Molecular characterization in lowland and medium land rice.

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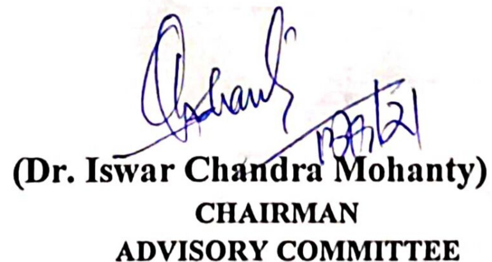

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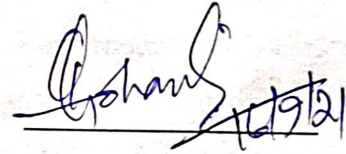

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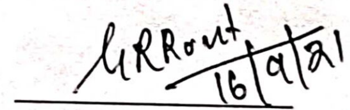

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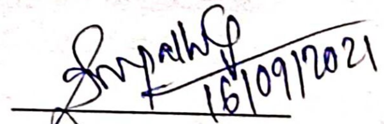

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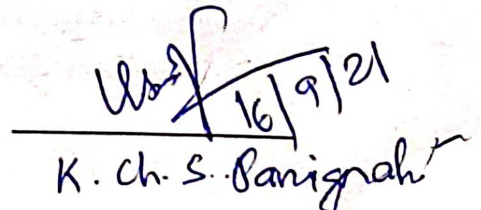

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ABBREVIATIONS

Fe	Ferrous
µg	Micro gram
%	Percentage
µl	Micro litre
µM	Micro mole
AFLP	Amplified Fragment Length Polymorphism
AM	Association Mapping
Bp	Base pair
Cm	Centimorgan
CTAB	N-Cetyl-N, N, N-trimethyl ammonium bromide
dNTP	Deoxy nucleotide triphosphate
EDTA	Ethylene Diamine Tetra Acetate
et al.,	*et alia* (Latin : and others)
EtBr	Ethidium Bromide
ISSR	Inter Simple Sequence Repeat
RFLP	Restriction Fragment Length Polymorphisms
AFLP	Amplified Fragment Length Polymorphisms
Mg	Milli gram
Min	Minute
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
rpm	Revolution per minute
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TBE	Tris boric acid EDTA buffer
TE	Tris EDTA
STS	Sequenced Tagged Sites
DUS	Distinctiveness, Uniformity, and Stability
LBI	Leaf Bronzing Index
DNA	Deoxyribo Nucleic Acid

ABSTRACT

A staple food in one third of the world's population is Rice, *Oryza sativa* (2n = 24) of the Poaceae Family and Oryzoidea Sub-family, and it occupies nearly one-fifth of the total area under cereals. Indian agroecology has various rice genotypes that value both traditional genotypes and strive towards the acceptance of modern genotypes that are high in yield, stress-solid and climate resilient. It is very important for agro-policies to be planned, assessed and replaced by efficient and conducive genotypes across a range of agro-ecologies. There exists huge germplasm in rice with rich diversity which forms great reservoir of agronomically important genes those need to be characterized to identify suitable donors for introgression breeding. Molecular markers have demonstrated to be powerful tools to evaluate genetic variation and to clarify genetic interactions between and within species. A number of molecular markers, including RFLP, RAPD, SSR, AFLP and SNP, are currently available to evaluate molecular variability and diversity. This study aims to accurately assess the phenotyping and molecular characterization of low land rice genotypes, that includes land races, obsolete variety and high yielding genotypes using SSR-marker for DNA fingerprinting. The morphological characteristics of all current and promising species may not be sufficient to discriminate and identify the inherent diversity present among them. DNA markers currently available, with their codominant segregation and ability to repeatedly, accurately and effectively detect large amounts of discrete allles, microsatellite(SSRs) are seen to be the markers of choice for varietal identification. Besides, DNA fingerprinting is crucial to protect the genotypes from biopiracy. The molecular characterization of released genotypes shall provide sufficient knowledge on diversity among them at molecular level. Cluster analysis shall facilitate to visualise genetic relationship among the elite breeding lines. In this study the morphological characters of prominent local lowland rice germplasms like number of tillers, Plant height, Panicle length, Number of grains per panicle, 1000 grains weight, Days to 50% flowering, Days to 50% panicle initiation, leaf bronzing index & Yield q/ha are used for analysis. A phylogenetic tree is obtained for cluster analysis and classification of distinct diverse traits among the selected 60 genotypes of local lowland and medium land rice. This study precisely analyses the DNA profile of these genotypes using 5 screened primers for molecular characterisation. This information shall act as a crucial fulcrum in background selection during backcross breeding programme. Cataloguing of a panel of 60 germplasm adaptable to low and medium land rice habitat prone to iron toxicity using morpho-molecular attributes has been done to provide a portfolio information on notified varieties and promising landraces for crop improvement, utilization and conservation by various stakeholders like breeders, farmers, researchers and field staff.

INTRODUCTION

Rice is a member of the Gramineae family *(Oryza sativa* L.). The main staple food crop, which is cultivated across various countries and feeds over half of the global population, is Rice (*Oryza Sativa* L.). India is the world's second biggest rice producer & consumer with a 22.4 percent share of global output, with a production of 118.87 million tonnes in 2019-20 and a productivity of 2578 kg per ha over 43.8 million hectares. As per the government data rice production is pegged at a record 121.46 million tonne in 2020-21. Seeds are the most significant of all agricultural inputs and ascertain the performance and effectiveness of other inputs to improve productivity and production and thus farmers' incomes. Quality seeds with strong genetic potential suitable to the agro-climatic conditions are therefore essential to be produced in sufficient quantities at an affordable price and to be provided for farmers in time for greater production.

Rice has been discovered to be quite simple to digest. It's low in fat, cholesterol, and saturated fat, but high in starch and nutritional value. Rice is a good source of energy as well. It has a carbohydrate content of 77.5 percent. Carbohydrate is one of the two primary fuel sources for the human body. The second source of energy is fat. Aside from being a good source of nutrients, Rice is a good source of thiamine, riboflavin, and niacin, as well as energy. Rice that hasn't been processed contains a substance called a substantial amount of dietary fibre Rice's amino acid profile reveals that it contains a lot of them. The limiting amino acids are glutamic and aspartic acid, while lysine is the most abundant.

There are twenty-one wild and two cultivated species in the *Oryza* genus to which rice is grown. Nine of the wildlife are tetraploid, the rest diploid. O. sativa is a variable species, more frequent because of high yield and improved grains quality. It has a global distribution, it originated in the moist regions of South and South East Asia. The two rice species are *O. sativa* (common rice) and *O. glaberrima* (Red African rice). In many characters, Indica and Japonica are different in typical genotypes but overlap variations.. Rice is a staple food for approximately 65% of people living in India. For life in India, rice is crucial. It belongs to almost every meal and is produced in most rural farms. It is the largest staple grain in the world, particularly in Eastern and South Asia, the Middle East, South America and West India, as a cereal grain. There

are many rice variants; rice is mainly long, medium and short grain for many purposes. Long grain rice (high amylose) are usually kept intact after cooking; medium grain rice (high amylopectin) is stickier.

A huge number of genotypes / hybrids have been developed to meet the various agro-climatic conditions of India through coordinated efforts and reasonable plant breeding programmes of ICAR and State Agricultural University. By adding variants to the national list each year, data generation is immediately needed that can distinguish between one cultivar and another (Faccioli et al., 1995). Increasing numbers of better genotypes are leading to a narrow genetic base, as phenotypic descriptors are limited when too closely related cultivars have to be identified from each other.

A tiny quantity of seed, known as nucleus seed, is accessible with the plant breeder at the moment of release, and commercial quantities of seed are created following a number of multiplication processes. Plant breeders, farmers, certification organisations, seed testing laboratories, and the seed industry need to know the specific morphological properties of the variety for identification at different growth stages of the crop during the seed multiplication process. The pedigree of superior crop genotypes is preserved through consecutive stages of controlled seed production in corporate seed production and certification, which is based on a generation system. A well-organized system can be thought of as a protector of the pure seed supply, which necessitates the use of criteria that allow genotypes to be recognized and their genetic purity to be precisely evaluated. As a result, having a reliable method to distinguish between cultivars and evaluate the genetic purity of seed samples will allow seed producers to monitor and manage satisfactory levels of genetic purity at every generation of seed production and multiplication, ensuring that farmers receive high-quality seeds. Furthermore, unmistakable identification of elite crop types and hybrids is required for their protection and to avoid illicit commercial use. This is particularly true in rice in India, where the private sector dominates seed manufacture and sale of public-bred cultivars.

Furthermore, having in mind that the “Protection of Plant Genotypes and Farmers Rights Act - 2001,” which establishes an effective framework for protecting plant genotypes, farmers' and plant breeders' privileges, and encouraging the production of new plant genotypes, will be in force very soon. Distinctness, Uniformity, and Stability (DUS) of newly produced genotypes will be critical for their licensing under

the Act. DUS testing is currently under progress using crucial morphological and biochemical markers.

Domestication & Cultivation of Rice: Brief History

Rice first was domesticated and cultivated along the Yangtze River valley in China, according to popular belief. The shift from wild rice harvest to domesticated rice production is readily seen in morphological examinations of rice phytoliths from the Diaotonghuan ancient excavation. The presence of a considerable amount of wild rice phytoliths at the Diaotonghuan level, dating from 12,000–11,000 CA, suggests that wild rice gathering was an important part of the local subsistence strategy. Rice had been domesticated by 10,000–8,000 CA, according to alterations in the morphology of Diaotonghuan phytoliths. Soon after, Central China began to develop the two principal kinds of indica and Japonica rice. Rice farming spread rapidly over mainland Southeast Asia and westwards across India and Nepal in the late third millennium BC.

Rice is referenced for the first time in the Yajur Veda and then repeatedly in Sanskrit writings. Rice grains, it is said in India, need to be like two brothers, connected but not glued together. Because rice is generally connected with affluence and fertility, it is customary to throw rice at ceremonies. China, India, Indonesia, Pakistan, Bangladesh, Vietnam, Thailand, Myanmar, the Philippines, and Japan now source the majority of the world's rice. Agriculturalists in Asia still produce 92 percent of the globe's rice. Thai fragrant rice, Basmati, Patna rice, Vietnamese fragrant rice, and a hybrid cultivar from America sold under the brand names Texmati are quite well aromatic rice cultivars. The aroma and flavour of Basmati and Texmati are similar to that of popcorn. In April 2002, draught genomes for the two most popular rice genotypes, indica and japonica, were released. Because of its short genome (430 megabase pairs), rice was picked as a model organism for grass biology research. Rice was the first crop to have its genome sequenced in its entirety.

The United Nations General Assembly designated 2004 to be the International Year of Rice on December 16, 2002. And over 40 countries signed on to the proclamation.

High Yielding Genotypes of Rice

The High Yielding Genotypes are a collection of crops developed specifically for increasing agriculture production during the Green Revolution. Rice, like corn and wheat, has been genetically modified to boost yields. As a result of this endeavour,

Asian labour markets have shifted away from agricultural and toward industrial industries. In 1966, the International Rice Research Institute in Los Banos, Philippines, created the first "Rice Car," the IR8. IR8 was developed by crossing an Indonesian variety known as "Peta" with a Chinese variety known as "Dee Geo Woo Gen."

Because rice kernels are devoid of vitamin A, those who eat rice for the majority of their calories are at danger of vitamin A insufficiency. Rice has been genetically modified by German and Swiss researchers to manufacture beta-carotene, a precursor of vitamin A, in the rice kernel. The beta-carotene in the processed (white) rice gives it the colour "gold," hence the name "golden rice." In humans who ingest rice, beta-carotene is transformed to vitamin A. Several of rice strains have been tested, and while some generate beta-carotene in the hull, no non-genetically engineered strains have been discovered to produce beta-carotene in the kernel. Subsequent efforts are being made to add the quantity and quality of other nutrients in golden rice.

Future prospectus

Molecular marker-based technologies have transformed crop plant genetic study, and their application can aid in genotype individualization and variety recognition. For exact identification and legal protection against unlawful commercial use, molecular fingerprinting is essential. Plant breeding would become more competitive, and public-private partnerships would flourish. With the establishment of plant variety protection rights and export under WTO regulation, full characterisation of high-quality rice germplasm is becoming more important, combining existing morphological descriptors with repeatable DNA-based marker profiles. The introduction of molecular marker technology has made it possible to quantify the type and degree of genetic variation as well as identify cultivars.

The ability to recognise genetic variation in plants is essential for breeding success. For optimal use of plant genetic resources in crop development, unambiguous, reliable, rapid, and cost-effective identification of genetic diversity in plant genotypes, breeding lines, and accessions is required. Traditional morphological and biochemical indicators have been proven to be insufficiently discriminatory, necessitating the employment of more precise approaches. Furthermore, because many of the traits of interest have poor heritability and are genetically complicated, these markers are unreliable.

It has long been known that genetic diversity is important in promoting plant breeding and conservation methods (Sehgal and Raina., 2008). Molecular markers are effective tools for detecting genetic variation and for linking phenotypic and genotypic variation (Varshney et al., 2005). Advancement in the creation of DNA-based marker systems has increased our understanding of genetic resources in latest years. These molecular markers are divided into three categories: I hybridization-based markers, such as restriction fragment length polymorphisms (RFLPs), and (ii) PCR-based markers, such as random amplification of polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) (AFLPs). Inter simple sequence repeats (ISSRs) and microsatellites or simple sequence repeats (SSRs), and (iii) sequence based markers i.e. single nucleotide polymorphism (SNPs) (Varshney *et al*., 2007; Sehgal and Raina., 2008). The most of these molecular markers were created using either a genomic DNA library (RFLPs or SSRs) or random PCR amplification of genomic DNA (RAPDs) or both (AFLPs) (Varshney et al., 2007). Owing to the presence of a variety of molecular marker techniques and their alterations, comparative studies in a variety of crops, including soybean, wheat, and barley, have been conducted (Powell et al., 1996).

SSR markers, in particular, have become increasingly important in plant genetics and breeding due to a number of desirable characteristics, including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage (including organeller genomes), chromosome particular location, automation, and high bandwidth genotyping (Parida et al., 2009). AT repeats are abundant in plants, but the AC repeat is the most prevalent in animals. This seems to be the general trait that distinguishes the genomes of plants and animals (Powell et al., 1996). SSRs are found in both coding and noncoding sections of the nuclear genome and are widely dispersed. The chloroplastic (Chung et al., 2006) and mitochondrial (Soranzo et al., 1999; Rajendrakumar et al., 2007) genomes both contain them. Because of the varied amount of repeats in the microsatellite areas, the high length polymorphism may be easily and reliably discovered using polymerase chain reaction (PCR).

In evaluating the genetic purity of crop types, PCR-based molecular markers are particularly rapid, consistent, and potent. According to Ravi et al. (2003), SSR marker analysis, which is centred on the availability of over 2500 SSR loci spanning

the entire rice genome (Temnykh et al., 2000; McCouch et al., 2002), can continue to be an effective method for marker-based varietal profiling and purity assessment in rice in the coming years. DNA Fingerprint refers to an identifiable specific pattern of cultivars produced by the use of DNA markers. Fingerprinting using molecular markers enables for exact, objective, and speedy cultivar identification, and has been shown to be an effective approach for agricultural germplasm characterisation and management (Chakravarthi and Naravaneni, 2006) and has specific edge over biochemical and morphological methods. Because of their abundance, co-dominant inheritance, high polymorphism, repeatability, and ease of assay by polymerase chain reaction (PCR), simple sequence repeat (SSR) markers have been widely utilised for genetic study and cultivar recognition (Kuleung et al., 2004). Microsatellite-based DNA fingerprinting of rice cultivars yielded useful information, which can be supplemented with other microsatellite markers. The information gathered can be utilised to safeguard plant genetic resources. For speedy and accurate cultivar identification, it is critical to create a fingerprinting database of the most common commercial cultivars in the market (Zhu et al., 2012).

These markers are suitable to high-throughput genotyping and have established to be a helpful tool for paternity testing, high-density genome mapping, gene mapping, marker-assisted selection, and identifying genetic and evolutionary relationships. For fully sequenced plant genomes like rice and Arabidopsis thaliana, a substantial number of microsatellite markers are now accessible.

Although there has been a lot of work done on rice using molecular techniques, there has been very little work done on studying molecular characterisation in lowland and medium land local rice genotypes. As a result, the following objectives guided the current investigation:

1. Phenotyping of low land and medium land rice variety.
2. Molecular characterization using SSR marker.
3. DNA Fingerprinting.

REVIEW OF LITERTURE

Rice (Oryza sativa L.) is a vital staple food crop that feeds the world's population. The genetic diversity of global rice germplasm is very high when compared to many other crop species. Three subspecies of rice, indica, japonica, and javanica, make up a significant reservoir of germplasm that includes local landraces and genotypes (Khush, 1997; Lu et al., 2005; Garris et al., 2005). Rice is farmed on around 154 million hectares yearly, yielding 600 million tonnes, and contributes for 35 percent to 75 percent of the total calories consumed by more than 3 billion Asians (Khush, 2005). Rice genotypes with higher yield potential and greater yield stability must be developed to supply an expected 5 billion rice eaters by 2025. (Khush, 2005). Rice is a staple food crop in many parts of the world. Rice is the primary source of calories for more than 90% of Asia's population. Rice demand has risen dramatically as a result of global population growth.

The rise in world population and the scarcity of agricultural resources compelled the search for other resources to counteract these trends. Developing countries are confronted with the task of rapidly increasing agricultural production and feeding their rapidly growing populations based on genetic potential. Rice is one of the most significant staple crops in most parts of the world, providing calories to 50 to 80 percent of the population (Khush, 2003).

In rice-genotype improvement over the last decades, conventional breeding has played an essential role. However, progress has been gradual and has been hampered by a number of obstacles, including the time-consuming nature of the selection process and difficulty in selecting optimal genotypes. Because most agronomic parameters are quantitative, breeders have been urged to use biotechnological techniques and molecular markers in rice breeding, which is known as molecular breeding. There are various types of molecular marker which may differ in costs, installations, reliability and individual differences (Schlötterer, 2004; Schulman, 2007).

Molecular germplasm study would provide knowledge about the genetic variance levels in and between species for plant enhancement programmes. This would be used in order to select diverse parents of the same species or to identify the closest interspecies crossing parents, enhance heterosis, and integrate desirable genes in the

finest germplasm, from the more diverse backgrounds (Henry, 1997). The maximum genetic performance potential refers to the phenotypic variation present in the original population and kept in the following selection cycles. Phenotypic differences are directly linked to genetic diversity even if they interact with environmental factors (Moose and Mumm, 2008).

For a very great many years, the characterization and quantification of genetic diversity in closely related plant germplasm has been an important objective for a proportioned application of genetic resources. A primary interest for breeders is the experiment of the genetic differences in nursery resources. This information is required for germplasm selection and supervising and to prevail genetic advantages (Chakravarthy and Rambabu, 2006). The environmental influence also exists on markers based on expressed genetic products, proteins and isozymes, which show low polymorphism and low abundance **(**Ravi et al., 2003). In contrast, molecular markers based on DNA have demonstrated that they are potent tools for evaluating genetic variation. Many unexplored environmental factors characterised the elaboration of genetic relationships within and among species (Powell et al., 1996). Ravi et al. (2003) reported that only a few primers covering all 12 chromosomes would be possible to produce a unique simple repetitive sequence (SSR) profile within the rice genotype. The microsatellites are repeated 1 to 6 bp motifs that occur frequently in all prokaryotic and eukaryotic genomes analysed until now (Zane et al., 2002).

Micro-satellite existence was validated in several eukaryotes by Hamada et al. (1982) and the most micro- satellites in plants have been confirmed by Tautz and Renz (1984). SSRs are present and are spread across the nuclear genome in both coding and non-coding areas (Kalia et al., 2011). This can also be found in the mitochondrial genome (Rijendrakuma et al., 2007) and chloroplastitic genomes (Provan et al., 2001; Chung et al., 2006). Due to the various numbers of repeats in microsatellite areas, SSR shows high polymorphisms of length (Zane et al. 2002). They can thus be readily detected through a polymerase chain reaction (PCR).Microsatellites have high throughput genotyping thus have assured to be an extremely significant tool for establishment of genetic relationships (Parida et al., 2009; Kalia et al., 2011).

Assessment of genetic diversity

For successful use of plant genetic resources, unambiguous, reliable, rapid, and cost-effective identification of genetic diversity in crop plants, breeding lines, and accessions is required. Several markers have been employed in the past to measure the genetic diversity of rice germplasm.

Biochemical markers

Proteins generated by gene expression are known as biochemical markers. Isozymes have been successfully exploited as biochemical markers in various elements of plant breeding and genetics in current history.

When particularly in comparison to morphological indicators, biochemical markers provide a lot of variety. Gleszmann used a multi variate approach to assess allelic frequencies at 15 isozyme loci in 1688 rice cultivars from various Asian nations. The results revealed that 95 percent of the cultivars are divided into six groups, with the remaining 5% dispersed over intermediate places. The Indica was in Group 1 and the Japonica was in Group 6. Aromatic rices are classified as Group 5.

Isozyme markers have been used to analyse genetic variation, but because they only cover a small fraction of the genome, they are unsuitable for practical breeding (Second, 1982). The restricted number of isozymes present in the germplasm is one of its drawbacks. As in the context of maize and tomato, the best defined genomes, only 30 to 40 loci may be mapped in a genome. Another drawback of isozyme markers is that they are also regarded phenotypic markers because the assay is for the gene product rather than the gene itself. As a result, when the expression of a specific isozyme is developmental or dependent on external inputs, this can pose issues.

Morphological markers

The qualitative trait that may be rated visually usually corresponds to morphological indicators. They've been discovered in nature or as a consequence of mutagenesis research.

For assessing diversity, morphological indicators, both qualitative and quantitative, have been used. In *Oryza sativa*, Chang (1976) and Takahashi (1984) identified two eco-species: Japonica and Indica. Oka (1953) used morphological and phonological

studies to separate these two eco-species genetically. Genetic diversity has long been studied using morphological indicators. However, the low number of morphological features is a significant drawback. As a result, various plant genotypes are sometimes difficult to discern. Because the genetic basis of many of these morphological variation is unidentified, these markers could not be demonstrated to be useful for genetic differentiation (Bachmann, 1983). Furthermore, morphological diversity estimates are less trustworthy due to stage-specific expression of characteristics and the effects of external environment.

Introduction to DNA Markers

Instantaneous instances of a single population with two or more genotypes are characterised as genetic polymorphism. Genetic polymorphism can be used as markers for the tagging of traits, chromosomes and/or DNA fragments at morphological, cytological and molecular levels. Genetic markers in linking analysis, gene mapping, gene transmission, and selection aids have been used for several décades. Several different kinds of markers, such as morphological variations, protein polymorphisms, like isozyme loci variation, and DNA polymorphisms, have been produced. Since the very first genetic map was drawn using a restricting fragment length polymorphism (RFLP), the DNA based markers received the most attention (Botstein et al., 1980) DNA markers mirror DNA genetic polymorphism resulting from potential nucleotide dissimilarities. DNA markers have nearly no practical limitation of number, often no direct phenotypic effect, nor are they affected by the environment, than other types of genetic markers.

DNA sequencing is costly and careful, but a straightforward approach to explore variations at a locus. Many techniques for the visualisation of DNA polymorphism have been devised over the last few years. DNA fingerprint characterises bar code such as multilocus sample fragmentation DNA patterns generated after electrophoretic segregation of genomic fragments of DNA (Jeffrey et al., 1985). The term fingerprinting and profiling for DNA has been used to explain combined application of various single locus detection systems and has been used to research various aspects of plant genomes as an inventive tool. These include genetic variability characterization, fingerprinting and genome location, genetic analysis,

population genetics, taxonomy, plant breeding, and diagnostics. The use of DNA-based markers to identify and authenticate plant genomes has grown increasingly prevalent.

Desirable Characteristics of Ideal DNA Markers :

- Affordable & the assay is simple and quick.
- Simple, quick, and low-cost
- Extremely polymorphic and repeatable
- Throughout the genome in an even distribution
- Ensure that genetic discrepancies are adequately resolved.
- Obtain multiple, independent, and dependable markers.
- Small amounts of tissue and DNA samples are required.
- Link to various phenotype.
- Recurring occurrence in genome and codominant inheritance
- Environmental circumstances or management techniques are selectively neutral.
- No prior knowledge of an organism's genome is required organism
- Data communication across laboratories should be simple.

However, no molecular marker can provide all of the benefits indicated.

Hybridization-based markers and polymerase chain reaction (PCR)-based markers are two types of molecular markers that are used to assess DNA polymorphism. In hybridization-based markers, DNA profiles are visualised by hybridising a restriction endonuclease digested DNA fragment to a labelled probe, which is a DNA fragment of known sequence, whereas in PCR-based markers, specific or arbitrarily selected oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme are used to amplify specific DNA sequences in vitro. The use of PCR in research and clinical laboratories has skyrocketed since the invention of thermostable DNA polymerase. The PCR method is exceedingly sensitive and operates at a high rate. Its various applications have opened up a plethora of new possibilities in the realm of molecular biology (Saiki et al., 1985; Saiki et al., 1988). The efficacy of various classes of PCR-based markers has recently been employed to describe barley and rice genotypes (Asker et al., 2005 and Irk et al., 2000).

Simple Sequence Repeats (SSR)

Microsatellites, also known as SSRs, are found in abundance in eucalyptus. Polymorphism depending on the number of repeat units in a specific area of the genome is known as SSR polymorphism (SSRP) (Jacob et al., 1991).

Plants and animals have different numbers and types of microsatellite repeats. Plants have a frequency of repeats longer than 20 bp reported to be every 33 kb, whereas mammals have a frequency of per 6 kb. AG or TC is a relatively standard repeat unit in humans, although AT is more frequent in plants, followed by AG or TC, however plants have around 10 times less SSRs than people. Nucleotide sequence flanking the repeat helps to construct the primers to amplify the various number of repeat units in distinct genotypes. This polymorphism is extremely reproducible. These primers are particularly suitable for analyzing polymorphic loci quickly and accurately, and the findings might be utilised to create a physical map using these sequence tags. Unfortunately, few primer sequences for identifying SSRs in plants have been produced.

Inter-Simple Sequence Repeats (ISSR)

ISSR-PCR solves several of the drawbacks of conventional DNA-based markers, like RAPD's low reproducibility, AFLP's huge costs, and the necessity to know flanking sequences in order to create particular primers for SSR evaluation (Zietkiewicz et al., 1994; Gupta et al., 1994).

Microsatellites, which are typically 16-25 bp in length, are used as primers in an one PCR reaction that targets numerous genomic loci to amplify inter-SSR sequences of various sizes. ISSR markers' amplified products are typically 200-2000 bp long and detectable on both agarose and polyacrylamide gel electrophoresis.

ISSR polymorphism is beneficial in a variety of crop species for genetic diversity, phylogenetic investigations, genome mapping, and evolutionary biology (Reddy et al., 2002). Chen et al. used ISSR and RAPD markers to identify the microspore origination of another culture-derived flax plant (1998). Polymorphic fragments in between two parents of F1 donor plants were found, and their segregation patterns in anther culture generated plants have been used to assess the source of those plants and the degree of independence of plants reproduced from the same callus. By using 1 ISSR primer

(UBC 889) and 2 RAPD primers (UBC 556 and 561), 12 out of 16 plants were unequivocally recognised as being obtained from microspores.

DNA Fingerprinting of Rice Germplasm

DNA fingerprinting, also known as profiling, is a well-known method of determining genuineness. It has emerged as a simple, yet appealing technique for addressing paternity issues, confirming quality criteria in plant biotech companies, describing crop genotypes, micro organisms, preserving breeders rights, and other uses in plant sciences, in addition to its forensic cases. It necessitates solutions able to reduce a genome's immense complexity by emphasising only just few polymorphic DNA sequences. With the advent of hyper dense genetic and molecular linkage maps in rice, now it is possible to select locus specific, extremely polymorphic, and co-dominant markers such as microsatellites to conduct more structured diversification and fingerprinting studies. This not only aids in the correct calculation of genetic similarity, but it also allows researchers to investigate the link between rice genotypes. Rice is among the few crops with a wide genetic range.

To utilise the hypervariable character of microsatellites for uses in plant genomics, a variety of hybridization-based and PCR-based approaches have been initiated. Even though there is no distinction in the length of the microsatellites carried by these fragments, the polymorphism found by this technique represents polymorphism due to variation in the width of restriction fragments holding the microsatellites. This method yielded high levels of variation between close genotypes, which has proven effective in paternity testing, genotype recognition, and population genetics (Weising et al., 1995).

Rice germplasms contain a wide pool of genetic variety. The amount of genetic variety in germplasm collections is crucial for selecting different genotypes for agricultural genetic development. PCR-based marker approaches such as RAPD, SSR (Microsatellite), ISSR, and AFLP have been used to investigate rice genetic variation.

Beverley et al. (1997) investigated Oryza sativa L. genetic variation. RAPD and ISSR-PCR, two PCR-based molecular marker methods, were used to collect data from 19 different locations. Using RAPD, a set of 14 arbitrary sequence deca nucleotides guided the production of 94 reproducible marker bands, 47 (50%) of which were polymorphic. Furthermore, a set of 9 ISSR primers guided the amplification of 71 PCR products, 40 (56%) of whom were polymorphic. Due to subtle discrepancies in the linkages

exhibited between rice groups utilising the two types of PCR-based markers, an intraspecific doubled haploid mapping population was used to investigate their map placements. The findings that the chromosomal locations of markers can have effect on diversity assessments is shown and the significance of this is described.

Blair et al. (1999) used 32 primers carrying distinct simple sequence repeat (SSR) motifs to study a total of 59 types, typical of the variety of cultivated rice (*Oryza sativa* L.). The ISSR findings indicated that the poly (GA) motif was much more frequent than the poly(GT) motif inside the di-nucleotide class, and that the frequency and clustering of certain tri- and tetra-nucleotide simple sequence repeats was diverse and motif-specific. Additionally, tri-nucleotide ISSR markers were shown to be lesser polymorphic than di-nucleotide or certain tetra-nucleotide ISSR markers, indicating which motifs would develop decent microsatellite marker targets.

Virk et al. (2000) found that using a significant amount of markers from four molecular marker systems (AFLP, isozymes, ISSR, and RAPD) for unveiling genetic diversity and distinguishing between infra-specific groups of Oryza sativa L. germplasm revealed genetic diversity and discriminated between infra-specific groups. When other markers were utilised, meanwhile, there was only limited agreement on relationships between individual accessions at the greatest levels of genetic similarity. Although the disparities were not substantial, when variance was divided among and within the three subspecific groups, significant variation was discovered among than within groups using AFLP and isozymes, with the opposite being true for RAPD and ISSR. Polymorphism was measured using average heterozygozygosity and optimal number of alleles, and the results were similar for each marker system.

Inter simple sequence repeat (ISSR) polymorphism was used by Joshi et al. (2000) to investigate genetic variation and evolutionary relationships in Oryza. To measure genetic variation, a total of 30 ISSR primers were examined, with 11 polymorphic and meaningful patterns chosen. ISSR research reveals that the Oryza genus developed along a polyphyletic pathway, with Oryza brachyantha (FF genome) being the most divergent species in the genus, and Oryza australiensis (EE genome) not belonging to the Officinalis complex. ISSR also identified 87 possible genome/species-specific molecular markers in eight of Oryza's nine genomes.

Non-anchored inter simple sequence repeats (ISSRs) are unpredictable multiloci markers created by PCR amplification with just a microsatellite primer, according to Bornet and Branchard (2001). Polymorphisms were found in abundance in seven dicot species when two tri-nucleotide and two tetra-nucleotide primers were used. As a result, non-anchored ISSR markers are ideal for DNA fingerprinting.

Nagaraju et al. (2002) investigated three rice groups using 19 simple sequence repeat (SSR) loci and 12 inter-SSR-PCR primers. In 24 variations from the three groups, a total of 70 SSR alleles and 481 inter-SSR-PCR markers were discovered. Traditional Basmati varietals were found to have the least genetic diversity. According to the findings, the fragrant rice types studied in this study are most likely descended from a single land race. Both marker assays clearly distinguished the classic Basmati (TB) and semi-dwarf NB rice cultivars employed in this investigation. These markers have the potential to be employed in Basmati rice breeding projects and to authenticate the traditional Basmati types used in this study.

Using ISSR- and SSR-PCR markers, Sarla et al. (2003) investigated the genetic diversity of 24 O. nivara accessions from 11 Indian states and four O. sativa types, one from each of Glaszmann's isozyme groups I, II, V, and VI. A accession had ten alleles out of 40 amplified at six loci that were unique to it. Jaya, Dular, Basmati 370, and Taipei 309 all have O. nivara alleles in common. That the use of informative primers reduces the amount of time, money, and effort required for phylogeny and germplasm management research.

Siwach et al. (2004) investigated allelic diversity and created a DNA fingerprint database of 24 rice genotypes, including three higher price traditional Basmati rice genotypes, nine cross-bred Basmati rice genotypes, a local scented selection, eight indica rice genotypes, and three japonica rice genotypes. At the 50 SSR loci, a total of 229 alleles were found, with 49 alleles occurring in only one of the 24 types. Traditional and cross-bred Basmati rice genotypes amplified different alleles than indica and/or japonica rice genotypes at 15 of the SSR loci. The three traditional Basmati rice genotypes are closely related and have varying degrees of similarity with other cross-bred Basmati rice genotypes, according to genetic relationships among rice genotypes determined by UPGMA cluster analysis and three-dimensional scaling based on Principal Component Analysis.

AFLP, ISSR, and SSR markers of genetic diversity and patterns of connections were utilised by Saini et al. (2004) to study the 18 Basmati and non-Basmati rice genotypes. Using five AFLP primer combinations, 25 UBC ISSR primers, and 30 widely dispersed, mapped SSR markers, a total of 171 (110 polymorphic), 240 (188 polymorphic), and 160 (159 polymorphic) bands were found, respectively. When employing the SSR data-set, AMOVA revealed more variation between groups than within groups, but the reverse was true for both ISSR and AFLP data-sets. For a comprehensive genetic investigation of Basmati rice germplasm, the study underlines the importance of using a mix of diverse marker systems.

SSR markers were employed by Jain et al. (2004) to investigate genetic variation in 69 rice genotypes. At the 30 simple sequence repeat (SSR) loci, a total of 235 alleles were found, with 62 (26.4%) of those found solely in Basmati and other scented/quality rice germplasm accessions. Twenty of the 30 SSR markers might be used to separate traditional Basmati rice genotypes, whereas a single panel of eight markers could be utilised to distinguish premium traditional Basmati, cross-bred Basmati, and non-Basmati rice types with differing commercial value in the marketplace.

For SSR assessment, Ye-yun et al. (2005) employed super hybrid rice combinations and their parental lines. A total of 144 SSR primer pairs were employed across 12 rice chromosomes, with 47 of them detecting polymorphism in the rice lines examined. In addition, five primer pairs were chosen as special primer pairings for each of the five hybrid rice combinations. SSR technology was indicated to have vast potential in variety authentication and purity determination by combining the speedy, easy method of DNA extraction.

Rota et al. (2005) used linkage maps premised on cDNA-RFLP markers to compare rice (Oryza sativa L.), barley (Hordeum vulgare L.), and wheat (Triticum aestivum L.) genomes. The low figure of polymorphic RFLP markers has hampered the construction of extensive wheat genetic maps and the numbers of anchor points in comparative maps. The objectives of the study were to determine in-silico rice genome locations for primer sets evolved for wheat and barley Expressed Sequence Tags, as well as to characterise the proportion of transcribed DNA sequences comprising simple sequence repeats (SSR or microsatellites) by magnitude and motif for wheat, barley, and rice.

SSR was utilised by LI Ya-li et al. (2006) to identify two subspecies (Indica and Japonica) of farmed rice (Oryza sativa L.) using 19 pairs of primers (simple sequence repeat). 17 of the 19 pairs of primers (89.47%) could only amplify one type of band type across all individuals, however primers RM251 and RM18 could amplify polymorphic band types. According to the findings, 84.21 percent of SSR loci in genomic DNA of common wild rice in Yuanjiang revealed indica-japonica differentiation, while 13.79 percent of the loci remained primitive, and the majority of the discovered loci in the natural population were homogenetic.

Using 30 SSR primers on chromosomes 7-12, Chakravarthi and Naravaneni (2006) demonstrated genetic diversity and DNA fingerprinting of 15 elite rice genotypes. The findings demonstrated that all of the primers displayed unique variability among the cultivars examined, demonstrating the power of microsatellites to detect polymorphism. Using 18 different SSR primers, the data from the DNA fingerprinting research enabled to distinguish and define nine variants. Backcross breeding programmes can use this information to make background selections.

Singh et al. (2007) revealed how to identify SSR markers from oil palm genomic sequences using a simple and successful technique. There were a total of 12 informative SSR markers identified. The markers discovered in Elaeis oleifera were shown to be applicable in a second species, Elaeis guineensis. SSR markers have also shown promise as molecular probes for DNA fingerprinting of oil palm tissue culture clones, in addition to genetic mapping. SSR markers can be utilised for clonal identification, line homogeneity monitoring between and among clones, and culture mix-up detection.

Giarrocco et al. (2007) used 26 simple sequence repeat (SSR) markers to examine sixty-nine accessions, indicating the genetic link between different rice cultivars. There were a total of 219 polymorphic bands found. The clusters match the lineage information on the accessions, and the Japonica cluster contains practically all Argentina-released cultivars. Genomic linkages in Argentine rice germplasm were discovered using DNA polymorphism analysis, resulting in a database that can be used for cultivar identification, local germplasm conservation, and breeding initiatives.

RAPD and ISSR–PCR were employed by Bhuyan et al. (2007) to detect genetic diversity in 24 rice genotypes using 10 primers. With 92.5 and 98 percent

polymorphism, a total of 81 RAPD and 201 ISSR markers were created. The averaged polymorphism information content (PIC) for RAPD markers was 0.741, while it was 0.888 for ISSR markers. Depending on RAPD and ISSR analyses, the Jaccards similarity coefficient was 0.480 and 0.257, respectively.

A segregating population was generated from a cross between Y-1281 (high yielding mutant variety) and Kalizira by Kibria et al. (2008) to boost the yield potential of the local aromatic variety Kalizira. In 32 rice lines, nine, twelve, and seventeen pedigree lines (PLs) with the fragrance gene (fgr) locus were discovered using three SSR markers, RM223, RM342A, and RM515, in homozygous condition. In PLs, the marker RM515 discovered the most fgr loci. Fourteen promising lines were found to have fragrance genes, resulting in greater yields, improved agronomic performance, and improved grain quality. These SSR markers could be used in (MAS) and could help identify the fgr locus in different rice genotypes.

Using inter-simple sequence repeat (ISSR) markers, Reddy et al. (2009) discovered genetic relationships among 12 rice genotypes, including 9 drought, flood, and salinity tolerant types. ISSR markers associated with (GA)8YG distinguished the three groups of stress-tolerant genotypes and can be utilised to identify genes/new alleles related with the three abiotic stresses in rice germplasm, according to the findings.

Gao et al. (2009) analysed eight somaclonal mutants produced from seven different cultivars using 120 SSR markers dispersed across all 12 chromosomes of rice. Thirteen SSRs found variations between the bacterial blight resistant mutant HX-3 and its wild-type counterpart Minghui 63. Ten SSRs revealed variations between the purple sheath mutant Z418 and the wild type C418. These findings indicated that some SSR markers in the rice genome may detect more polymorphisms than others. In addition, the DNA alterations of eight mutants were evaluated using a transposon display (TD) of five active rice transposons, Tos17, Karma, mPing, nDart, and dTok.

Girma et al. (2010) used inter simple sequence repeats (ISSRs) as a molecular marker to investigate the genetic diversity of three wild rice genotypes. Using four di-nucleotide and two tetra nucleotide primers, a total of 93 clear and repeatable bands were produced. The trees and PCA clearly identified six unique categories based on origin populations. Shannon's diversity index also confirmed that wild rice populations have more diversity than cultivated rice populations. Furthermore, the Shannon's

diversity was partitioned, revealing that the majority of the variances were seen among populations (63 percent). Similarly, ANOVA shows that there are more genetic differences between populations (72.9) than within populations (P = 0.05). (27.1).

Kanagaraj et al. (2010) used 23 recombinant inbred (RI) lines of IR20/Nootripathu, two Indica ecotypes with exceptional drought response, to investigate SSR markers for markers connected to drought resilience. 1206 rice microsatellite primer pairs were used to screen the parents for polymorphism. Three primers revealed polymorphism between bulks out of 134 SSR polymorphic primers between parents. These three primers were found to co-segregate among the various RI lines that made up the bulks.

Singh et al. (2010) estimated genetic diversity among 20 rice to salt tolerance using simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) markers. SSRs had a polymorphism rate of 1.1 percent, but ISSRs had a polymorphism rate of 90.7 percent. SSRs had a mean genetic similarity of 0.88 while ISSRs had a mean genetic similarity of 0.85. Estimates of ISSR genetic similarity of 20 rice cultivars based on 39 polymorphic markers ranged from 0.55 for PR108/CSR19 to 0.94 for Pokkali/CSR20, with an average of 0.81. By involving parents from several molecular clusters, the discoveries are anticipated to speed up the breeding of novel salt resistant cultivars.

Youssef et al. (2010) employed RAPD-PCR and inter simple sequence repeats (ISSRs) markers to identify genetic variation among 6 novel rice lines and 4 cultivars with varied drought tolerance responses and to identify particular DNA markers linked with drought tolerance. The dendrogram divided all cultivars and new lines into two clusters, with the cross of tolerant line (P-5-3-b) and susceptible cultivar (Giza 172) being proposed as the best cross for drought tolerance studies because it has the lowest similarity value (0.44) and is also grouped in its own cluster. They can be deemed favorable drought tolerant markers because two fragments of around (315 and 505 bp) were seen in the genomic DNA of the drought resistant lines using HP15 primer whereas they were lacking in the susceptible cultivars.

Florence et al. (2010) used ten morpho-agronomic characters to characterise 32 traditional upland rice cultivars in Kihan, Malapatan, Sarangani Province, and assessed the overall genetic diversity pattern of traditional upland rice cultivars based on morpho-agronomic variations that can be subjected to more robust tests using molecular markers.

Wellington et al. (2011) used qualitative and quantitative agromorphological descriptors to characterise 146 upland rice (O. sativa L.) accessions. Polymorphism was found in 12 of the 14 qualitative features studied, whereas significant differences (p 0.05) were found in 11 of the 14 quantitative variables studied.

71 aromatic rice germplasm from IGKV, Raipur, was described by Parikh et al. (2012). Anthocyanin pigmentation, plant habit, and awning feature were used to characterise and group these germplasm. The genetic characteristics for the 10 agronomic qualities revealed that genotypes for fertile spikelets per panicle, spikelet density, spikelet sterility per cent, and hundred seed weight could be chosen.

Anjan and Mishra (2013) investigated the agromorphic characterization and relationship between 20 West Bengal rice landraces using 20 qualitative and 13 quantitative morphological morphological traits and 82 agromorphic descriptors. They found that morphological traits were useful for preliminary crop improvement programme evaluation and can be used to assess genetic diversity among morphologically dif-ferent landraces.

According to DUS test guidelines, Mallick et al. (2013) classified newly released rice variety BKNR-1 as a late maturing semi dwarf, semi-erect rice variety with medium thick stem and medium tillering ability. It has no anthocyanin coloration on its nodes and internodes, light green leaves that are medium broad and medium long with late leaf senescence, and no anthocyanin coloration on the leaf sheath. However, the auricles were a pale purple colour.

Sarawgi et al. (2013) used 29 morphological and eight agronomical parameters to characterise 782 rice germplasm accessions. Except for leaf collar, leaf ligule, and ligule form, most morphological features varied between accessions. For most of the agronomical parameters studied, there was a large level of variation. On the basis of mean values, the top ten accessions for yield ancillary features were found after evaluating 782 accessions for eight quantitative characters.

Sinha et al. (2013) looked into 23 agromorphological characteristics of rice landraces. In 18 of the 23 qualitative qualities studied, polymorphism was discovered. Color of coleoptile, presence of leaf collar, presence of leaf ligule, form of ligule, and presence of secondary branching in panicle were non-polymorphic features. Colorless coleoptile,

leaf collar, leaf ligule, and secondary branching in panicle are all present in 100 percent of the total variations.

Vinita et al. (2013) used 24 different rice microsatellite markers to examine the genetic relatedness of Indian rice cultivars. Various morphological criteria such as number of grains per panicle, thousand grain weight, length and breadth of whole and milled grains, cooking properties such as grain elongation ratio, grain aroma, and alkali spreading value were also assessed.

The landraces were categorised into 26 groups by Krishna and Nidhi et al. (2014), who based their classification on pigmentation on 12 plant sections. Rice germplasm was split into three groups based on plant habit, and three groups were also created based on awning character.

Shrivastava et al. (2015) used DUS test criteria for rice of 37 morphological and agronomical parameters to characterise 30 lines of rice generated from Indica into Japonica derived crosses. Leaf pubescence on the blade surface, culm attitude, and heading time are all traits to look out for. stem length, spikelet density of pubescence of lemma, spikelet colour of stigma, spikelet density of pubescence of lemma panicle: length of main axis, flag leaf attitude of blade (late observation), panicle: curvature of main axis, panicle number per plant, spikelet-color of tip of lemma, lemma and palea colour, panicle awns, panicle: colour of awns, panicle: distribution of awns, panicle secondary branching, panicle: attitude of branches, panicle exertion, panicle exertion, time maturity The genotypes of grain weight, grain length, and grain width exhibited considerable differences in 1000 completely matured grains.

Sinha et al. (2015) evaluated grain morphological features in fifty-five traditional rice cultivars from West Bengal. The examined types had a wide range of grain properties, including grain size and shape, anthocyanin coloration of the lemma-palea and kernel, fragrance presence or absence, and awning features. Extensive genetic variation among these kinds was revealed by the wide range in grain morphological features, which may be used to select parents for plant breeding and the development of new improved genotypes.

Following the Distinctiveness, Uniformity, and Stability test, Kalyan et al. (2017) investigated the characterisation of 35 rice landraces (O. sativa L.) using 29 agro-morphological variables (DUS). On the basis of twenty-nine fundamental features, 22

of the 35 landraces tested were deemed to be distinct. Awns were absent in 29 cultivars, while six cultivars had awns predominantly at the tips. 15 cultivars had semi-erect to spreading culms, two of which were erect to semi-erect and 14 of which were spreading.

On the basis of 31 morphological parameters, Kujur et al. (2017) analysed 47 rice genotypes. Except for coleoptile colour, leaf auricle, leaf anthocyanin coloration of auricles, leaf collar, leaf anthocyanin coloration of collar, leaf ligule, panicle secondary branching, and decorticated grain fragrance, most morphological features varied between accessions. For most of the agronomical parameters studied, there was a large level of variation.

Eight rice genotypes were described for 56 characters by Komala and Gurumurthy (2018). Variation was found in 37 of the 56 characters analysed. Only the genotypes Intan and Asha had anthocyanin coloration in their leaf sheaths, while the remaining six genotypes did not. Intan and Asha had purple coleoptiles, while the other six genotypes had green coleoptiles.

The study by Parthiban et al. (2018) aimed to create and describe descriptors for casuarina genetic resources, which would allow for the identification of genotypes for varietal registration. After assessing the 17 clones based on physical character, descriptors of casuarina genetic resources were created. 15 descriptors, consisting of 7 quantitative and 8 qualitative features, were established and reported for the clone investigated.

Venkataramana et al. (2018) investigated morphological characterization utilising 40 different morphological features, as described by the Distinctiveness, Uniformity, and Stability test (DUS). Six DUS characters, namely the presence of leaf auricles, the presence of leaf collar, the presence of leaf ligule, the shape of leaf ligules, male sterility, and the presence of secondary branching in panicles, showed no variability among the 40 DUS characters used to characterise 399 rice genotypes. Nine characters had the most variability. Maximum variability aids in germplasm selection, and rare classification in morphological features aids in germplasm identification.

Molecular characterization

Markers based on individual variances in DNA sequences identify more polymorphisms than previous approaches, and they represent a new generation of genetic markers (Bostein et al.,1980; Tanksley et al.1989). All of the constraints associated with morphological and biochemical indicators could possibly be addressed by varietal profiling methods that directly use DNA. As the depth of knowledge about plant genomes and technologies for unleashing the information contained within those genomes grows, DNA methods have become an increasingly important future technology for varietal identification, purity testing, and evaluation of genetic diversity among cultivars. For genetic diversity analysis, varietal classification, genetic purity evaluation, and DUS testing, DNA markers are thought to be the best (Weising et al., 1991).

Nagaraju et al. (2002) used 19 simple sequence repeat (SSR) loci and 12 inter-SSR-PCR primers to investigate the potential use of SSR markers in Basmati rice breeding programmes and authentication of traditional basmati genotypes in a subset of three rice groups: Traditional Basmati (TB), Evolved Basmati (EB), and Semidwarf Non-Basmati (NB) rice genotypes. In 24 types from the three groups, a total of 70 SSR alleles and 481 inter SSR PCR markers were discovered. Traditional basmati genotypes had the lowest genetic diversity, whereas developed basmati genotypes had the highest genetic diversity, according to both marker assays.

Using 30 fluorescently labelled rice microsatellite markers, Jain et al. (2004) examined 69 rice genotypes, comprising 52 genotypes of basmati and quality rice germplasm available in India. At the 30 simple sequence repeat (SSR) loci, a total of 235 alleles were found, with 62 (26.4%) of them found solely in basmati and other scented/quality rice germplasm accessions. The average number of alleles per locus was 7.8, and polymorphism information content (PIC) values ranged from 0.2 to 0.9. Only 20 of the 30 SSR markers could tell the difference between typical basmati rice cultivars.

Simple sequence repeat (SSR) marker analysis was used by Siwach et al. (2004) to analyse allelic diversity and create a DNA fingerprint database of 24 rice genotypes. At the 50 SSR loci, a total of 229 alleles were found, with 49 alleles occurring in only one of the 24 types. The average polymorphism information content (PIC) per marker was 0.62, with values ranging from 0.0 (RM 167) to 0.78 (RM 170). The findings suggest

that these primers could be used for genotype identification, purity and adulteration monitoring, and plant variety protection.

Using 30 SSR primers on chromosomes 7-12, Kalyan Chakravarthi et al. (2006) investigated the genetic diversity and DNA fingerprinting of 15 elite rice genotypes. The findings demonstrated that all of the primers displayed unique variability among the cultivars examined, demonstrating the power of microsatellites to detect polymorphism. The rice genotypes were divided into ten classes by cluster analysis, with japonica types DH-1 (Azucena) and Moroborekan clustering apart from indica genotypes. According to this study, utilising microsatellites to measure genetic diversity and linkages, a higher range of similarity values for related cultivars provides greater confidence. Using 18 different RM primers, the information gained from the DNA fingerprinting investigations allows to distinguish and define 9 types. Backcross breeding programmes can use this information to make background selections.

For the characterisation and classification of 21 rice genotypes, Hossain et al. (2007) used a total of 30 microsatellite molecular markers. The number of alleles per locus varied from three to nine (RM 165, RM 219, RM 248, RM 463, RM 470, and RM 517), with an average of 4.53 alleles throughout the 30 loci studied. All 30 loci had polymorphism information content (PIC) values ranging from 0.30 (RM 219) to 0.84 (RM 223). According to PIC values, RM 223 was shown to be the best marker for identifying 21 genotypes. At each site, the frequency of the most common allele ranged from 24% (RM 223 and RM 334) to 81 percent (RM 219).

Through DNA fingerprinting utilising five microsatellite marker loci, Jalaluddin et al. (2007) discovered the genetic diversity of 22 indica rice (Oryza sativa L.) genotypes (RM 1, RM 3, RM 168, RM 226, and OSR 19). For these loci, the number of alleles found in the 25 rice cultivars ranged from 2 to 4, with an average of 2.8. The locus RM 1 had the highest number of alleles (4.0), whereas locus RM 3 and OSR 19 had the lowest number of alleles (2.0).

Nipon et al. (2007) used ISSR PCR using 10 primers to analyse genetic heterogeneity across 24 rice genotypes from Assam. A total of 201 ISSR markers with 98 percent polymorphism were created. ISSR markers had an average polymorphism information content (PIC) of 0.88. According to famer's categorization, cluster analysis and cophenetic correlation values based on ISSR data discretely segregate the accession.

Multiplex primer pairs BF-STS-401 and BF-STS-402 were suggested by Nirmala Rajendran et al. (2007) for testing genetic purity in commercial seed lots of CMS lines and hybrids of rice.

Using microsatellite (SSR) markers scattered across the rice genome, Ram et al. (2007) genotyped 35 rice accessions. The average number of alleles per locus was 4.86, indicating a polymorphism rate of 95.2 percent and a polymorphism information content of 0.707. Landraces, cultivars, and wild relatives were clearly divided into groups using cluster analysis based on microsatellite allelic diversity. Allelic variability of SSR markers was strong enough to classify rice cultivars, landraces, and wild relatives, as well as record the genetic variability found for future use.

Kibria et al. (2009) used simple sequence repeat (SSR) markers to evaluate the genetic variability among aromatic rice genotypes. Three SSR markers connected to the aroma gene (RM 223, RM 342A, and RM 515) yielded 46 bands within genotypes. The number of alleles per locus varied from one to two, with an overall number of effective alleles of 1.78 to 2.49.

Rahman et al. (2009) evaluated the detection and discrimination of 17 HYVs and 17 local rice cultivars, comprising two wild rice cultivars, using three rice SSR markers (RM 11, RM 151, and RM 153) markers. With 6.33 alleles per locus, all markers were proven to be polymorphic. 15 local rice cultivars and 11 HYVs were identified using these three markers. For the BR-11, Badshabhog, and BR-19 cultivars, three variety distinctive alleles, RM 11/147, RM 151/289, and RM 153/178, were discovered.

Molla et al. (2010) identified ten farmed groundnut cultivars in Bangladesh using three SSR markers: PM 36, PM 50, and PM 238. These three SSR primers correctly differentiated all of the cultivars. PM 36/227, PM 50/110, PM 50/116, PM 50/118, PM 50/137, and PM 238/200 were discovered as variety-specific alleles. The three primers yielded a total of 13 alleles ranging in size from 109 to 241 bases. In complement to the breeder's morphological features, the data derived from this research could be used for IPR to safeguard Bangladeshi groundnut genotypes.

Rahman et al. (2010) reported on DNA fingerprinting with 7 SSR markers on 28 local rice types. The loci were polymorphic (P95) in all of the types after PCR amplification. The primer RM 335 with motif (CTT) 20 produced the most alleles (15) and the greatest PIC value (0.909). Gene flow (Nm) values ranged from 0.047 to 0.00 with an

average of 0.02. Genetic differentiation (Fst) values ranged from 0.84 to 1.00 with an average of 0.92. Nei's genetic distance value (D) ranged from 0 to 2.56 across 378 varietal pairs, resulting in a permutation combination of 28 rice genotypes.

Kanupriya et al. (2011) used 23 microsatellite markers to define 9 guava cultivars. The number of alleles per locus ranged from 3 to 12, with an average of 6.39. The markers' polymorphic information content ranged from 0.340 to 0.900, with an average of 0.749. With a mean of 0.824, the anticipated heterozygosity ranged from 0.392 to 0.961. With a mean of 0.143, the probability of identity ranged from 0.031 to 0.487. The overall likelihood of identity was 2.73 10-22.

Yanfang Zhu et al. (2011) used 5 ISSR and 2 sets of fluorescence-labeled TP M13-SSR markers to distinguish 8 wheat cultivars that were closely related. The number of polymorphic bands discovered by each ISSR primer ranged from 3 to 8, with an average of 4.8 per primer, yielding a total of 43 unique reproducible bands. All eight wheat genotypes were identified using the ISSR primer UBC 849. In the eight wheat cultivars, the two sets of fluorescence-labeled TP-M13-SSR markers yielded a total of 29 alleles, with an average of 14.5 per locus. Finally, the 8 closely related wheat cultivars were fingerprinted using two fluorescence-labeled TP-M13-SSR markers.

Deepti Anand et al. (2012) fingerprinted commercially grown Indian rice hybrids using fifty-eight SSR markers. There were forty polymorphic amplifying 121 alleles with molecular weights ranging from 70bp to 280bp among them. In addition, twenty SSR sites yielded hybrid-specific unique alleles that will be beneficial in determining the hybrid's identification.

Hashemi Petroudi et al. (2012) identified variation between eight rice hybrids and their parental lines using fourteen microsatellite markers. Ten of the markers were discovered to be polymorphic. In addition, polymorphic primers were used to detect off-type seeds among F1 hybrids, ensuring genetic purity. As a result, microsatellite markers can be used to reliably profile DNA and evaluate seed quality in rice types, particularly hybrids.

Lakshmana Reddy and colleagues (2012) looked at eggplant. For maximal discrimination and repeatability at 35 loci, Molecular profiles of genotypes were constructed using 39 EST-SSR primers. There were a total of 181 alleles, ranging from 9.0 (EEMS 24) to 3 (EEMS 46 and EEMS 20), with a mean of 5.02 alleles per locus

ranging from 85 to 300 bp and a PIC of the markers ranging from 0.343 to 0.794. The likelihood of identity ranged from 0.010 to 0.376, with expected heterozygosity ranging from 0.560 to 0.880. The findings show that "IIHR-3" and "IIHR-7" came from the same parent, while "Arka Anand" is a cross between "IIHR-3" and "SM-6-6," suggesting a strong fit with genetic similarity values.

Rahman et al. (2012) used thirty-four microsatellite markers to characterise 21 types of rice genotypes, and eight of them were found to be polymorphic (RM 10713, RM 424, RM 289, RM 20244, RM 5371, RM 6266, and RM 1155) and could be used for varietal identification and DNA finger printing of these aromatic rice genotypes.

Yan-fang Zhu et al. (2012) employed 32 SSR markers to fingerprint 48 rice cultivars and distinguish indica and japonica subspecies, as well as eight groupings of cultivars that were closely related. With an average of 2.33 bands per primer pair, 18 of the thirty-two SSR primer pairs were polymorphic, yielding a total of 42 unique reproducible bands. With each primer pair, the number of polymorphic bands observed ranged from 1 to 4, with an average of 2.28 per primer pair. Each primer pair's polymorphic information content (PIC) ranged from 0.10 to 0.50, with an average of 0.31. Monica Harta et al. (2013) used six SSR loci (SS2, MD5, MD7, MD 27, ZAG 62, and ZAG 79) to define four grapevine cultivars (Babeasca neagra, Feteasca regala, Franacusa, and Grasa de Cotnari), as well as four foreign genotypes (Cabernet Sauvignon, Chardonnay Blanc, Riesling and Merlot Noir) A barcode system was built using DNA microsatellites. Integrating such DNA barcodes into national and international databases could improve the accuracy with which Romanian grapevine genetic resources are maintained.

Kumar et al. (2014) used a molecular (SSR) marker to characterise a group of 72 rice genotypes gathered from different villages in Chhattisgarh state. On a 2.5 percent agarose gel, SSR analysis with 15 polymorphic SSR primers revealed 44 distinct alleles with an average of 2.93, ranging from 1 to 4 alleles per locus.

Naga chaitanya et al. (2015) used 13 SSR markers to create a DNA fingerprint of 79 guava accessions. CERVUS 3.0 software was used to conduct the statistical analysis. A total of 86 alleles were amplified, with a mean of 6.5 and a range of 5 to 9 alleles per locus. The observed heterozygosity values ranged from 0.00 to 0.364, while allelic sizes ranged from 86 to 256 bp. The heterozygosity expected ranged from 0.689 to

0.821. PIC values ranged from 0.630 to 0.789. The chances of being right ranged from 0.05 to 0.12. The created DNA barcode is extremely discriminative.

Vanisri et al. (2018) used the distinctive pattern of SSR polymorphism from allelic variation data to produce DNA barcodes for 14 visually identical medium grained rice genotypes and eight visually similar long slender grain types. To make variety recognition easy and precise, single-tube multiplex assays and DNA barcodes were created using existing evidence on 32 markers for medium slender genotypes and 35 markers for long slender genotypes, which can supplement traditional standard practises in determining purity and certification

MATERIAL AND METHOD

The current investigation entitled "Molecular Characterisation in lowland and medium land Rice," has been carried out at the Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar, Odisha during the year 2020-2021. The materials employed, the experimental procedures followed, and the techniques used during this inquiry are briefly discussed in the following sections of this chapter.

3.1 Collection of Germplasm lines

Sixty genotypes of rice (*O. sativa*) used in the present study, were procured from the Rice Research Station under Genetics Division, College of Agriculture, OUAT, Bhubaneswar during 2021.

Sl no	Genotype	Sl no	Genotype
1	SANKARBAKO	31	JUNGAJHATA
2	KALAKRUSHNA	32	RANGASIULI
3	ASSAM CHUDI	33	SALUAGAJA
4	GELEI	34	BASUDHA
5	GURUMUKHI	35	KALAJEERA
6	JUBRAJ	36	GOBINDABHOG
7	DHINKISIALI	37	AGNISAR
8	DHABALABHUTA	38	KABIR
9	LATAMAHU	39	NADALGHANTA
10	MAIDA	40	LATA CHAUNRI
11	DHUSURA	41	NALI KALAMA
12	JALPEYA	42	SARUBHAJANA
13	CHUDI	43	LUNA
14	JUIPHULA	44	ABHIRAM
15	KARPURAKRANTI	45	SEBATI
16	RAMAKRUSHNABILASH	46	AHIRMAN
17	SUNAPANI	47	BHUTMUNDI
18	CHAMPEISIALI	48	MAKARKANDA

19	NALIJAGANNATH	49	JATA
20	MAHSURI	50	KHAJURI KANDI
21	RANISAHEBA	51	TULASIMALI
22	JAIPHULA	52	NALIBAUNSAGAJA
23	PIPALBASA	53	PATENI
24	BUDIDHAN	54	NIKIPAKHIA
25	BASAPATRI	55	JHILLI
26	BAGADACHINAMALA	56	HUNDER
27	BIRIDI BANKOI	57	DHOJABANKOI
28	JAGABALIA	58	KORKAILI
29	DHOIAMADHOI	59	KALAMULIA
30	BISHNUPRIYA	60	KUSUMAKUNDA

Table: 3.1 List of selected rice genotypes used in Investigation.

3.1.1 Phenotyping

Tillers per hill, Plant height, Panicle length, Number of grains per panicle, 1000 grains weight, days to 50% flowering, days to 50% panicle initiation, Leaf bronzing index and Yield q/ha were all considered when phenotyping 60 rice lines. These observations were made using the Rice Standard Evaluation System as a guide (IRRI, 2013).

Tillers per Hill :	Tillers
Plant Height (in cm) :	PH
Length of Panicle (in cm) :	PL
Number of Grains per Panicle :	GN
Weight of 1000 Grains (in gms) :	GW
Days to 50% Flowering :	DAF
Days to 50% Panicle Initiation:	DAP
Leaf Bronzing Index :	LBI
Yield (in Q/Ha) :	Yield

Table: 3.2 Morphological characters for analysis

3.1.2 Material for genotyping

3.1.2.1. Chemicals used

Different chemicals utilized for genotyping analysis (DNA extraction, DNA purification, PCR analysis and Quantification).

Sr.No.	Chemical	Manufacturer
1	Cetyltrimethyl ammonium bromide (CTAB)	HiMedia
2	B-mercaptoethanol	HiMedia
3	Boric acid	HiMedia
4	Ethylenediamine-tetraacetic acid (EDTA)	HiMedia
5	2-Propanol	HiMedia
6	Chloroform	HiMedia
7	Ethidium bromide	HiMedia
8	Bromophenol blue	HiMedia
9	Glacial acetic acid	HiMedia
10	Isoamyl alcohol	HiMedia
11	Phenol	HiMedia
12	RNase A	Thermo fisher
13	Sodium Acetate	HiMedia
14	Sodium Chloride	HiMedia
15	Sodium hydroxide	HiMedia
16	Tris- HCL	HiMedia
17	Sucrose	HiMedia
18	Glycerol	HiMedia
19	Taq DNA polymerase	Promega
20	dNTPs mix	Promega
21	Primers	IDT, Eurofins,GeNeiTM
22	Molecular grade water	Promega
23	Agarose	GeNeiTM
24	Magnesium chloride	Promega
25	Tag Buffer	Promega
26	Ethanol	-

Table: 3.3 Chemical used for genotyping

3.1.2.2. Primers used

Five SSR Primers were selected from the sequence information available in literature of L Chrisnawati et al. (2016), E Matthus et al. (2015),Anuradha et al. (2012) and were synthesized from Integrated DNA Technologies (IDT ®)

Sr. No	Primer	Sequence (5'-3')	Chromosome No	Reference
1.	OsIRT 1	CGTCTTCTTCTTCTCCACCACGAC GCAGCTGATGATCGAGTCTGACC	3	L Chrisnawati et al. (2016)
2.	RM243	GATCTGCAGACTGCAGTTGC AGCTGCAACGATGTTGTCC	1	Anuradha et al. (2012)
3.	RM260	ACTCCACTATGACCCAGAG GAACAATCCCTTCTACGATCG	12	Anuradha et al. (2012)
4.	RM574	GGCGAATTCTTTGCACTTGG ACGGTTTGGTAGGGTGTCAC	5	Anuradha et al. (2012)
5.	RM248	TCCTTGTGAAATCTGGTCCC GTAGCCTAGCATGGTGCATG	7	Anuradha et al. (2012)

Table: 3.4 List of primers along with sequence, chromosome No and reference.

3.2 Seed germination

Each variety was chosen for ten healthy seeds, which were then sown in pots. Germination was seen at every two-day period. Fresh and young leaves were removed after two weeks, rinsed, weighed, and ground to a fine powder under liquid nitrogen.

Fig.1 Seed Germination

Fig.2 Rice Seedlings

Fig.3 14-Day old Seedlings

3.3 Crushing of leaves

The leaves were crushed in liquid nitrogen using a pestle and mortar, taking care not to melt the material. The sample was placed into a 50ml centrifuge tube and maintained at $-20^{0}C$ before thawing.

3.4 DNA extraction and purification

3.4.1. DNA Isolation using CTAB Method :

The total rice genomic DNA was isolated from five-week-old rice germplasm line and variety plants using a stepwise CTAB technique (Doyle and Doyle, 1987).

1. To prevent nuclease contamination, the CTAB extraction buffer was autoclaved at 121°C for 15 minutes at 15 psi.
2. Two grammes of leaf samples from each genotype were gathered from the field and after cleaning with tap water, it was sterilised with 70% ethanol. These are the leaves in a sterile, pre-cooled mortar pastel were ground to a fine powder with the use of liquid nitrogen
3. Pre-warmed (65-67 0C for 1 hour) extraction was added twice as much in the case of a powdered sample Incubate for 1-1.5 hours at 65°C after thoroughly mixing.
4. The mixture was then cooled to room temperature and equalised. A 24:1 ratio of chloroform to isoamyl alcohol was added and stirred by gently shaking it fully
5. In a centrifuge, the emulsion was centrifuged at 10000 rpm for 20 minutes at 4 0C.
6. The supernatant was collected in a new sterile centrifuge tube, which was then filled with an equivalent volume of cold isopropanol and gently mixed. After a while, the DNA began to form threads.
7. In an eppendorftube, the precipitated threads were taken out and cleaned with 70% ethanol.
8. Using a lyophilizer, the extracted DNA was dried.
9. 100 µl of TE buffer was used to dissolve the lyophilized DNA powder.
10. Using TBE buffer, DNA was measured on a 0.8 percent agarose gel electrophoresis. The existence of DNA was confirmed after electrophoresis, and a considerable amount of RNA was discovered. As a result, the samples were purified using RNase.

3.4.2. Purification of DNA

1. Then 3µl RNase was added, mixed well, and incubated for 1 hour at 37^0C. An equal volume of phenol, chloroform, and isoamyl alcohol (25:24:1) was added and thoroughly mixed for 2 minutes before spinning for 5 minutes.

2. The DNA supernatant was removed, and an equal volume of chloroform:isoamyl alcohol was added, mixed thoroughly, and centrifuged for 10 minutes.
3. To make a creamy colour solution, the aqueous layer was eliminated and the process repeated.
4. Then, to separate the aqueous layer, 1/10th volume of 3M Sodium acetate and 2.5 volume of absolutely chilled alcohol were poured, gently mixed so that DNA may precipitate down, and maintained overnight or for an hour at -20oC if no precipitation was seen.
5. The solution was then centrifuged for 5 minutes at 8000 rpm, with the supernatant decanted off.
6. Two washes with 70% ethanol were used to eliminate any remaining salts. The DNA was then vacuum-sealed, dissolved in TE (10:1) buffer at room temperature, and kept frozen at -20°C.

3.5 DNA Quantification by agarose gel electrophoresis through Band-brightness

The genomic DNA isolated and purified earlier was quantified by agarose gel electrophoresis using a series of graded known concentrations of a reference DNA and comparing with the band brightness. Using varied concentrations of agarose gel, DNA fragments of various sizes can be resolved. Small DNAs can be separated more easily with a higher concentration of agarose gel, while larger DNAs can be separated more easily with a lower concentration of gel. The running buffer, 0.5 X TBE, supplies ions to facilitate electric conductivity while also establishing the pH of the medium for DNA movement. Ethidium bromide (EtBr) is a fluorescent dye that intercalates between the bases and allows the mobility of DNA fragments in gel to be easily detected. Besides, the genomic DNA could also be quantified accurately by spectrophotometric analysis.

3.5.1. Quantification procedure:

1. An 0.8 percent Agarose gel was produced in 0.5X TBE buffer and cast in a tray after 5µl of EtBr (10 mg/ml) was added.
2. In the gel, a comb was introduced and allowed to solidify at room temperature.
3. After the gel had completely solidified, the comb and castors were carefully removed.
4. The horizontal tank was filled with 0.5X TBE and the gel was placed in it.

5. 3µlitres of DNA were combined with 2µlitres of loading dye (6X bromophenol blue dye) and put into the comb-made wells in the agarose gel.
6. The gel was operated for 1 hour on a 65V electric source.
7. In the UVI Tech Cambridge gel documentation system, the gel was then viewed under UV light.

The embedded camera captured an image of clear bands of high molecular weight DNAs right below the loaded well.

3.6 Dilution of DNA

A portion of the stock DNA samples was diluted with a suitable amount of TE buffer to obtain a working concentration of 10mg/µl and stored at 4ºC.

3.7 SSR Optimisation of condition for PCR

A factorial experiment was conducted in a volume of 25µl with three concentrations of MgCl2 (2.0mM, 2.5mM, and 3.0mM), 1U of Taq DNA polymerase, three concentrations of template DNA (10mg, 20mg, 30mg), and 0.5µM primer to find optimal amplification reaction conditions for 30 rice genotypes. The annealing temperatures were 5 degrees Celsius above and below the melting temperature, Tm, of the primer in question. Finally, the conditions were tuned as shown in Table 3.2, resulting in the amplification of clear and consistent bands. The amplification of available SSR primers was examined, and the ones that exhibited greater resolution were chosen for the current investigation.

Table 3.5. PCR constituents optimized for SSR analysis

Component	Stock	Final conc. per reaction
10X buffer	1x	2 µl
MgCl2 (25mM)	2mM	1.2µl
dNTPs (10mM)	200µM	2µl
Primer	5µM	2µl
Taq polymerase	1 unit	1µl
Deionised water distilled	-	13.8µl
Total	-	22µl
DNA	10-50ng	3µl

3.7.1 Thermocycler conditions for SSR

I. Pre-denaturation at 95ºC for 5min.

II. Forty cycles of denaturation, annealing and extension was as follow:

Table 3.6 PCR Programme Components

40 cycles	**Temp ºC**	Duration
Denaturation	95ºC	1min
Annealing	ATº	1min
Extension	72ºC	1min

Note: ATº= Annealing temperature which varies with primer to primer.

III. Followed by final extension at 72ºC for 8 min.

3.7.2 PCR Reaction mix (Master Mix)

Total volume of PCR reaction mix was made to 25µl of which 22.0µl was master and remaining 3µl was the individual DNA of the genotype.

3.7.3 Primer Screening

Sixty SSR primers were screened, out of which five SSR gave satisfactory amplification. These five SSR primers are enlisted in Table 3.3.

3.7.4 Agarose gel Electrophoresis

Before agarose gel electrophoresis, reaction products were mixed
with 2.0µl of 10x loading dye (Annexure-II) and spun for a while before loading.

3.7.5 Preparation of Agarose gel

For SSR 2.5% agarose gel was prepared. 5.0gm agarose was mixed well in 200ml of 1X TBE buffer and boiled in oven till it completely dissolved. During warming intermittent shaking were done 4-5 times to prevent formation of clumps of agarose. After complete dissolving the gel was kept for cooling at 50-60ºC.

The gel casting tray was cleaned by 70% ethyl alcohol and was levelled. Then poured the molten gel in the casting plate containing 26 well comb and was kept for solidification. Gel was transferred to electrophoresis unit containing 1X TBE buffer and DNA samples were loaded along with 1kb/20bp DNA ladder (20 bp DNA ruler plus).

3.7.6 Staining DNA

The gel was transferred to the staining tray once electrophoresis was completed, which contained 10 µl EtBr per 100 ml distilled water. The needed amount of water was then added and the shaker was left for 30-40 minutes to stain.

Destaining was done using distilled water to remove the excess stain from the gel.

3.7.7 Gel photography

Gel Doc. technology was used to photograph stained DNA/gels under UV light (UVITECH, Cambridge, UK).

3.7.8 Scoring and Data Analysis

The amplification product was scored as "1" if the allele was present, "0" if the allele was absent, and "3" if the sample was lost. The distance of migration of amplified DNA fragments according to their molecular weights in the gel was used to determine allele homology.

In SSR, each amplification product was treated as a single SSR marker, and all samples were scored.

3.7.9 Similarity coefficient

The selected genotype was compared to the remainder of the genotypes for the similarity coefficient. The higher the coefficient value, the more similar the comparison variety is to the selected variety. Furthermore, the value of the coefficient denotes the degree of resemblance between the two types. According to Jaccard (1908), the similarity coefficient was calculated as follows:

$$\text{Jaccard's similarity Coefficient} = \frac{n_{xy}}{n_1 - n_2}$$

n_{xy} = Number of common bands in the sample a and b

n_1 = No of total bands present in all samples

n_2 = Number of bands not present in the sample a or b but present in other samples

The similarity matrix was exposed to produce a dendrogram by software programme NTSYS pc Version 2.2. Exeter Software, N.Y. (Rohlf, 2005).

3.8 DNA profiling

- A 15ml/tube master mixture was prepared and thoroughly mixed.
- Divided into the needed number of thin-walled flat-headed PCR tubes, together with 1.5µ l of template DNA (20-50 mg) and mixed thoroughly.

- Placed all of the PCR tubes in the peltier blocks of the thermocycler (Applied Biosystems Veriti) in a harmonic or even pattern, and the lid was correctly closed.
- The samples are then electrophoresed in 0.5 percent TBE as tank buffer for 2.5 hours on a 3 percent agarose gel at 90 V.
- The gel was then examined for template DNA amplification using a gel-documentation system (UVI-tech Cambridge)
- .With the inbuilt camera, a photograph was obtained for additional study. The photograph was then analysed for molecular weight analysis in relation to the ladder using the integrated UVI Tech programme (Himedia 50bp Set Uptm).
- The thermocycling method was set to (2 min, 95 °C), followed by 35 cycles of denaturation (30 s, 95 °C), anneling temp. (a range of 4-50C lower than Tm of each primer) for 30 sec, extension for 2 minutes at 72 0C, and final extension at 72 0C for 5 min and store at 4 C.

Number of tillers, Plant height, Panicle length, Number of grains per panicle, 1000 grains weight, days to 50% flowering, days to 50% panicle initiation and Yield q/ha were all considered when phenotyping 60 rice lines. These observations were made using the Rice Standard Evaluation System as a guide (IRRI, 2013).

3.9 Data Analysis

In an attempt to make morphological and molecular characterisation of selected 60 local rice genotypes out of 150 lowland and medium land rice genotypes the morphological traits like tillers per hill, plant height, length of panicle, grains per panicle, weight of 1000 grains, days to 50% flowering, days to 50% panicle initiation, leaf bronzing index and yield per hectare was taken into consideration from the assessed data of previous one year. And the DNA profile of the gene specific primer RM 243 was generated for the 60 rice genotypes. Further the morphological data is used for binary coding and generation of phylogenetic tree for cluster analysis and morphological characterisation of diverse genotypes of rice such that it ensures detection and removal of similar and inferior genotypes that are incompetent to act as donor during gene introgression for crop improvement.

RESULT

4.1. Phenotypic variation of rice genotypes

4.1.1. Natural diversity among rice genotypes in field condition

Previous one year data was taken into consideration for morphological trait analysis, as the current data couldnot be generated due to outbreak of the 2nd wave of Covid 19. A binary coding of phenotypic characters was done to generate dendrogram for analysis of diversity among the selected local rice genotypes.

During the Kharif season of 2019-20, a set of 60 rice genotypes, comprising landraces and high yielders, was tested for several morphological traits in soil in natural habitat at the experimental site of the Regional Research and Technology Transfer station, OUAT, Bhubaneswar. A random sample of rice plants from the plot was used to determine all of the morphological attributes.

There were visible phenotypic differences among the genotypes examined. ANOVA analysis revealed significant differences between individuals for all evaluated variables. All the morphological traits studied here such as, Numbers of days to 50% flowering after sowing, plant height, panicle length, number of grains per panicle, 1000 grain weight, (seed index), leaf bronzing index, yield in Q/ha, number of tillers per hill have shown diversity. In replications of each rice genotype, all morphological features were scored. The results of the morphological analysis are shown as follows:

Table 4.1 Result of Phenotyping screening

Sl. No.	Tiller	PH	PL	GN	GW	DAF	DAP	LBI	YIELD
1	5.75	93.5	17.25	75	22.75	82.75	71	2.75	13.24
2	6.25	112.25	21.25	128.25	13.5	104.5	99	2.75	12.57
3	5.75	108	22.25	86.75	22.25	105.25	97	2.25	17.94
4	5.25	105.25	19.5	102.25	16.75	106	95	1.25	18.22
5	8.25	102.25	19.5	59.25	22.25	106.5	93.25	5.75	13.74
6	5	101	25.5	78.25	17	108	96.25	7.75	18.65
7	5.5	107	22.75	101	13.5	104	96.75	2.25	24.47
8	5.25	109.25	16.75	81.75	17.5	102	92.5	2	24.08

9	6.75	104.75	17.75	75	14.75	108	95.5	4.25	12.77
10	6.25	116.75	20.5	99	21.25	105.5	96.75	4.25	17.33
11	8	120.75	24	66.5	22.25	108.5	101	6.75	13.68
12	5.25	111	22.75	66.25	15.25	107.75	96.5	4.5	10.78
13	7.5	109.75	21.5	120.5	21	108.5	102	2.75	20.18
14	6.25	116	20.75	123.75	9.75	102.25	94.5	6	9.76
15	5.5	121.5	20.5	99.5	12	102.5	93.25	6.5	15.04
16	4.5	119.5	21	125.25	8	104.75	97.75	1.5	11.6
17	7	97.25	19.75	97.75	21.75	102.75	93.5	5	24.62
18	5.5	123.75	22.5	90.75	25.5	108.75	101	4.5	16.93
19	6.5	121	23.5	112.5	21.75	109	100.25	3	16.51
20	5.75	109.75	17.5	185.5	14.25	106	101.75	1.5	25.15
21	7.25	111.75	23	130.5	18.5	115	108	1.5	20.75
22	8.75	116	20.75	101.75	17.5	107.75	98.75	3.5	19.05
23	5.25	123	27	69.5	21.75	105.25	101	4.5	10.7
24	4.75	109.75	16.25	178.5	14	109.25	104.25	7	13.83
25	7	115.5	19.5	89.25	12.75	105.5	101.25	6.75	11.95
26	6.75	98.75	22.5	95.5	17	104.5	102	8.75	15.38
27	5.25	113	22	79.25	22	105.75	97.5	2.5	15.14
28	6	106.25	22.75	144.5	17	101.25	93.75	3	17.14
29	8.75	116.75	21	146	19.25	106	101.75	2.25	21.23
30	6	66.25	13.75	108.5	13	105	97.75	8.75	10.89
31	6.25	115.75	19.25	99.75	18.25	110.5	104.5	4	14.97
32	4.25	112	21	132.25	18.75	116.5	109.75	4.25	13.74
33	9	96.25	26	141.5	17.25	114.5	108.25	6	14.02
34	11	96.5	19	141.5	14	110.5	102.5	6.25	16.92
35	7.5	119.75	23	138.5	9	106	98.5	0.75	11.26
36	7.75	101.25	21.75	160.25	16	113.5	106	4.25	14.46
37	3.25	111.25	21.75	95.75	15	119	110.75	2	14.88
38	6	89	18.5	115.75	18	115.5	106.5	2	16
39	8	124.25	21	98.25	17.5	111.75	101.75	1.75	16.59
40	6.25	115.5	22.75	161.75	14	107.75	101.75	2.25	21.85
41	5	96.25	16.75	146.5	17	117	109.75	5.25	19.86
42	5	94	19.75	127	18.25	113	103.25	4.75	23.82
43	6.75	130.25	16.75	100.5	19	114	106	6	18.35
44	5.75	120.75	14.5	124	22	110	101.75	3	16.69
45	6.5	84	19.75	76	9.75	110	100.75	6	17.04
46	4.75	115.25	18.5	87	17	107.75	100.5	3.75	15.68
47	7	121.25	22.5	100.5	21.75	109	100.75	2.25	20.63
48	9.25	116.25	20.75	92.75	17.5	108	103.25	1.75	18.35
49	7.25	126.5	18.75	103.75	18.25	111.75	100.75	4.75	21.3

50	7.5	122.75	15.5	97.75	16.5	109.75	101.5	4.5	21.51
51	5	121.75	21.75	95.25	18.5	114.5	105.5	2.5	12
52	5.5	129.25	22.25	67.05	20	111.75	102.5	2.25	18.1
53	5.75	119	22.5	166.75	21.75	112.5	106.25	2.75	16.71
54	4.25	94	21.75	166.50	12.25	119.75	110.75	2.75	23.53
55	6	109.5	16.5	173.25	16.5	108	101.5	4.25	13.84
56	3.5	114.75	17.75	115.25	16	109	99.75	6	20.03
57	7	108.75	23.75	87.50	18.75	109	103.75	2	21.74
58	4.75	123.25	22.75	87.75	13	120.5	111.5	1.5	13.88
59	8.5	115.75	20.75	69.25	17.75	119.75	114	1.75	15.39
60	5.75	105.5	17.25	101.24	16.25	111	103.25	3.25	13.1
CD$_{(0.5)}$	1.56	10.23	4.28	9.85	3.5	6.75	6.54	0.55	5.80
CV%	10.5	12.85	8.74	11.95	7.85	11.94	10.26	8.46	9.78

Further the morphological data is used for binary coding and generation of phylogenetic tree for cluster analysis and morphological characterisation of diverse genotypes of rice such that it ensures detection and removal of similar and inferior genotypes that are incompetent to act as donor during gene introgression for crop improvement.

4.2 Molecular characterization

In this investigation, a total of 5 SSR markers were employed to characterise and analyse the diversity among 60 rice genotypes, including landraces and HYVs. All 60 genotypes chosen for finding connection between markers showed significant diversity. Calculating allele number, size, and PIC values was used to assess the extent of polymorphism among rice germplasms. The next sections examine the key features of allelic diversity data for all polymorphic SSR markers across all 60 rice genotypes.

4.2.1 Allelic Diversity generated by polymorphic SSR marker

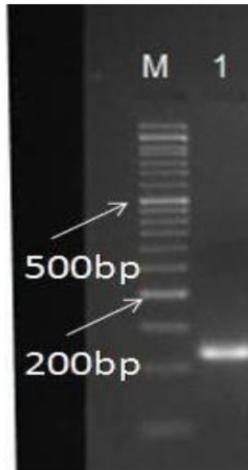
A panel of 60 genotypes, including germplasms and high-yielding rice genotypes, were examined for genetic diversity using five SSR markers. In the table, SSR markers are included together with their genetic diversity factors. Five SSR markers were used to amplify a few alleles. The number of alleles ranged between two and five, with an average of 2.5. In a panel of 60 rice genotypes, the RM260 marker had the most alleles. In RM260, the main allele frequency average was 0.6610, with a minimum value of 0.3182. All of the markers examined had a heterozygosity value of less than 0. The

average gene diversity among all SSR markers analysed was 0.4284, with RM 260 showing the highest gene diversity at 0.7587.

Sl.No	Marker	Min allele size (bp)	Max allele size (bp)	Major Allele Frquency	Allele No	Gene Diversity	Hetero-zygosity	PIC	Ta (˚C)
1	**RM243**	90	120	0.6596	2.000	0.4491	0.0851	0.3482	55
2	**RM574**	100	150	0.6909	2.000	0.4271	0.6182	0.3359	55
3	**RM248**	70	100	0.5611	2.000	0.4925	0.2333	0.3712	55
4	**RM260**	70	260	0.3182	5.000	0.7587	0.3766	0.7173	55
	Mean			0.5575	2.750	0.5318	0.3283	0.4432	55.

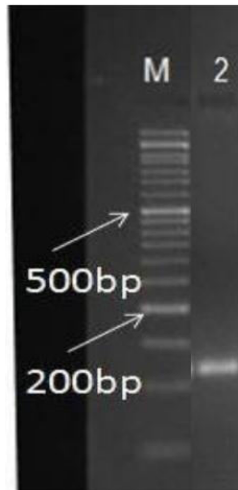
Table4.2 Details of SSR loci used for genotyping a set of 60 rice genotypes and their genetic diversity parameters.

In an attempt to make morphological and molecular characterisation of selected 60 local rice genotypes out of 150 lowland and medium land rice genotypes the morphological traits like tillers per hill, plant height, length of panicle, grains per panicle, weight of 1000 grains, days to 50% flowering, days to 50% panicle initiation, leaf bronzing index and yield per hectare was taken into consideration from the assessed data of previous one year. And the DNA profile of the gene specific primer RM 243 was generated for the 60 rice genotypes and is depicted hereunder along with their panicle, seeds and morphological data as assessed.



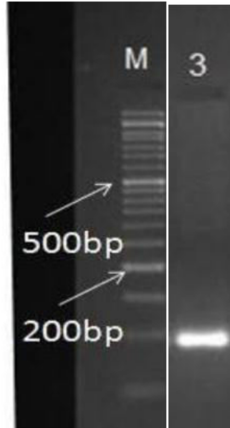
Tillers per Hill :	5.75
Plant Height (in cm) :	93.5
Length of Panicle (in cm) :	17.25
Grains per Panicle :	75
Weight of 1000 Grains (in gms) :	22.75
Days to 50% Flowering :	82.75
Days to 50% Panicle Initiation:	71
Leaf Bronzing Index :	2.75
Yield (in Q/Ha) :	13.24

1. SANKARIBAKO



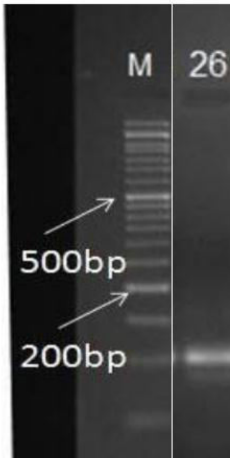
Tillers per Hill :	6.25
Plant Height (in cm) :	112.25
Length of Panicle (in cm) :	21.25
Grains per Panicle :	128.25
Weight of 1000 Grains (in gms) :	13.5
Days to 50% Flowering :	104.5
Days to 50% Panicle Initiation:	99
Leaf Bronzing Index :	2.75
Yield (in Q/Ha) :	12.57

2. KALAKRUSHNA



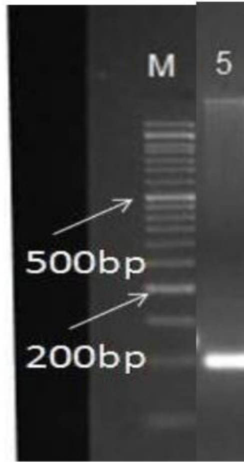
Tillers per Hill :	5.75
Plant Height (in cm) :	108
Length of Panicle (in cm) :	22.25
Grains per Panicle :	86.75
Weight of 1000 Grains (in gms) :	22.25
Days to 50% Flowering :	105.25
Days to 50% Panicle Initiation:	97
Leaf Bronzing Index :	2.25
Yield (in Q/Ha) :	17.94

3. ASSAMCHUDI



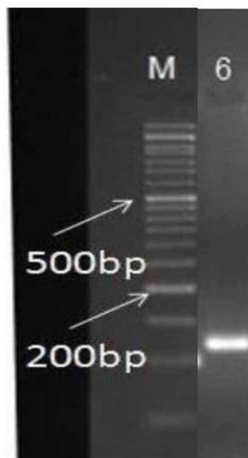
Tillers per Hill :	5.25
Plant Height (in cm) :	105.25
Length of Panicle (in cm) :	19.5
Grains per Panicle :	102.25
Weight of 1000 Grains (in gms) :	16.75
Days to 50% Flowering :	106
Days to 50% Panicle Initiation:	95
Leaf Bronzing Index :	1.25
Yield (in Q/Ha) :	18.22

4. GELEI



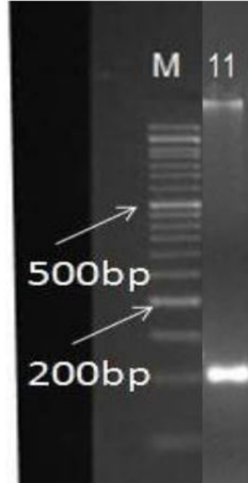
Tillers per Hill :	8.25
Plant Height (in cm) :	102.25
Length of Panicle (in cm) :	19.5
Grains per Panicle :	59.25
Weight of 1000 Grains (in gms) :	22.25
Days to 50% Flowering :	106.5
Days to 50% Panicle Initiation:	93.25
Leaf Bronzing Index :	5.75
Yield (in Q/Ha) :	13.74

5. GURUMUKHI



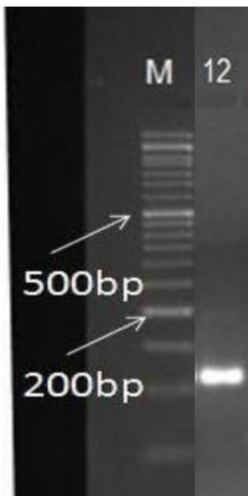
Tillers per Hill :	5
Plant Height (in cm) :	101
Length of Panicle (in cm) :	25.5
Grains per Panicle :	78.25
Weight of 1000 Grains (in gms) :	17
Days to 50% Flowering :	108
Days to 50% Panicle Initiation:	96.25
Leaf Bronzing Index :	7.75
Yield (in Q/Ha) :	18.65

6. JUBARAJ



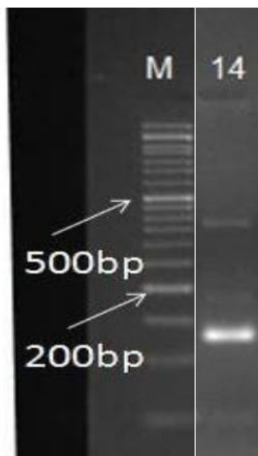
Tillers per Hill :	5.5
Plant Height (in cm) :	107
Length of Panicle (in cm) :	22.75
Grains per Panicle :	101
Weight of 1000 Grains (in gms) :	13.5
Days to 50% Flowering :	104
Days to 50% Panicle Initiation:	96.75
Leaf Bronzing Index :	2.25
Yield (in Q/Ha) :	24.47

7. DHINKISIALI



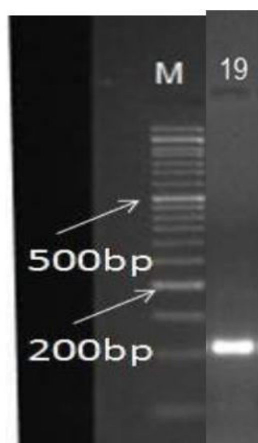
Tillers per Hill :	5.25
Plant Height (in cm) :	109.25
Length of Panicle (in cm) :	16.75
Grains per Panicle :	81.75
Weight of 1000 Grains (in gms) :	17.5
Days to 50% Flowering :	102
Days to 50% Panicle Initiation:	92.5
Leaf Bronzing Index :	2
Yield (in Q/Ha) :	24.08

8. DHABALABHUTA



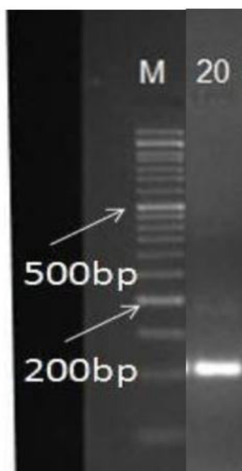
Tillers per Hill :	6.75
Plant Height (in cm) :	104.75
Length of Panicle (in cm) :	17.75
Grains per Panicle :	75
Weight of 1000 Grains (in gms) :	14.75
Days to 50% Flowering :	108
Days to 50% Panicle Initiation:	95.5
Leaf Bronzing Index :	4.25
Yield (in Q/Ha) :	12.77

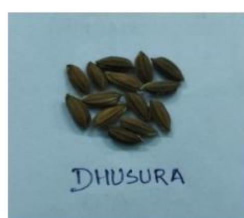
9. LATA MAHU



Tillers per Hill :	6.25
Plant Height (in cm) :	116.75
Length of Panicle (in cm) :	20.5
Grains per Panicle :	99
Weight of 1000 Grains (in gms) :	21.25
Days to 50% Flowering :	105.5
Days to 50% Panicle Initiation:	96.75
Leaf Bronzing Index :	4.25
Yield (in Q/Ha) :	17.33

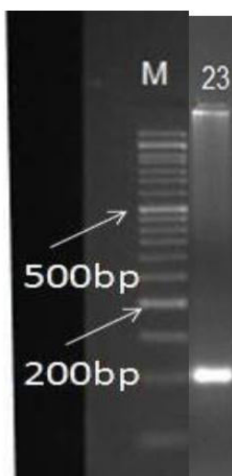
10. MADIA





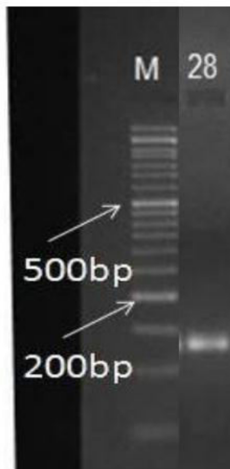
Tillers per Hill :	8
Plant Height (in cm) :	120.75
Length of Panicle (in cm) :	24
Grains per Panicle :	66.5
Weight of 1000 Grains (in gms) :	22.25
Days to 50% Flowering :	108.5
Days to 50% Panicle Initiation:	101
Leaf Bronzing Index :	6.75
Yield (in Q/Ha) :	13.68

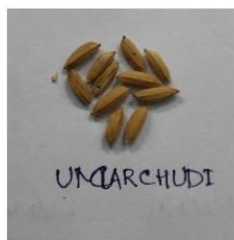
11. DHUSURA



Tillers per Hill :	5.25
Plant Height (in cm) :	111
Length of Panicle (in cm) :	22.75
Grains per Panicle :	66.25
Weight of 1000 Grains (in gms) :	15.25
Days to 50% Flowering :	107.75
Days to 50% Panicle Initiation:	96.5
Leaf Bronzing Index :	4.5
Yield (in Q/Ha) :	10.78

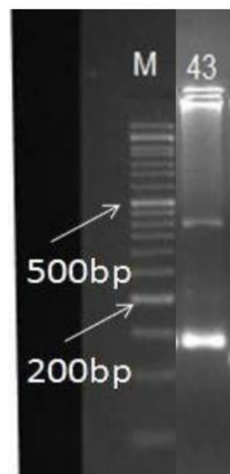
12. JALPEYA





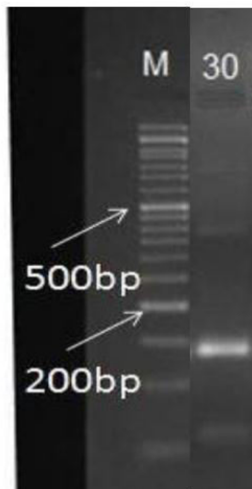
Tillers per Hill :	7.5
Plant Height (in cm) :	109.75
Length of Panicle (in cm) :	21.5
Grains per Panicle :	120.5
Weight of 1000 Grains (in gms) :	21
Days to 50% Flowering :	108.5
Days to 50% Panicle Initiation:	102
Leaf Bronzing Index :	2.75
Yield (in Q/Ha) :	20.18

13. UMARCUDI



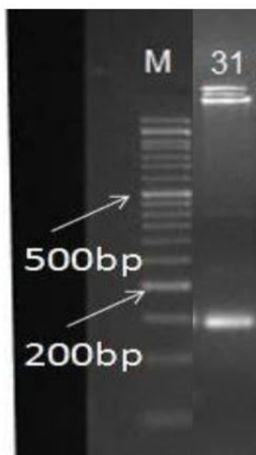
Tillers per Hill :	6.25
Plant Height (in cm) :	116
Length of Panicle (in cm) :	20.75
Grains per Panicle :	123.75
Weight of 1000 Grains (in gms) :	9.75
Days to 50% Flowering :	102.25
Days to 50% Panicle Initiation:	94.5
Leaf Bronzing Index :	6
Yield (in Q/Ha) :	9.76

14. JAIPHULA



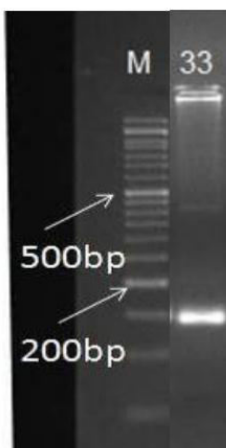
Tillers per Hill :	5.5
Plant Height (in cm) :	121.5
Length of Panicle (in cm) :	20.5
Grains per Panicle :	99.5
Weight of 1000 Grains (in gms) :	12
Days to 50% Flowering :	102.5
Days to 50% Panicle Initiation:	93.25
Leaf Bronzing Index :	6.5
Yield (in Q/Ha) :	15.04

15. KARPURAKANTI



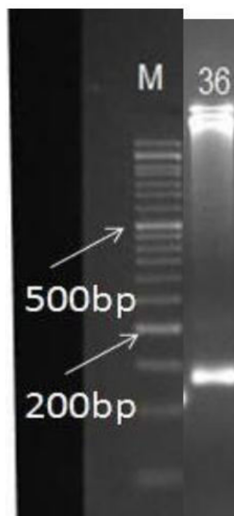
Tillers per Hill :	4.5
Plant Height (in cm) :	119.5
Length of Panicle (in cm) :	21
Grains per Panicle :	125.25
Weight of 1000 Grains (in gms) :	8
Days to 50% Flowering :	104.75
Days to 50% Panicle Initiation:	97.75
Leaf Bronzing Index :	1.5
Yield (in Q/Ha) :	11.6

16. RAMAKRUSHNABILAS



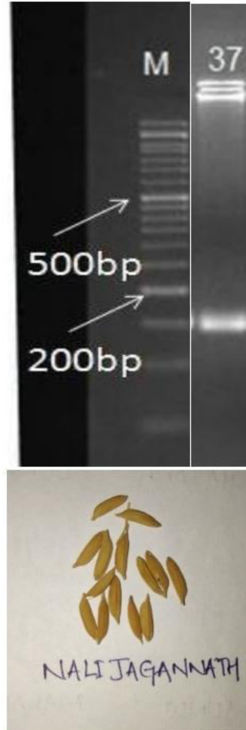
Tillers per Hill :	7
Plant Height (in cm) :	97.25
Length of Panicle (in cm) :	19.75
Grains per Panicle :	97.75
Weight of 1000 Grains (in gms) :	21.75
Days to 50% Flowering :	102.75
Days to 50% Panicle Initiation:	93.5
Leaf Bronzing Index :	5
Yield (in Q/Ha) :	24.62

17. SUNAPANI



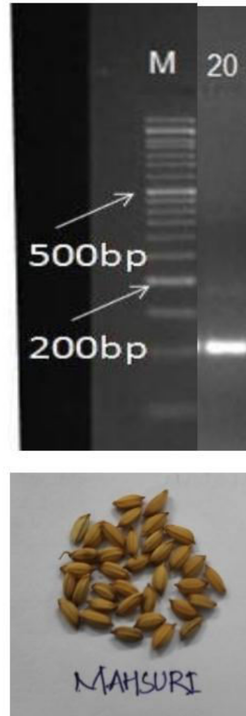
Tillers per Hill :	5.5
Plant Height (in cm) :	123.75
Length of Panicle (in cm) :	22.5
Grains per Panicle :	90.75
Weight of 1000 Grains (in gms) :	25.5
Days to 50% Flowering :	108.75
Days to 50% Panicle Initiation:	101
Leaf Bronzing Index :	4.5
Yield (in Q/Ha) :	16.93

18. CHAMPEISIALI



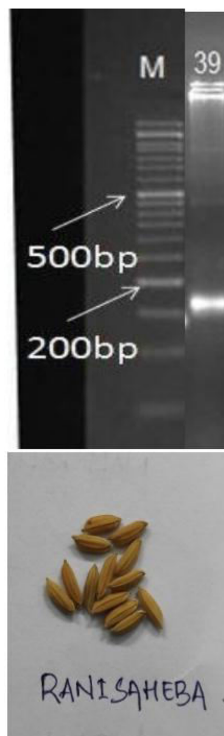
Tillers per Hill :	6.5
Plant Height (in cm) :	121
Length of Panicle (in cm) :	23.5
Grains per Panicle :	112.5
Weight of 1000 Grains (in gms) :	21.75
Days to 50% Flowering :	109
Days to 50% Panicle Initiation:	100.25
Leaf Bronzing Index :	3
Yield (in Q/Ha) :	16.51

19. NALIJAGANNATH



Tillers per Hill :	5.75
Plant Height (in cm) :	109.75
Length of Panicle (in cm) :	17.5
Grains per Panicle :	185.5
Weight of 1000 Grains (in gms) :	14.25
Days to 50% Flowering :	106
Days to 50% Panicle Initiation:	101.75
Leaf Bronzing Index :	1.5
Yield (in Q/Ha) :	25.15

20. MAHSURI



Tillers per Hill :	7.25
Plant Height (in cm) :	111.75
Length of Panicle (in cm) :	23
Grains per Panicle :	130.5
Weight of 1000 Grains (in gms) :	18.5
Days to 50% Flowering :	115
Days to 50% Panicle Initiation:	108
Leaf Bronzing Index :	1.5
Yield (in Q/Ha) :	20.75

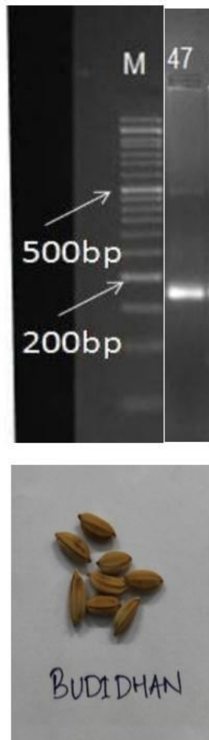
21. RANISAHEBA

Tillers per Hill :	8.75
Plant Height (in cm) :	116
Length of Panicle (in cm) :	20.75
Grains per Panicle :	101.75
Weight of 1000 Grains (in gms) :	17.5
Days to 50% Flowering :	107.75
Days to 50% Panicle Initiation:	98.75
Leaf Bronzing Index :	3.5
Yield (in Q/Ha) :	19.05

22. JABAPHULA

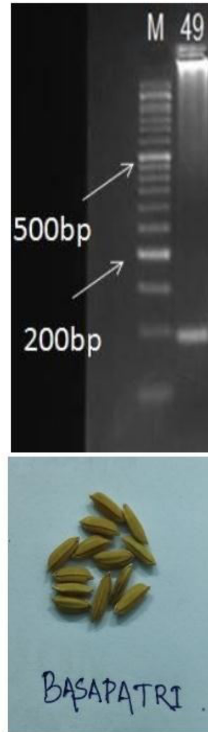
Tillers per Hill :	5.25
Plant Height (in cm) :	123
Length of Panicle (in cm) :	27
Grains per Panicle :	69.5
Weight of 1000 Grains (in gms) :	21.75
Days to 50% Flowering :	105.25
Days to 50% Panicle Initiation:	101
Leaf Bronzing Index :	4.5
Yield (in Q/Ha) :	10.7

23. PIPALBASA



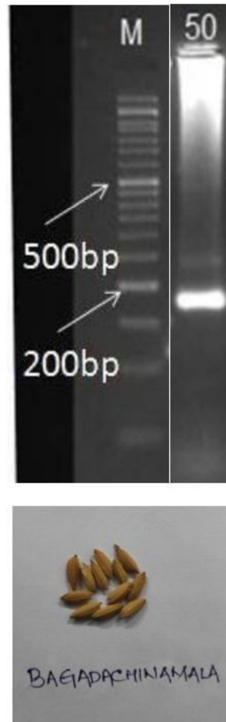
Tillers per Hill :	4.75
Plant Height (in cm) :	109.75
Length of Panicle (in cm) :	16.25
Grains per Panicle :	178.5
Weight of 1000 Grains (in gms) :	14
Days to 50% Flowering :	109.25
Days to 50% Panicle Initiation:	104.25
Leaf Bronzing Index :	7
Yield (in Q/Ha) :	13.83

24. BUDHIDHAN



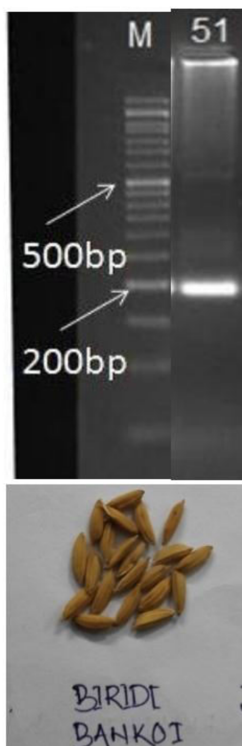
Tillers per Hill :	7
Plant Height (in cm) :	115.5
Length of Panicle (in cm) :	19.5
Grains per Panicle :	89.25
Weight of 1000 Grains (in gms) :	12.75
Days to 50% Flowering :	105.5
Days to 50% Panicle Initiation:	101.25
Leaf Bronzing Index :	6.75
Yield (in Q/Ha) :	11.95

25. BASAPATRI



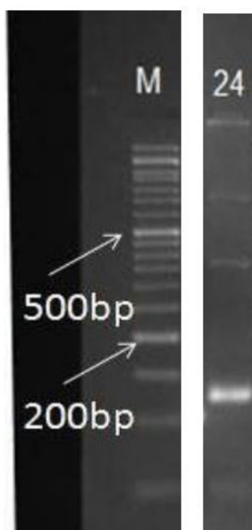
Tillers per Hill :	6.75
Plant Height (in cm) :	98.75
Length of Panicle (in cm) :	22.5
Grains per Panicle :	95.5
Weight of 1000 Grains (in gms) :	17
Days to 50% Flowering :	104.5
Days to 50% Panicle Initiation:	102
Leaf Bronzing Index :	8.75
Yield (in Q/Ha) :	15.38

26. BAGADACHINAMALA

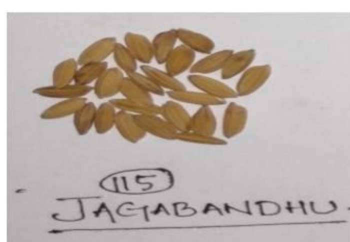


Tillers per Hill :	5.25
Plant Height (in cm) :	113
Length of Panicle (in cm) :	22
Grains per Panicle :	79.25
Weight of 1000 Grains (in gms) :	22
Days to 50% Flowering :	105.75
Days to 50% Panicle Initiation:	97.5
Leaf Bronzing Index :	2.5
Yield (in Q/Ha) :	15.14

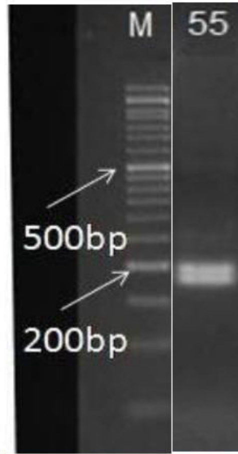
27. BIRIDIBANKOJ

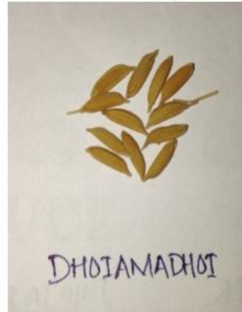


Tillers per Hill :	6
Plant Height (in cm) :	106.25
Length of Panicle (in cm) :	22.75
Grains per Panicle :	144.5
Weight of 1000 Grains (in gms) :	17
Days to 50% Flowering :	101.25
Days to 50% Panicle Initiation:	93.75
Leaf Bronzing Index :	3
Yield (in Q/Ha) :	17.14



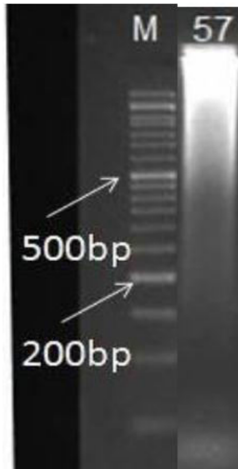
28. JAGABANDHU





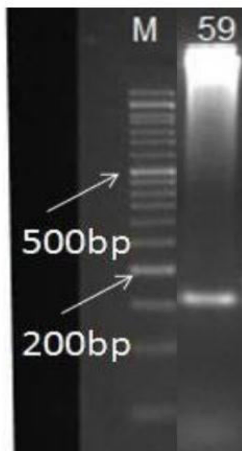
Tillers per Hill :	8.75
Plant Height (in cm) :	116.75
Length of Panicle (in cm) :	21
Grains per Panicle :	146
Weight of 1000 Grains (in gms) :	19.25
Days to 50% Flowering :	106
Days to 50% Panicle Initiation:	101.75
Leaf Bronzing Index :	2.25
Yield (in Q/Ha) :	21.23

29. DHALAMADHOBI



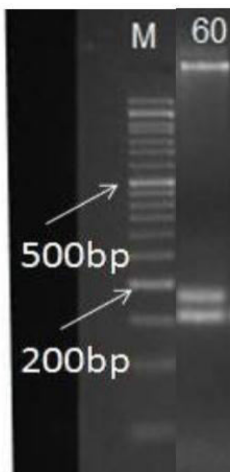
Tillers per Hill :	6
Plant Height (in cm) :	66.25
Length of Panicle (in cm) :	13.75
Grains per Panicle :	108.5
Weight of 1000 Grains (in gms) :	13
Days to 50% Flowering :	105
Days to 50% Panicle Initiation:	97.75
Leaf Bronzing Index :	8.75
Yield (in Q/Ha) :	10.89

30. BISHNUPRIYA



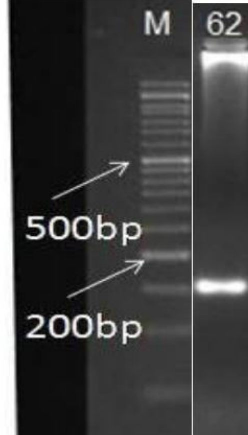
Tillers per Hill :	6.25
Plant Height (in cm) :	115.75
Length of Panicle (in cm) :	19.25
Grains per Panicle :	99.75
Weight of 1000 Grains (in gms) :	18.25
Days to 50% Flowering :	110.5
Days to 50% Panicle Initiation:	104.5
Leaf Bronzing Index :	4
Yield (in Q/Ha) :	14.97

31. JUNGAJHATA



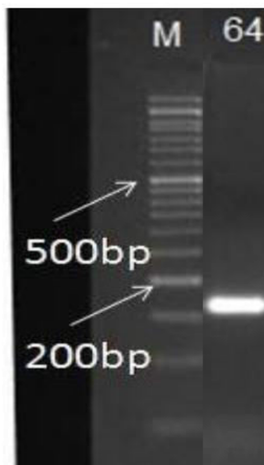
Tillers per Hill :	4.25
Plant Height (in cm) :	112
Length of Panicle (in cm) :	21
Grains per Panicle :	132.25
Weight of 1000 Grains (in gms) :	18.75
Days to 50% Flowering :	116.5
Days to 50% Panicle Initiation:	109.75
Leaf Bronzing Index :	4.25
Yield (in Q/Ha) :	13.74

32. RANGASIULI



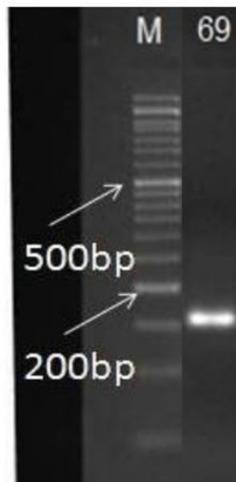
Tillers per Hill :	9
Plant Height (in cm) :	96.25
Length of Panicle (in cm) :	26
Grains per Panicle :	141.5
Weight of 1000 Grains (in gms) :	17.25
Days to 50% Flowering :	114.5
Days to 50% Panicle Initiation:	108.25
Leaf Bronzing Index :	6
Yield (in Q/Ha) :	14.02

33. SALUAGAJA



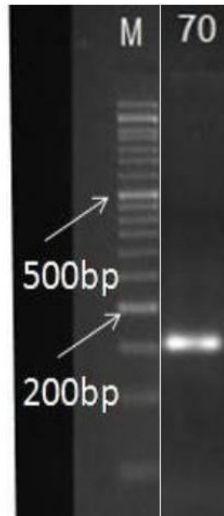
Tillers per Hill :	11
Plant Height (in cm) :	96.5
Length of Panicle (in cm) :	19
Grains per Panicle :	141.5
Weight of 1000 Grains (in gms) :	14
Days to 50% Flowering :	110.5
Days to 50% Panicle Initiation:	102.5
Leaf Bronzing Index :	6.25
Yield (in Q/Ha) :	16.92

34. BASUDHA



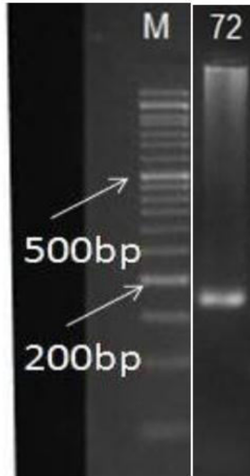
Tillers per Hill :	7.5
Plant Height (in cm) :	119.75
Length of Panicle (in cm) :	23
Grains per Panicle :	138.5
Weight of 1000 Grains (in gms) :	9
Days to 50% Flowering :	106
Days to 50% Panicle Initiation:	98.5
Leaf Bronzing Index :	0.75
Yield (in Q/Ha) :	11.26

35. KALAJEERA



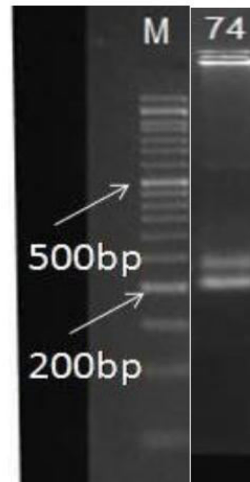
Tillers per Hill :	7.75
Plant Height (in cm) :	101.25
Length of Panicle (in cm) :	21.75
Grains per Panicle :	160.25
Weight of 1000 Grains (in gms) :	16
Days to 50% Flowering :	113.5
Days to 50% Panicle Initiation:	106
Leaf Bronzing Index :	4.25
Yield (in Q/Ha) :	14.46

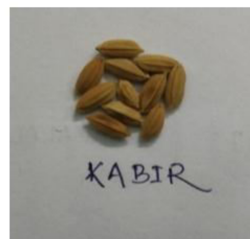
36. GOBINDABHOGA



Tillers per Hill :	3.25
Plant Height (in cm) :	111.25
Length of Panicle (in cm) :	21.75
Grains per Panicle :	95.75
Weight of 1000 Grains (in gms) :	15
Days to 50% Flowering :	119
Days to 50% Panicle Initiation:	110.75
Leaf Bronzing Index :	2
Yield (in Q/Ha) :	14.88

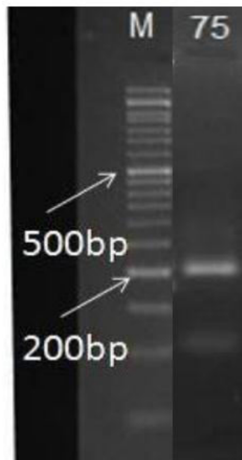
37. AGMISAR





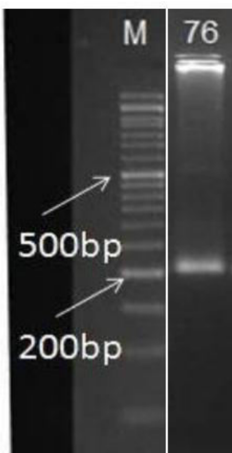
Tillers per Hill :	6
Plant Height (in cm) :	89
Length of Panicle (in cm) :	18.5
Grains per Panicle :	115.75
Weight of 1000 Grains (in gms) :	18
Days to 50% Flowering :	115.5
Days to 50% Panicle Initiation:	106.5
Leaf Bronzing Index :	2
Yield (in Q/Ha) :	16

38. KABIR



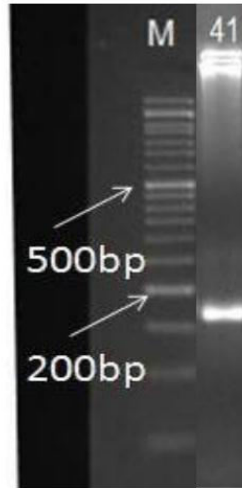
Tillers per Hill :	8
Plant Height (in cm) :	124.25
Length of Panicle (in cm) :	21
Grains per Panicle :	98.25
Weight of 1000 Grains (in gms) :	17.5
Days to 50% Flowering :	111.75
Days to 50% Panicle Initiation:	101.75
Leaf Bronzing Index :	1.75
Yield (in Q/Ha) :	16.59

39. NADALGHANTA



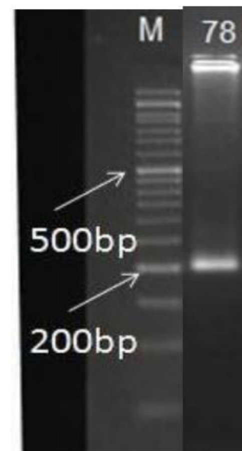
Tillers per Hill :	6.25
Plant Height (in cm) :	115.5
Length of Panicle (in cm) :	22.75
Grains per Panicle :	161.75
Weight of 1000 Grains (in gms) :	14
Days to 50% Flowering :	107.75
Days to 50% Panicle Initiation:	101.75
Leaf Bronzing Index :	2.25
Yield (in Q/Ha) :	21.85

40. LATACHAUNRI



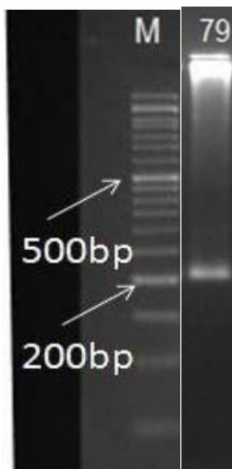
Tillers per Hill :	5
Plant Height (in cm) :	96.25
Length of Panicle (in cm) :	16.75
Grains per Panicle :	146.5
Weight of 1000 Grains (in gms) :	17
Days to 50% Flowering :	117
Days to 50% Panicle Initiation:	109.75
Leaf Bronzing Index :	5.25
Yield (in Q/Ha) :	19.86

41. NALIKAMALA



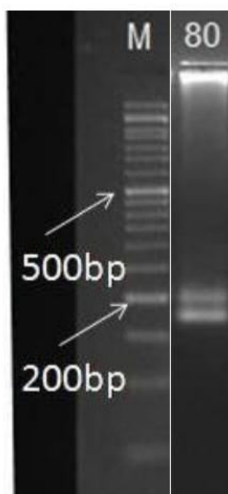
Tillers per Hill :	5
Plant Height (in cm) :	94
Length of Panicle (in cm) :	19.75
Grains per Panicle :	127
Weight of 1000 Grains (in gms) :	18.25
Days to 50% Flowering :	113
Days to 50% Panicle Initiation:	103.25
Leaf Bronzing Index :	4.75
Yield (in Q/Ha) :	23.82

42. SARUBHAJANA



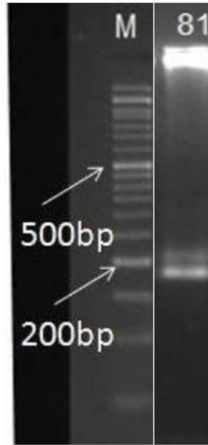
Tillers per Hill :	6.75
Plant Height (in cm) :	130.25
Length of Panicle (in cm) :	16.75
Grains per Panicle :	100.5
Weight of 1000 Grains (in gms) :	19
Days to 50% Flowering :	114
Days to 50% Panicle Initiation:	106
Leaf Bronzing Index :	6
Yield (in Q/Ha) :	18.35

43. LUNA



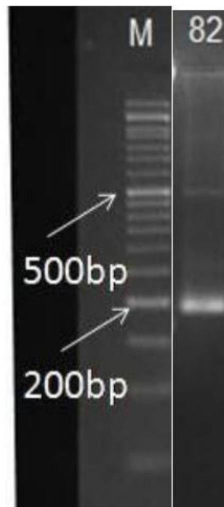
Tillers per Hill :	5.75
Plant Height (in cm) :	120.75
Length of Panicle (in cm) :	14.5
Grains per Panicle :	124
Weight of 1000 Grains (in gms) :	22
Days to 50% Flowering :	110
Days to 50% Panicle Initiation:	101.75
Leaf Bronzing Index :	3
Yield (in Q/Ha) :	16.69

44. ABIRAM



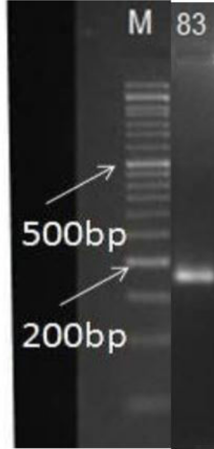
Tillers per Hill :	6.5
Plant Height (in cm) :	84
Length of Panicle (in cm) :	19.75
Grains per Panicle :	76
Weight of 1000 Grains (in gms) :	9.75
Days to 50% Flowering :	110
Days to 50% Panicle Initiation:	100.75
Leaf Bronzing Index :	6
Yield (in Q/Ha) :	17.04

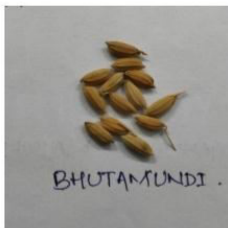
45. SEBATI



Tillers per Hill :	4.75
Plant Height (in cm) :	115.25
Length of Panicle (in cm) :	18.5
Grains per Panicle :	87
Weight of 1000 Grains (in gms) :	17
Days to 50% Flowering :	107.75
Days to 50% Panicle Initiation:	100.5
Leaf Bronzing Index :	3.75
Yield (in Q/Ha) :	15.68

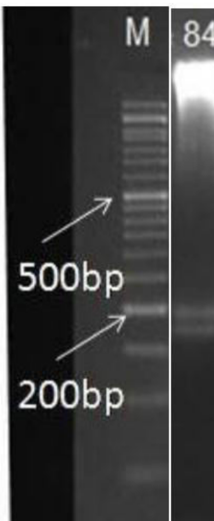
46. AHIRAM





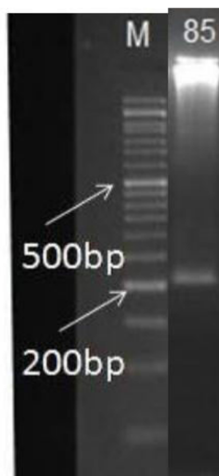
Tillers per Hill :	7
Plant Height (in cm) :	121.25
Length of Panicle (in cm) :	22.5
Grains per Panicle :	100.5
Weight of 1000 Grains (in gms) :	21.75
Days to 50% Flowering :	109
Days to 50% Panicle Initiation:	100.75
Leaf Bronzing Index :	2.25
Yield (in Q/Ha) :	20.63

47. BHUTAMUND



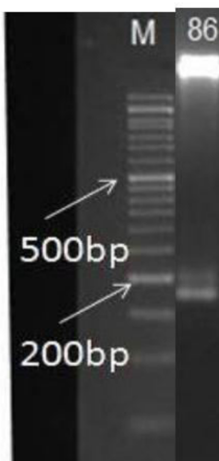
Tillers per Hill :	9.25
Plant Height (in cm) :	116.25
Length of Panicle (in cm) :	20.75
Grains per Panicle :	92.75
Weight of 1000 Grains (in gms) :	17.5
Days to 50% Flowering :	108
Days to 50% Panicle Initiation:	103.25
Leaf Bronzing Index :	1.75
Yield (in Q/Ha) :	18.35

48. MAKARAKANDA



Tillers per Hill :	7.25
Plant Height (in cm) :	126.5
Length of Panicle (in cm) :	18.75
Grains per Panicle :	103.75
Weight of 1000 Grains (in gms) :	18.25
Days to 50% Flowering :	111.75
Days to 50% Panicle Initiation:	100.75
Leaf Bronzing Index :	4.75
Yield (in Q/Ha) :	21.3

49. JATA

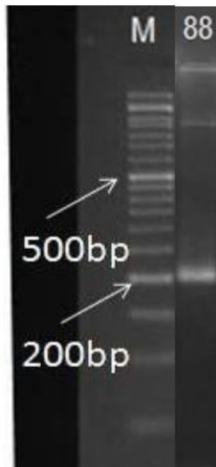


Tillers per Hill :	7.5
Plant Height (in cm) :	122.75
Length of Panicle (in cm) :	15.5
Grains per Panicle :	97.75
Weight of 1000 Grains (in gms) :	16.5
Days to 50% Flowering :	109.75
Days to 50% Panicle Initiation:	101.5
Leaf Bronzing Index :	4.5
Yield (in Q/Ha) :	21.51

50. KHAJURAKANDI

Tillers per Hill :	5
Plant Height (in cm) :	121.75
Length of Panicle (in cm) :	21.75
Grains per Panicle :	95.25
Weight of 1000 Grains (in gms) :	18.5
Days to 50% Flowering :	114.5
Days to 50% Panicle Initiation:	105.5
Leaf Bronzing Index :	2.5
Yield (in Q/Ha) :	12

51. TULASIMALI

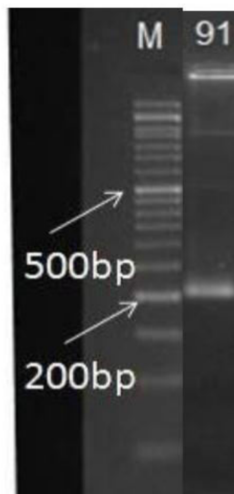


Tiiiers per Hiii :	5.5
Plant Height (in cm) :	129.25
Length of Panicle (in cm) :	22.25
Grains per Panicle :	67
Weight of 1000 Grains (in gms) :	20
Days to 50% Flowering :	111.75
Days to 50% Panicle Initiation:	102.5
Leaf Bronzing Index :	2.25
Yield (in Q/Ha) :	18.1

52. NALIBAUNSAGAJA

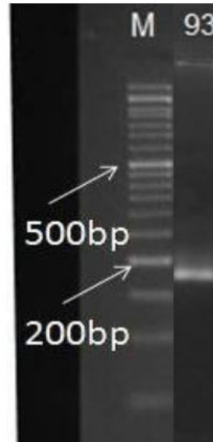
Tillers per Hill :	5.75
Plant Height (in cm) :	119
Length of Panicle (in cm) :	22.5
Grains per Panicle :	166.75
Weight of 1000 Grains (in gms) :	21.75
Days to 50% Flowering :	112.5
Days to 50% Panicle Initiation:	106.25
Leaf Bronzing Index :	2.75
Yield (in Q/Ha) :	16.71

53. PATENI



Tillers per Hill :	4.25
Plant Height (in cm) :	94
Length of Panicle (in cm) :	21.75
Grains per Panicle :	166.5
Weight of 1000 Grains (in gms) :	12.25
Days to 50% Flowering :	119.75
Days to 50% Panicle Initiation:	110.75
Leaf Bronzing Index :	2.75
Yield (in Q/Ha) :	23.53

54. NIKIPAKHIA



Tillers per Hill :	6
Plant Height (in cm) :	109.5
Length of Panicle (in cm) :	16.5
Grains per Panicle :	173.25
Weight of 1000 Grains (in gms) :	16.5
Days to 50% Flowering :	108
Days to 50% Panicle Initiation:	101.5
Leaf Bronzing Index :	4.25
Yield (in Q/Ha) :	13.84

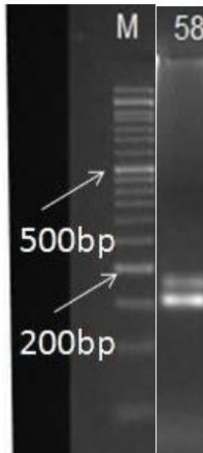
55. JHILLI

Tillers per Hill :	3.5
Plant Height (in cm) :	114.75
Length of Panicle (in cm) :	17.75
Grains per Panicle :	115.25
Weight of 1000 Grains (in gms) :	16
Days to 50% Flowering :	109
Days to 50% Panicle Initiation:	99.75
Leaf Bronzing Index :	6
Yield (in Q/Ha) :	20.03

56. HUNDER

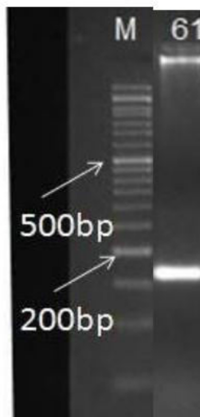
Tillers per Hill :	7
Plant Height (in cm) :	108.75
Length of Panicle (in cm) :	23.75
Grains per Panicle :	87.5
Weight of 1000 Grains (in gms) :	18.75
Days to 50% Flowering :	109
Days to 50% Panicle Initiation:	103.75
Leaf Bronzing Index :	2
Yield (in Q/Ha) :	21.74

57. DHOIABANKOI



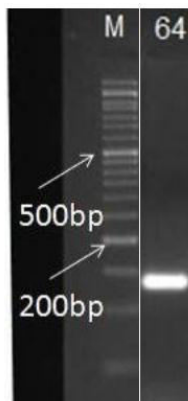
Tillers per Hill :	4.75
Plant Height (in cm) :	123.25
Length of Panicle (in cm) :	22.75
Grains per Panicle :	87.75
Weight of 1000 Grains (in gms) :	13
Days to 50% Flowering :	120.5
Days to 50% Panicle Initiation:	111.5
Leaf Bronzing Index :	1.5
Yield (in Q/Ha) :	13.88

58. KORKAILI



Tillers per Hill :	8.5
Plant Height (in cm) :	115.75
Length of Panicle (in cm) :	20.75
Grains per Panicle :	69.25
Weight of 1000 Grains (in gms) :	17.75
Days to 50% Flowering :	119.75
Days to 50% Panicle Initiation:	114
Leaf Bronzing Index :	1.75
Yield (in Q/Ha) :	15.39

59. KALAMULIA

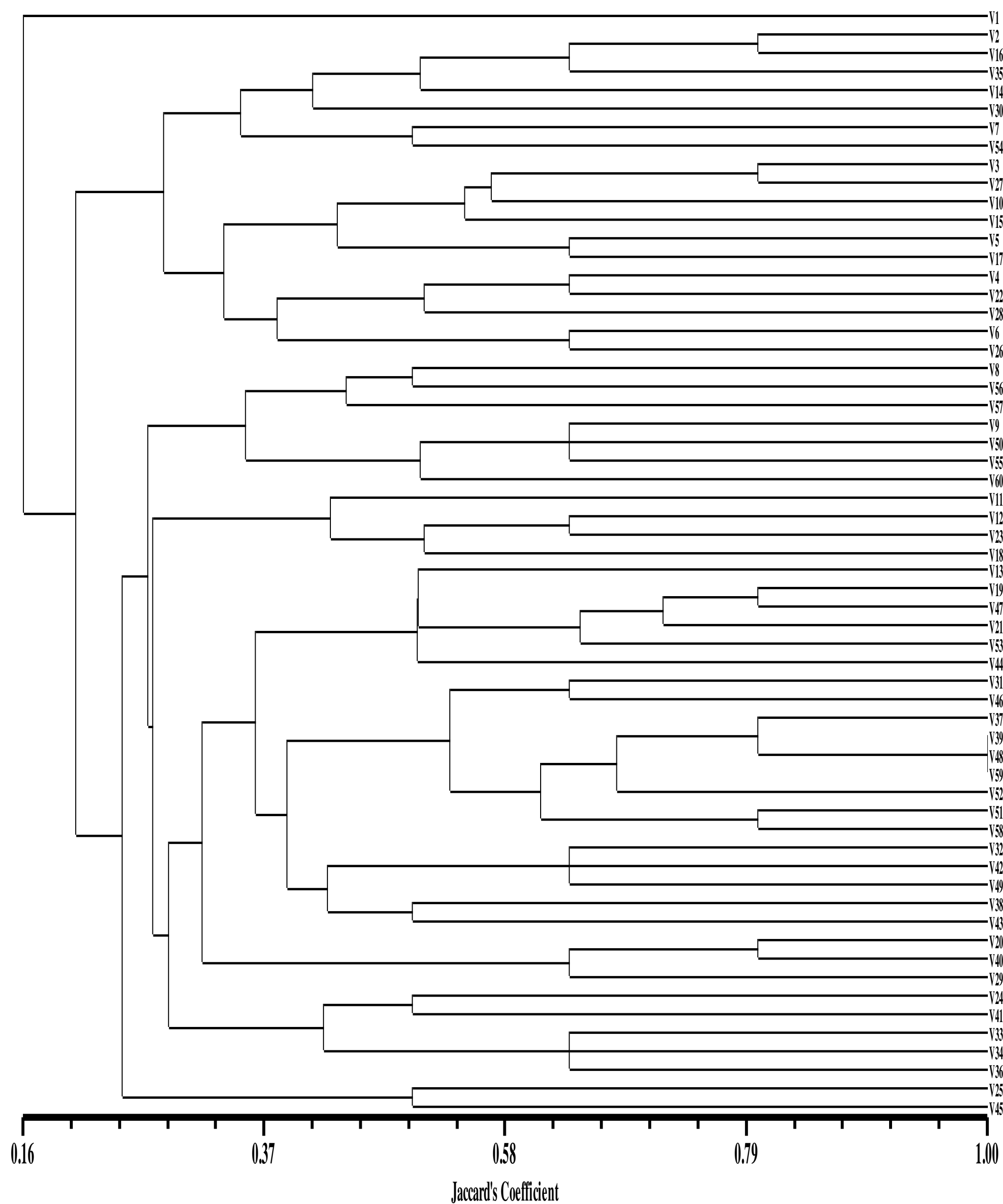


Tillers per Hill :	5.75
Plant Height (in cm) :	105.5
Length of Panicle (in cm) :	17.25
Grains per Panicle :	101
Weight of 1000 Grains (in gms) :	16.25
Days to 50% Flowering :	111
Days to 50% Panicle Initiation:	103.25
Leaf Bronzing Index :	3.25
Yield (in Q/Ha) :	13.1

60. KUSUMKUNDA

4.2.2. Cluster analysis

UPGMA created a dendrogram based on the genetic similarity matrix among the rice germplasms to demonstrate the genetic relatedness of the germplasms studied. Using Jacard's Coefficient of Similarity, the cluster analysis revealed significant genetic heterogeneity among the genotypes, with similarity co-efficients ranging from 0.14 to 0.96. As previously stated, the UPGMA-based dendrogram divided all 60 genotypes into two major and seven sub groups.



Phylogenetic tree constructed by UPGMA

DISCUSSION

Rice (*Oryza sativa* L.) is a monocotyledonous crop that is a staple meal in Asia and consumed by a huge portion of the world's population (Xipeng et al., 2011). It produced 99.15 million tonnes in 2008-09, according to estimates. In 2008-09, it accounted for 42% of total food grain production (Ricepedia 2013). However, over the last ten years, demand for rice has expanded in tandem with population growth. To meet the growing demand of foodgrains in commensurate with the population growth, there is need to have constant effort to develop high yielding rice varieties through introgression breeding. But rice cultivation is subjected to a variety of stresses, resulting in yields that are never realised in the field in comparison to the potential yield as developed by the breeder. The rice germplasm is a major source of agronomically important genes which can be exploited as donors for developing HYVs through various breeding approaches. Therefore, assessment of genetic diversity existing amongst the available germplasm pool is of prime importance. In the present study, an attempt has been made to study the genetic diversity existing in the available germplasm through morpho-molecular trait analysis.

The morphological data presented here has been statistically analysed to have a better insight into the diversity of rice germplasm. The analysis of variance showed that genotypes were highly significant ($p < 0.05$) for all the characters like plant height, number of grains per panicle,leaf bronzing index, days to maturity, thousand-seed weight, panicle length, number of tillers per hill , number of grains per panicle and grain yield indicating the existence of variability among the tested genotypes.

The phylogenetic analysis of the sixty rice genotypes has put all those into seven clusters which depicted the natural diversity existing among the test genotypes and also indicated that the genotypes of heterogeneous origin were present in same cluster. Therefore, the selection of parental material for hybridization programme simply based on geographic diversity may not be a rewarding exercise (Fellahi et al., 2013). The choice of suitable diverse parents based on genetic divergence analysis would be more fruitful than the choice made on the basis of geographical distances. This cluster analysis may help in selecting ideal donor parents for an introgression breeding programme as optimium genetic divergence is desired between the parents for obtaining higher frequency of desirable recombinants, the

chances of obtaining good segregants by crossing the little diverse genotypes belonging to same cluster are very low. In order to increase the possibility of isolating good segregants in the segregating generations, it would be reasonable to attempt crosses between the diverse genotypes from inter clusters and not from intra-clusters.

The morphological traits of all present and potential species may not be enough to distinguish and identify them, necessitating the use of more precise approaches. With its codominant segregation and capacity to identify vast numbers of distinct allles regularly, correctly, and effectively, microsatellite DNA markers are recognised as the markers of choice for varietal identification. Using the SSR-marker for DNA fingerprinting, this research attempts to ascertain the phenotyping and molecular characterization of low land rice genotypes, their germplasm, land races, obsolete genotypes, and high yielding genotypes.

To conserve the variety, DNA fingerprinting is essential. The molecular characterisation and fingerprinting of released cultivars will offer enough information on molecular variation between them. It was proposed to characterize atleast 60 nos of genotypes using some SSR markers which could not be possible except for one due to outbreak of Corona pandemic situations.

Germplams is the basic material for launching a crop improvement programme. Germplasm evaluation that refers to screening of gemplasms in respect of morphological, genetical, economic, biochemical, physiological, pathological and entomological attributes, is essential to get a clear picture about the significance of individual germplasm line. Besides, various stakeholders like breeders and farmers need a portfolio of diverse crop varieties with agro-morphological and agronomic traits. It is very essential then to provide a catalogue of notified varieties and promising landraces for crop improvement, utilization and conservation. The catalogue’s compilation of agromorphological characteristics and digital pictures aids in distinguishing the varieties, providing datasets that include the crop varieties and their trait combinations. An honest attempt has been made to catalogue a panel of 60 germplasm adaptable to low and medium land rice habitat prone to iron toxicity.

SUMMARY AND CONCLUSION

The results of the present investigations designated "*Molecula*r ***characterization in lowland and medium land rice"*** was undertaken at the Department of Agricultural Biotechnology, College of Agriculture, Bhubaneswar are summarized as hereunder.

- A total of 60 rice (*Oryza sativa*. L) genotypes representative of local lowland and medium and germplasms were taken for the morphological investigation.
- In the morphological study, the different genotypes were scored on the basis of their bronzing scale, plant height, panicle length, No of tillers per hill, grain no, grain weight, yield Q/Ha.
- Phenotypic characterization with respect to leaf bronzing associated with yield loss has classified the set of genotypes into three classes. i.e. Susceptible, moderately resistant and resistant.
- Further the morphological data is used for binary coding and generation of phylogenetic tree for cluster analysis and morphological characterisation of diverse genotypes of rice such that it ensures detection and removal of similar and inferior genotypes that are incompetent to act as donor during gene introgression for crop improvement.
- The cluster analysis carried out here in this investigation may be useful in selecting ideal donor parents for getting better gene seggregants.
- An honest attempt has been made to catalogue a panel of 60 germplasm adaptable to low and medium land rice habitat prone to iron toxicity to provide a catalogue of notified varieties and promising landraces for crop improvement, utilization and conservation by various stakeholders like breeders, farmers, researchers and fieldstaff.

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