some modification.

PRINCIPLE:

DNA extraction from plant tissue can vary depending on the material used. Essentially any mechanical means of breaking down the cell wall and membranes to allow access to nuclear material, without its degradation is required. For this, usually an initial grinding stage with liquid nitrogen is employed to break down cell wall material and allow access to DNA while harming cellular enzymes and chemicals remain inactivated .once the tissue has been sufficiently ground, it can then be resuspended in a suitable buffer, such as CTAB. In order to purify DNA, insoluble particulates are removed through centrifugation. DNA must then be precipitated from the aqueous phase and washed thoroughly to remove contaminating salts. The purified DNA is then resuspended and stored in TE buffer or sterile distilled water. This method has been shown to give intact genomic DNA from plant tissue. To check the quality of the extracted DNA, a sample is run on an agarose gel, stained with ethidium bromide, and visualized under UV light.

REAGENTS REQUIRED FOR DNA EXTRACTION:-

Extraction buffer (1L):-

Nacl	0.5M Tris(pH 8)	12.1 gm
Tris	0.1M Nacl	29.2 gm

EDTA 0.05M EDTA 18.6 gm

β-Mercaptoethanol (0.01M) 200 µl/100ml

Nacl, Tris EDTA was added and pH adjusted to 8. Then β-Mercaptoethanol was added and autoclaved.

CTAB buffer(1L):

Nacl 2M Tris (pH 8) 46.8 gm

Tris 0.2M Nacl 24.2 gm

EDTA 0.05M EDTA 18.6 gm

CTAB 2 % (2g/100ml)

pH was adjusted to 8

SDS or Sodium Lauryl sulphate (10% w/v)- 1L:-

SDS 100gm

Ddw up to 1000ml

Potassium acetate (KOAc , 5M)- 200mL:-

Potassium acetate 98.14gm

ddW up to 200ml

Autoclave (121 psi, 20 min)

Chloroform-amyl alcohol (24:1)- 100mL:-

Chloroform 96ml

Iso-amyl alcohol 4ml

TE Buffer(T_{10},E_1 ,PH 8.0)-1L:-

TRIS-HCL (1M, PH 8.0) 10mL

EDTA (0.25 M) 4mL

Volume was made up to 1000 ml with ddw.

PROTOCOL FOR DNA EXTRACTION BY CTAB METHOD:

1) 25-35 days old seedlings were used for DNA extraction.
2) Young and healthy leaves (5gm) were collected from 25 days old plants.
3) Leaves were cut into pieces and homogenized completely with liquid nitrogen using mortar and pestle.
4) Leaf powder is transferred to 50 ml falcon tube containing 6ml ice-cold extraction buffer.
5) The samples are then incubated for 30 min at 65^0c after the addition of 0.8ml of 10% SDS. During incubation, the samples are shaken at regular intervals.
6) After incubation, 2ml of ice cold 5/3 potassium acetate (KAC) is added and samples are vigorously mixed and incubated on ice for 30 min.
7) Samples are centrifuged for 10 min at 4000 rpm.
8) The supernatant is filtered through mira cloth into another centrifuge tube.

9) 6ml of isopropanol is added to samples and then incubated at room temperature for 5 min for the precipitation of DNA.
10) The tubes are centrifuged for 10 min at 4000rpm.
11) Supernatant was discarded and the DNA pellet was dried overnight.
12) The dried DNA pellet was dissolved in 200-400µl TE buffer depending on size of the DNA pellet.
13) The dissolved DNA was transferred into fresh eppendrof tubes and incubated for 20 min at 37 ^{0}C after adding 2µl RNAse and mixing.
14) 400ml of CTAB buffer was added and the tubes were incubated for 15 min at 65^{0}c (tubes were periodically shaken).
15) 800 ml of chloroform-iso amyl alcohol was added and the tubes were centrifuged in minifuge for 5 min.
16) The supernatant was transferred to the fresh eppendrof tube and 1.4ml of ethanol (96%) was added and the mixture was incubated at room temperature for 15 min for DNA precipitation.
17) The tubes were centrifuged for 10 min in a minifuge and the supernatant was discarded.
18) The DNA pellet was washed with 70 % ethanol.
19) Ethanol was then removed by drying the pellet.
20) Finally, the DNA pellet was dissolved in 200µl of TE buffer and stored at -20^{0}C until use.

QUANTIFICATION OF GENOMIC DNA BY AGAROSE GEL ELECTROPHORESIS:

PRINCIPLE:

Electrophoresis through agarose or poly acrylamide gels is the standard method used to separate, identify and purify DNA fragments. The technique is simple, rapid to perform and capable of resolving fragments of DNA that cannot be separated adequately by other procedures such as density gradient centrifugation. The location of DNA within the gel can be determined directly by staining with low concentrations of the fluorescent intercalating dye ethidium bromide. Bands containing 1-10ng of DNA can be detected by direct examination of the gel in UV light (sharp et al., 1973).

REAGENTS REQUIRED FOR AGAROSE GEL ELECTROPHORESIS

Tank buffer-Tris Acetate EDTA (TAE, 20X) for 1L buffer:-

Tris base	9.65gm
Glacial Acetic acid	2.28mL
(EDTA) 0.5M, pH 8.0	4.00mL
Distilled water (ddW)upto	1000mL

It was Autoclaved and stored at RT

100mL stock was diluted to 1L with ddW to get 1X buffer

Loading /Tracking dye (10X BPB)-1mL

Source	60.7mg
Bromophenol blue	4.2mg
ddW up to	1000mL

Stored at 4^0 C

EthidiumBromide (10 mg/mL)-1mL

EtBr	10.0mg
Distilled water	1000mL

Dissolved and wrapped with aluminium foil and stored at 4 ^{0}c diluted to ~500ng/l with distilled water for staining.

PROTOCOL FOR AGAROSE GEL ELECTROPHORESIS OF GENOMIC DNA:

1) A suitable gel tray and combs were cleaned with a tissue paper soaked in rectified spirit.
2) The ends of the gel tray were sealed with an adhesive tape.
3) It was then placed on a level surface and leveled using a leveling bubble.
4) 0.8% agarose gel mix (0.8g agarose in 100mL of 1X TAE) was prepared and boiled in a microwave oven till a homogenous, clear, boiling solution was formed.
5) The gel solution was cooled to ~55^0 C.
6) Ethidium bromide (DNA intercalating agent) was added when temperature reached 55-60^0 C as staining agent.
7) Gel was poured into the gel mould with the combs avoiding trapping of air bubbles. Persistent air bubbles were removed with a pipette tip.
8) It was allowed to set for at least 30 min at room temperature.
9) 1X TAE buffer was poured into the buffer-tank of the electrophoresis unit; the unit was leveled using a leveling bubble.
10) The comb was removed carefully from the gel and the tapes were pulled off the gel tray and the gel tray was immersed in the buffer tank.
11) A suitable molecular weight marker was loaded into the first or the last lane (~250ng/lane). DNA sample dissolved in TE was pipetted onto a parafilm and mixed well with 3 µl of 10X loading dye by pipetting up and down several times and the samples were loaded into the gel wells.
12) The lid was closed and the electrodes were fixed (it was made sure that the negative terminal is at the same end of the unit as the sample loading wells are)
13) The power supply was turned off and the constant voltage was adjusted: run at a maximum of 5 V/cm of gel for 1-1.5 hours.
14) The gel was run till the dye front was ~2cm from the opposite end (put off the power supply before removing the lid of the electrophoresis set-up)
15) The DNA bands were observed under UV Trans illuminator.

SSR analysis:

Eleven SSR markers distributed on the chromosomes 1, 2, 6, 7, 9, 11 were employed to analyze the Genetic diversity. All of the SSR markers were obtained from GRAMENE (http://www.gramene.org/). PCR amplification of SSR markers was carried out using eleven primers listed in table 1.

Table 1: Primer sequence, chromosome location (CL), product range (PR), annealing temperature for 11 simple sequence repeats (SSR) markers used in the study

PRIMERS	SEQUENCES	CL	PR (bp)	ANNEALING TEMPERATURE
RM 144	F: tgccctggcgcaaatttgatcc	11	214-255	55
	R: gctagaggagatcagatggtagtgcatg			
RM 166	F: ggtcctgggtcaataattgggttacc	2	321	61

	R: ttgctgcatgatcctaaaccgg			
RM 190	F: ctttgtctatctcaagacac	6	104-124	55
	R: ttgcagatgttcttcctgatg			
RM 201	F: ctcgtttattacctacagtacc	9	144-158	55
	R: ctacctcctttctagaccgata			
RM 234	F: acagtatccaaggccctgg	7	133-163	55
	R: cacgtgagacaaagacggag			
RM 278	F: gtagtgagcctaacaataatc	9	131-146	55
	R: tcaactcagcatctctgtcc			
RM 302	F: tcatgtcatctaccatcacac	1	120-191	55
	R: atggagaagatggaatacttgc			
RM 315	F: gaggtacttcctccgtttcac	1	133-163	55
	R: agtcagctcactgtgcagtg			
RM 318	F: gtacggaaaacatggtaggaag	2	134-154	55
	R: tcgagggaaggatctggtc			
RM 351	F: ccatcctccaccgcctctcg	1	129-134	55
	R: tggaggaaggaaaggggacg			
RM 526	F: cccaagcaatacgtccctag	2	240-266	55
	R: acctggtcatgacaaggagg			

PCR amplification:-

Microsatellite analysis was done with the following steps:

1) PCR amplification of genomic DNA was done using forward and reverse
2) Microsatellite primers.
3) Resolution of polymorphism through Agarose gel electrophoresis.
4) Staining the gel.

5) Analysis of banding pattern.

PRINCIPLE:

The purpose of PCR is to make several copies of a specific gene or a specific sequence of DNA/RNA from a specified minimum amount of starting template for sequencing.

Chain reaction: In a chain reaction there are 3 major steps which are repeated for 30-40 cycles. This can be brought about by an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

Materials required to set-up a PCR reaction:-

1) PCR tubes or plates.
2) Thermal cycler.
3) dNTP mixture (dATP, dTTP, dGTP, dCTP) of 10µM concentration.
4) DNA -5-50ng/µL
5) 10X buffer -10mM tris-Hcl,50mM Nacl,15mM Mg Cl_2,0.01% gelatin.
6) Primer-forward and reverse of 10-15p moles each.
7) Taq 5 units/µL
8) Double distilled water sterile water.
9) Micropipettes and tips.
10) Agarose
11) Gel plate and combs.
12) Gel tank
13) TBE/TAE buffer of 1X concentration

PROCEDURE FOR POLYMERASE CHAIN REACTION:

1) The DNA samples, primer, buffer, Taq polymerase, dNTP mixture was taken and all the components were placed inside crushed ice.
2) The PCR plates were kept in ice pack.
3) 1.5µl of DNA was dispensed in each tube.
4) In a separate 1.5ml eppendrof tube cocktail was prepared using the following components for each tube.

Table 2: The cocktail for PCR amplification

Components	Quantity (µl/tube)	Quantity (µl/30 tubes)
Ddw	4.78µl	143.4µl
Taq buffer	1µl	30µl
dNTPs	1µl	30µl
Primers –Forward	0.8µl	24µl
Reverse	0.8µl	24µl
Taq polymerase	0.12µl	3.6µl

5) All the contents were mixed using a vortex machine and 8.5µl of cocktail was added to each PCR plate containing DNA.
6) The plate was kept in thermal cycler which had the following programmes.

Table 3: Thermo profile for the PCR

Profile	Step	Temperature (°C)	Duration (min)
1	Denaturation	95	5
2	Denaturation	94	1
	Annealing	56	1
	Primer Extension	72	1
3	Final extension	72	7
4	Dump	4	

Profiles 2 was programmed to run for 30 cycles.

Agarose gel electrophoresis of SSR-PCR products: GEL DOC

After PCR amplification, the PCR products are mixed with 2µl loading dye, and loaded in the wells of 3% Agarose gel.

Agarose gel electrophoresis:-

Agarose gel electrophoresis was performed to separate amplified products.

Materials Required:-

PCR amplified samples

2µl Loading dye: Bromophenol blue 0.5% (w/v):

3% Agarose

100bp ladder-6µl

Tank buffer:-

For 300ml, add 6ml of 50X TAE buffer and make upto 300ml with distilled water and add 9 gm of agarose.

Protocol:-

1) Clean a suitable gel tray and combs with a tissue paper soaked in rectified spirit.
2) Open ends of the gel casting plate were sealed with cello tape and placed on a horizontal perfectly leveled platform.
3) 3% agarose was added to 1X TAE buffer and boiled till the agarose dissolved completely and then cooled. Ethidium bromide was used as a staining agent at the final concentration of 1 µg/ml.
4) Agarose gel was poured in to the gel casting tray. The comb was placed properly and allowed to solidify.
5) After solidification of the agarose, the comb and cello tape were removed.
6) 2µl of loading dye was added to DNA samples and were loaded into the gel wells.
7) The gel was run at 110 volts for 2.5 hours and bands were visualized and documented in gel documentation system.

IV. RESULTS AND DISCUSSION

Results:

- Total genomic DNA was isolated by CTAB method and DNA was quantified by agarose gel electrophoresis.

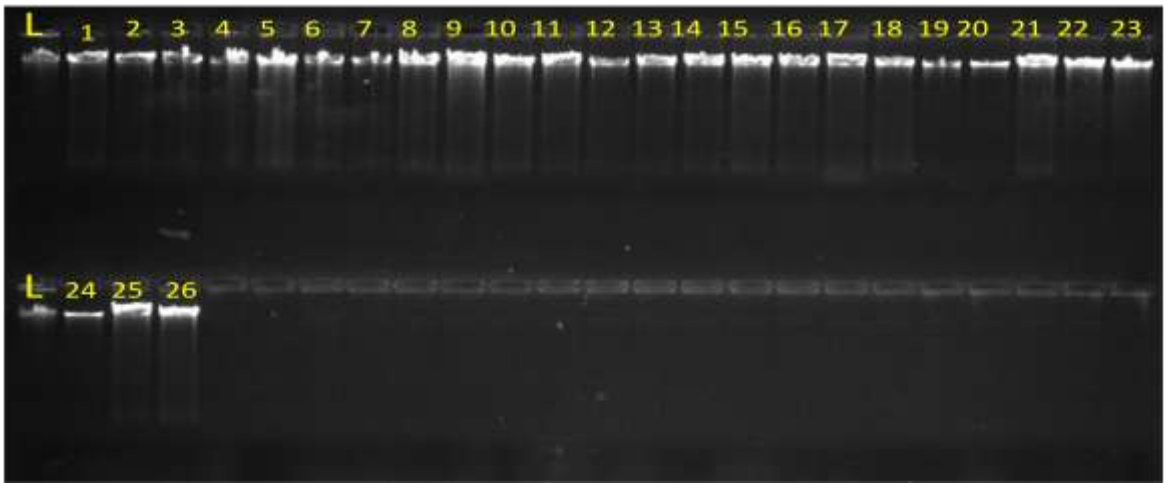

Fig 1: Genomic DNA quantification using 100bp ladder

TRAIT SPECIFIC MARKERS FOR LANDRACES:

A total of 11 microsatellite markers were used to assess the extent of genetic diversity across the 26 genotypes.

Table 4: Trait specific primers

Sr. No.	SSR Primer	Trait
1	RM 144	Blast resistance
2	RM 166	Root length
3	RM 190	Grain number
4	RM 201	Root length
5	RM 234	Root length
6	RM 278	Grain yield
7	RM 302	Root length
8	RM 315	Plant height
9	RM 318	Panicle length
10	RM 351	Seed dormancy
11	RM 526	Grain yield

Trait: Blast resistance

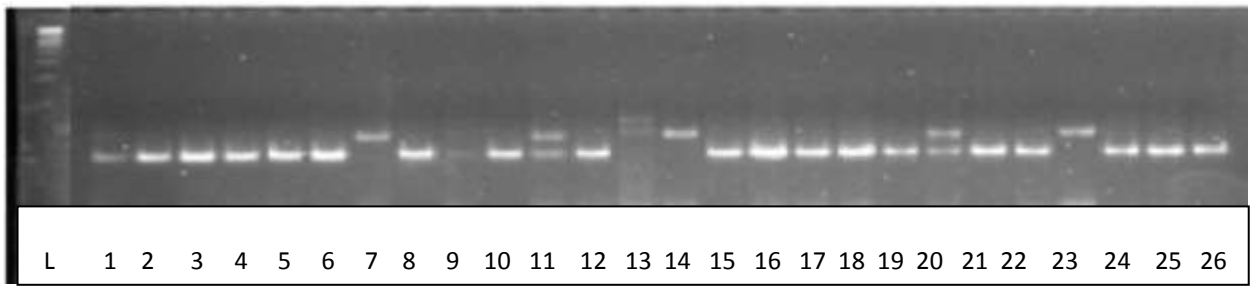

Fig 2: DNA profile of the 26 rice land races with SSR marker RM 144. L= Ladder marker (100bp)

Trait: Root length

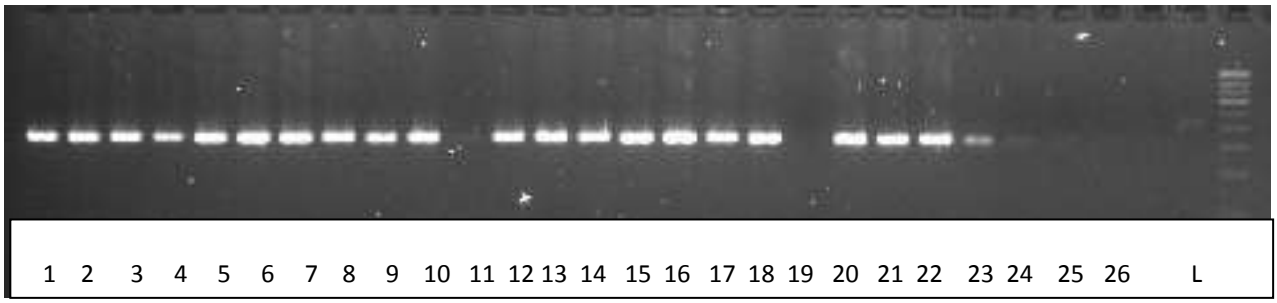

Fig 3: DNA profile of the 26 rice land races with SSR marker RM 166. L= Ladder marker (100bp)

Trait: Grain number

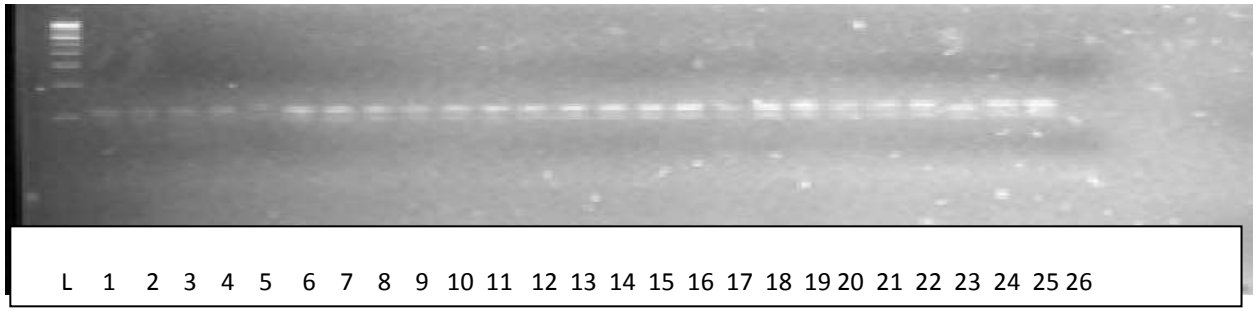

Fig 4: DNA profile of the 26 rice land races with SSR marker RM 190. L= Ladder marker (100bp)

Trait: Root length

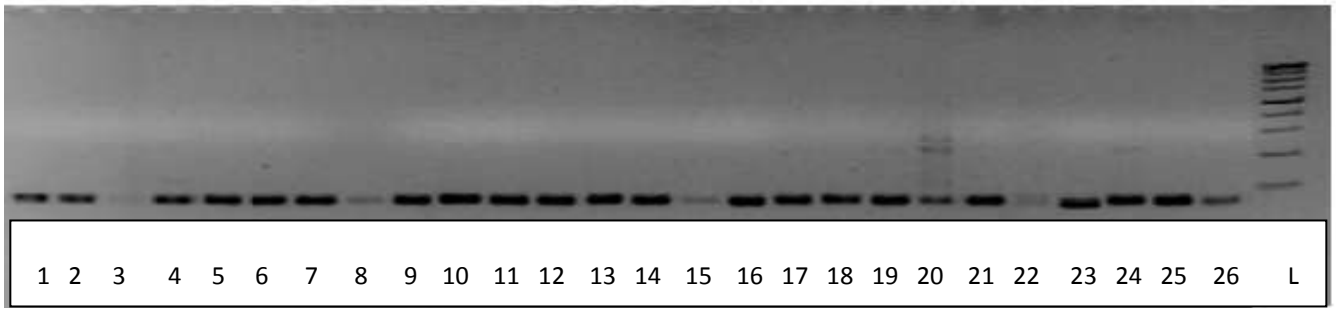

Fig 5: DNA profile of the 26 rice land races with SSR marker RM 201. L= Ladder marker (100bp)

Trait: Root length

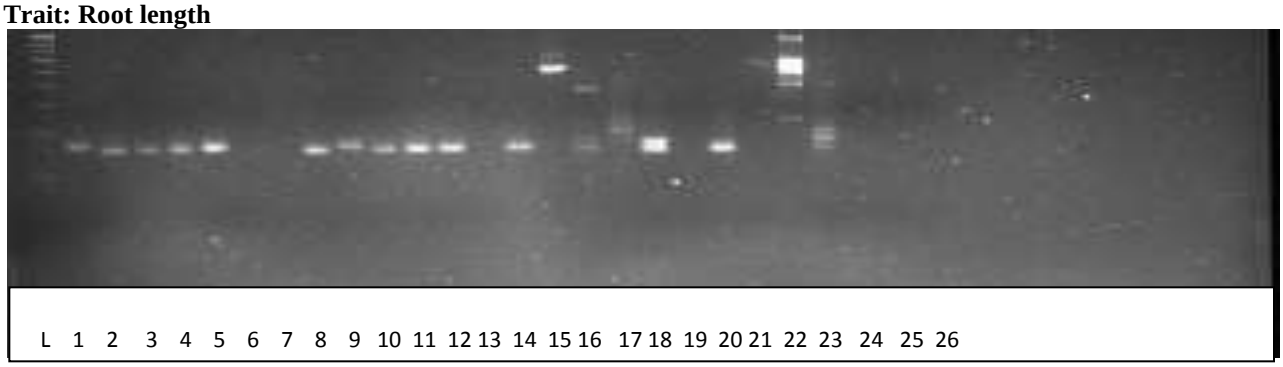

Fig 6: DNA profile of the 26 rice land races with SSR marker RM 234. L= Ladder marker (100bp)

Trait: Grain yield

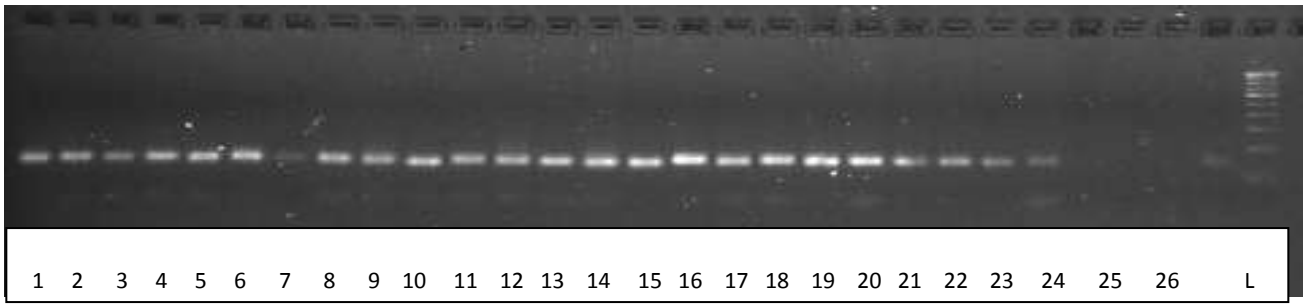

Fig 7: DNA profile of the 26 rice land races with SSR marker RM 278. L= Ladder marker (100bp)

Trait: Root length

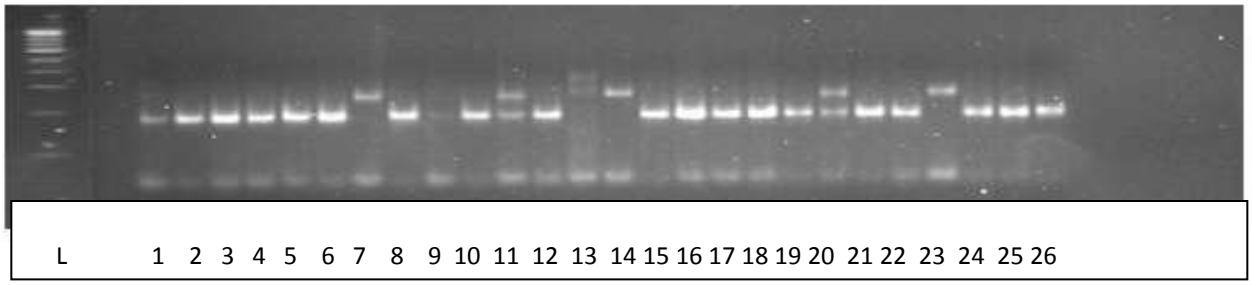

Fig 8: DNA profile of the 26 rice land races with SSR marker RM 302. L= Ladder marker (100bp)

Trait: Plant height

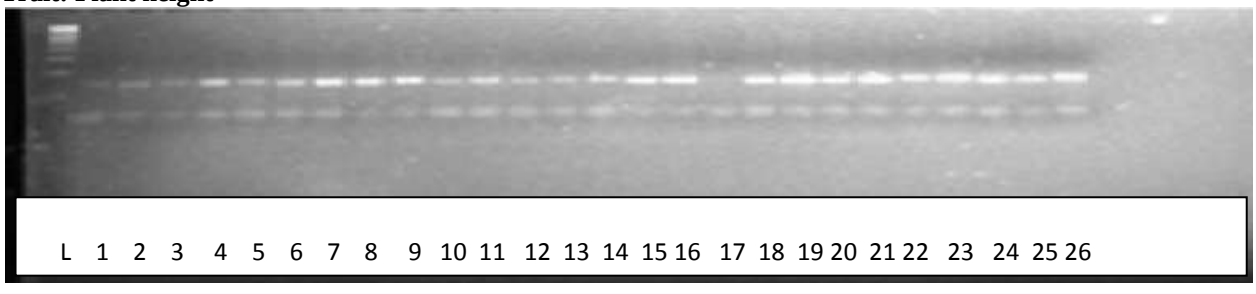

Fig 9: DNA profile of the 26 rice land races with SSR marker RM 315. L= Ladder marker (100bp)

Trait: Panicle length

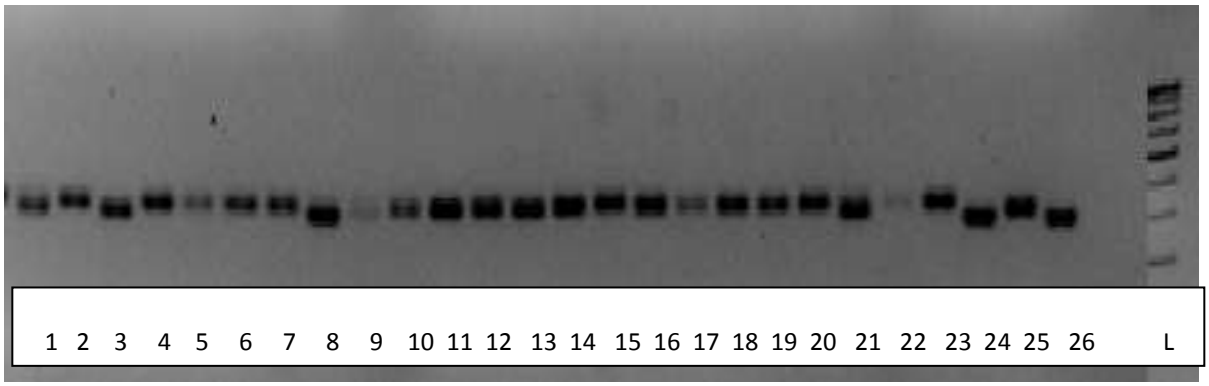

Fig 10: DNA profile of the 26 rice land races with SSR marker RM 318. L= Ladder marker (100bp)

Trait: Seed dormancy

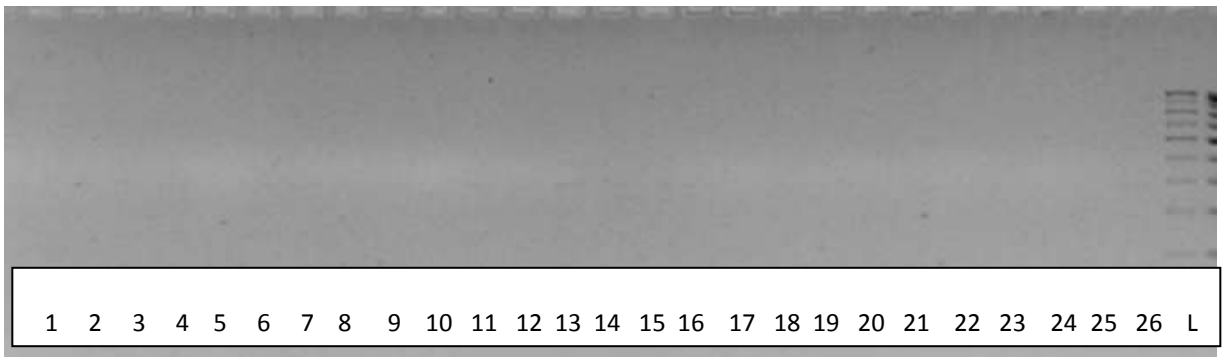

Fig 11: DNA profile of the 26 rice land races with SSR marker RM 351. L= Ladder marker (100bp)

Trait: Grain yield

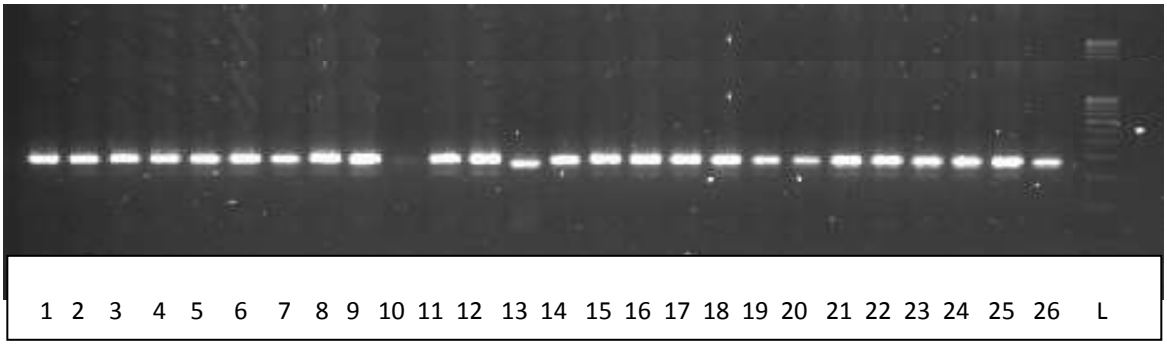

Fig 12: DNA profile of the 26 rice land races with SSR marker RM 526. L= Ladder marker (100bp)

SCORING DATA:

A total of 251 useful bands were scored from the amplification products with the eleven random SSR primers from 26 rice varieties. The number of amplification products generated by each primer ranged in size from 104 bp to 321 bp. Genetic diversity analyses were conducted on the basis of the scores.

Table 5: Scoring data of 26 Rice genotypes against 11 microsatellite markers. LR: Landrace

Genotypes	LR 101	LR 102	LR 103	LR 104	LR 105	LR 106	LR 107	LR 108	LR 109	LR 110	LR 111	LR 112	LR 113	LR 114	LR 115	LR 116	LR 117	LR 118	LR 119	LR 120	LR 121	LR 122	LR 123	LR 124	LR 125	LR 126
RM 144	1	1	1	1	1	1	2	1	0	1	2	1	0	2	1	1	1	1	1	0	1	1	3	1	1	1
RM 166	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	0	0
RM 190	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1
RM 201	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2
RM 234	1	2	2	2	1	0	0	2	1	1	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0
RM 278	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
RM 302	1	1	1	1	1	1	2	1	1	1	0	1	0	0	1	1	1	1	1	0	1	1	2	1	1	1
RM 315	1	1	2	2	2	2	2	2	3	2	2	2	2	3	2	2	0	3	3	3	3	4	4	3	3	4
RM 318	1	1	2	1	2	1	1	1	1	2	2	2	2	2	2	2	1	1	1	1	1	1	1	2	1	2
RM 351	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RM 526	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1

CLUSTER ANALYSIS:

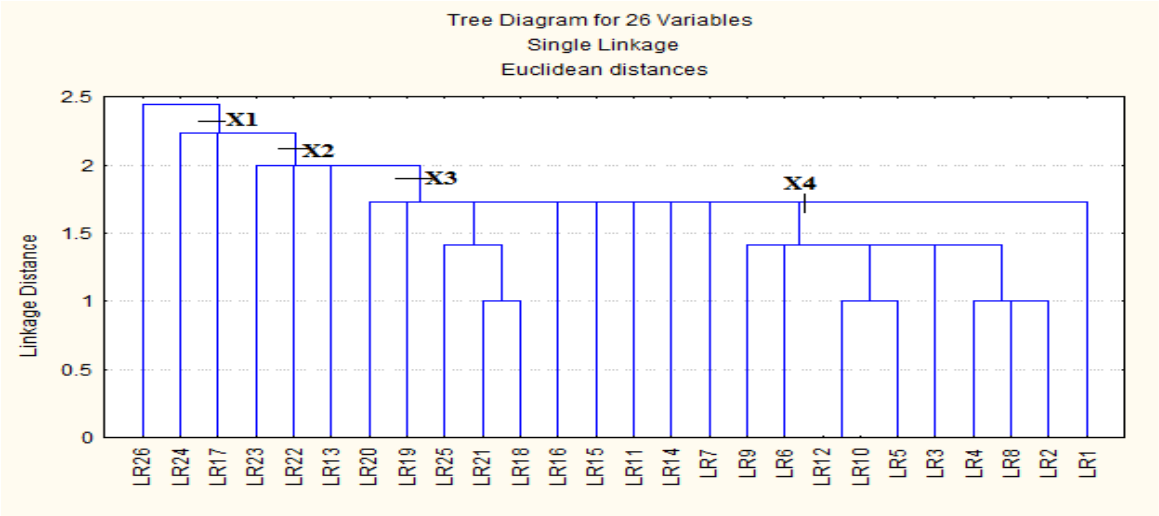

Fig 13: The UPGMA dendrogram showing the genetic relationships among 26 rice accessions based on 11 SSR markers.

Table 6: Composition and size of clusters along with percentage dissimilarity values among rice accessions.

Cluster no.	No. of genotypes	Percentage dissimilarity
1	1	0.90
2	2	3
3	3	5
4	20	8.37

Cluster analysis was performed to construct genetic relationship tree diagrams among studied rice varieties using an Unweighted Pair-Group Method with Arithmetic Average (UPGMA). The software used was STASTICA. The UPGMA

analysis revealed 4 super clusters i.e. X_1, X_2, X_3 and X_4. Highest inter-cluster distance was found in cluster X_4 and the lowest in X_1. X_1 cluster includes LR26 having 0.90% dissimilarity from the remaining and linkage distance of 2.4. X_2 cluster includes LR17 and LR24 having 3% dissimilarity and linkage distance of 2.2. X_3 cluster includes LR23, LR22, LR13 having 5% dissimilarity and linkage distance of 2. X_4 is the largest cluster which includes 10 major clusters i.e., $X_4$1 to $X_4$10. It is having linkage distance of 1.7 and 8.37% of dissimilarity. Among them $X_4$1 contain LR20, $X_4$2 contain LR19 and $X_4$3 is divided in to 3 sub clusters which include LR25, LR21 and LR18. $X_4$4 contain LR16, $X_4$5 contain LR15, $X_4$6 contain LR11. Sub cluster $X_4$7 contain LR14 and $X_4$8 contain LR7. Major cluster $X_4$9 is divided into 5 sub clusters which include LR9, LR6, LR12, LR10, LR5, LR4, LR3, LR2, LR8 and $X_4$10 contain LR1. LR 1 and LR 26 show higher level of dissimilarity and hence can be used as a donor lines for the particular traits.

V. DISCUSSION

Rice is a highly domesticated crop, and domestication processes are reported to be accompanied by genetic erosion, which causes a reduction in genetic diversity among traditional varieties and gradual loss of landraces from the fields. Modern rice cultivars have been developed through the hybridization of elite lines and subsequent selection for yield and quality traits, which resulted in a loss of useful genes to combat biotic and abiotic threats. Traditional rice varieties, or landraces, have a high level of genetic heterogeneity compared to modern cultivars. This genetic variability is very important for the sustainability of small farmers, because despite the low yield capacity, these varieties present high yield stability (Oka, 1991). Landraces are adapted to local, small scale, low-input environments where the plant ideotype may differ considerably from that developed for modern agricultural systems (Veteläinen *et al*., 1997). Another important aspect to consider is the differentiation of characters that are highly subject to natural selection, such as tolerance to biotic and abiotic stresses occurring during the cultivation of rice landraces.

The assessment of genetic diversity is an essential component in germplasm characterization and conservation. The results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially grown varieties.

SSRs were chosen for the analysis of genetic diversity of Karnataka rice cultivars because several works have showed these markers are very powerful for differentiating individual germplasm accessions, particularly when they are closely related (Bligh et al. 1999; Xu et al. 2004; Jeung et al. 2005). Additionally, SSRs show a series of advantages when compared with other DNA-based markers, such as abundance in the genome, high level of polymorphism, repeatability, co-dominance and cost-effectiveness (Ni et al. 2002).

Subspecies level differentiation has occurred in rice during evolution, there exist residual overlaps of key genetic differences in primitive landforms. Landraces are old cultivated genotypes that have not been bred through strict breeding principles, therefore can be relatively closer to such primitive forms. Useful genetic variation in the landforms can therefore be used more judiciously in breeding programmes without narrowing down the existing genetic variation in the cultivated germplasm. In the present study, we have tested this hypothesis in a small set of under exploited landraces of Karnataka, India, where rice cultivation has been in practice for thousands of years. Ubiquity of the SSR polymorphism in the rice genome offer unique opportunity of studying rice genotypes for phylogenetic and evolutionary comparisons. Some of these markers are more specific to subspecific genomes than the other. Coburn *et al.* (2002) suggested that those markers that were highly variable at both the inter-subspecific and intra subspecific levels, makes them very useful for distinguishing closely related genotypes.

The sequences of primers were obtained from the public website (http: //www. gramene.org/microsat/microsats.txt). SSR markers used in studies were synthesized by Bangalore Genie, India, were distributed on chromosome numbers 1, 2, 6, 7, 9, 11.

The dendrogram (Figure 13) resulting from UPGMA analysis revealed 4 major clusters of various sizes. Based on the present study, the large range of dissimilarity values for related genotypes using microsatellites provide greater confidence for the

assessment of genetic diversity and relationships. Varietal profiling based on SSR markers will be more reliable as compared to other markers, since SSR markers detect finer levels of variations among closely related lines.

VI. SUMMARY AND CONCLUSION

SUMMARY:

Genetic diversity underlies the improvement of crops by plant breeding. Landraces of rice (*Oryza sativa* L.) can contain some valuable alleles not common in modern germplasm. The aim here was to study the genetic diversity among rice landrace genotypes grown in Karnataka.

A total of 26 local rice (*Oryza sativa* L.) varieties of Karnataka were selected for genetic diversity study with 11 microsatellite DNA markers. Upon PCR amplification the bands were separated on Agarose gel using a gel electrophoresis system. The bands generated by the SSR primers were scored. A dendrogram based on this scoring data grouped 26 genotypes into four major clusters X1, X2, X3 and X4.

It was observed that LR 26 and LR 1 are distantly related and show higher level of dissimilarity. Thus hybridization between genotypes under the highly divergent clusters should result in maximum hybrid vigour and highest numbers of useful segregants for the trait studied.
It inferred that Karnataka landraces have diverse genetic bases and can be utilized in future breeding programs.

CONCLUSION:

To conclude, we could observe substantial genetic diversity in an under exploited set of landraces, in spite of using a limited number of genotypes and markers. Genetic diversity among 26 rice accessions (landraces) was determined using 11 SSR markers. The results reveal that Karnataka landraces have a great genetic diversity. SSR markers provided very useful data to exploit landraces for future research on rice breeding. LR 1 and LR 26 show higher level of dissimilarity and hence can be used as a donor lines for the particular traits.

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