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## REVIEW ARTICLE

## Utilization of Plant Genetic Resources and Diversity Analysis Tools for Sustainable Crop Improvement with special emphasis on Rice

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### Abstract

Plant genetic resources, in all forms, are valuable for the study of genetic diversity and analysis of genetic variation for crop improvements as they form the basis of world's food security. The value of conserved germplasm can be assessed for the useful traits in plant breeding and the economic impact on germplasm utilization in crop production and productivity. The objective of this review summarizes the importance of genetic diversity, and utilization of genetic resources in plant development through the advance molecular marker technology. The application of molecular markers, functional genomics and genetic engineering technologies will help to understand the genetic diversity and germplasm management will help to reveal the molecular basis of various biological phenomena in plants. Molecular marker technology is well developed over the last few decades and is beginning to be incorporated into breeding programs worldwide. The effective and complementary use of all technological tools and resources will be required for meeting the challenge imposed by the world's expanding food demand and scarcity as well. Molecular technologies have been applied in the analysis of specific genes, as well as to increase the understanding of gene action, generate genetic maps and assist in the development of gene transfer technologies.

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## INTRODUCTION

The challenge of feeding the expected 9 billion world population by 2050 in a sustainable manner can be met, among other measures, by rescuing and using more diversity in agricultural and food production systems, both in terms of crop as in terms of varieties within any given crop [1]. Present day agriculture is attributed with the both inter- and intra-specific reduced crop plant's diversity. It was estimated that today's amongst the total of approximately 30 thousand crop species, the human population today derives most of its calories from only 30 species known to feed the world, with the three top most cereal crops: rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) [2 - 4] and those species providing 95% of the global food energy [5, 6]. On the contrary, over 7,000 species are known as edible and are either partly or fully domesticated, suggesting that a large share of potential food sources is underutilized [7, 8].

In this adverse period, plant breeding contributes to diminution of the plant diversity through the development of adapted breeding populations with higher biological yield, potential best genotypes selection, genotypic

homogeneous cultivars development and promotion of few widely adapted varieties [3]. The basis for raising crop production and improving crop quality is to breed new varieties. The key to breed new varieties is largely depended on the breakthrough of mining the plant germplasm resources. The management of genetic resources is a complex, multi-faceted process [9]. Therefore, the collection, conservation, research and utilization of crop genetic resources should be an important content for the agricultural sustainable developmental research. The diverse population is providing the genetic barriers against different abiotic and biotic stresses [10, 11]. The plant genetic diversity plays an important role in present ongoing and future crop improvement. Crop breeding technology has helped plants to evolve and develop against biotic and abiotic stress condition. Hajjar et al. [11] showed that increasing genetic diversity is important for pest and disease management and provides chances for further improvement of the species. Beside these applied aspects, molecular marker-based genetic diversity is necessary for genetic mapping and marker-assisted selection in breeding programs [12]. Genetic diversity in crop plants may be analyzed at different levels: individual genotypes such as inbreed lines or pure lines or clones, populations, germplasm accessions, and species. Diversity can occur at three levels: genetic diversity (variation in genes and genotypes), species diversity (species richness) and ecosystem diversity (communities of species and their environment). The importance of biodiversity for humankind has been well recognized in the recent decades and it is essential for allowing sustainable development of various human activities.

Plant breeding has a long history of integrating the latest innovations in agro-biology and genetics to enhance crop improvement. Plant breeding with agri-horticultural crops has typically aimed at improved yields, nutritional qualities and other traits of commercial values. The plant breeding paradigm has been enormously successful on a global scale, with such examples of the development of hybrid maize (*Zea mays*), the introduction of wheat (*Triticum aestivum*) and rice (*Oryza sativa*) varieties that spawned the Green Revolution [13, 14] and the recent commercialization of transgenic crops [15]. Many of these products have been contributed numerous benefits to the global society through the plant breeding approaches.

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population and is one of the most important food crops grown and cultivated worldwide [16]. Both agronomically and nutritionally, such cereal occupies an important place among the food crops. It is well known that during early domestication and evolutionary selection, cultivated rice differentiated into two major subspecies, *indica* and *japonica* [17 - 19]. Although significant differences exist in morphological and physiological characters between the two subspecies [20] of *indica* and *japonica* varieties, it appears to be a major source of genetic diversity in the cultivated rice gene pool. Such *indica*-*japonica* differentiation has been detected in various rice samples using several classes of markers including isozymes [21, 22], protein markers [23], restriction fragment length polymorphism (RFLP) [24, 25], simple sequence repeats (SSRs) [26 - 28], and randomly amplified polymorphic DNA (RAPD) [29 - 31], amplified fragment length polymorphism (AFLP) [32, 33], sequence-tagged sites (STS) [34, 35], single nucleotide polymorphism (SNP) [36], and Chloroplast DNA [37, 38].

Recent trends of molecular tools hold the promise of allowing the identification of genes involved in a number of traits, including adaptive traits, and polymorphisms causing functional genetic variation (QTN – Quantitative Trait Nucleotides). Since the advent of molecular markers, researchers and breeders have aimed to identify functional markers associated with diverse biotic and abiotic stress condition. Molecular techniques are currently being utilized and developed for the assessment of genetic diversity, functional analysis of genes and targeted towards the diverse population. This review aims towards utilization of genomic resources for their importance in genetic diversity and prospecting of novel genes/alleles through the molecular approaches.

## GENETIC RESOURCES FOR GERMPLASM COLLECTION

Genetic resources will be fundamental in our efforts to improve agricultural productivity for the increasing world population. Genetic resources can be defined as all materials that are available for improvement of a cultivated plant species [39]. Agriculture productivity, before the 18<sup>th</sup> century completely depended on landraces for new varieties. During the industrial revolution, the entire nature and practice of agriculture was transformed forever. The discovery of genetics by Mendel in the mid-19<sup>th</sup> century and the subsequent work of other plant geneticists provided the basic knowledge that made dramatic increases in agricultural productivity. Plant breeding evolution had achieved two major events i.e., pre- (before 1965) and post- (after 1965) green revolution [40, 41]. The breeding and molecular marker technology has been frequently affected by genome studies. The discovery of each novel technique convened great hopes and perceptions for the improvement of crop yield. Wild species of *Oryza* are an important reservoir of useful genes and can be exploited both to broaden the existing narrow genetic base and enrich the existing varieties with desired agro-economically important traits. Today, plant breeders have gained achievement in molecular breeding through the advancement and accumulation of knowledge in the field of molecular genetics and biochemistry. Landraces and traditional varieties have been replaced by less diverse modern cultivars and hybrids.

The collection of major crop plants is held in international centers around the world. They are repositories of the biodiversity available for each species and are a valuable source of useful genes for plant breeders.

The priority of breeders has been, achieving the demonstrable short-term gains rather than long-term germplasm development using exotic germplasm. Thus, the large pool of variability instead of prompting more use has created a situation of not knowing where to begin. This has arisen due to an incomplete knowledge of germplasm accessions and the relationships among them, the unavailability of descriptive characters, and an uncertainty about the best evaluation methods for tapping the germplasm resources. The development of core collections has been suggested as means to enhance the use of genetic resources in the improvement programs. A core collection is a subset of accessions from the entire collection that captures most of available genetic diversity of the species [42]. The core subset can be evaluated extensively, and the information derived could be used to guide more efficiently the utilization of the entire collection [43, 44].

The expression crop genetic resource usually refers to the sum total of genes, gene combinations, or genotypes available for the genetic improvement of crop plants. Establishment of a core collection based on genetic variation shown by accessions would be obviously advantageous, but clear and detailed assessments of diversity within germplasm collections are not usually available. Such information can be obtained using molecular markers which afford many benefits for identifying variation and estimating biological diversity. The structure of the genetic diversity is also important when considering the development of 'core collections' in a genetic resource context [45]. Further, without determining diversity reliably it would not be possible to identify molecular marker/quantitative trait associations which have been shown to be useful in the process of germplasm evaluation [46]. Molecular dissection is much more powerful for determining the usefulness of a species than casual analysis at the morphological or physiological level. Useful alleles exist in both the related and unrelated species of all crop plants.

## CLASSICAL METHODS OF PLANT BREEDING BY USING CROP GENETIC RESOURCES

There are three ways of using Plant genetic resources in plant breeding [47, 48]:

- a. '**Introgression**' - involves the transfer of one or few genes or gene complexes (chromosome segments) from the genetic resources into breeding materials.
- b. '**Incorporation**' (also named 'genetic enhancement' or 'base broadening') - describes the development of new, genetically broad, adapted populations with large variation and acceptable performance level.
- c. '**Pre-breeding**' - refers to more basic research activities with the goal of facilitating use of 'difficult' materials.

Plant genetic resources are essential for sustainable agriculture and food security. Genetic resources may be located *in situ* i.e. within their natural ecosystems and habitats, (often referred to as wild genetic resources) or, in the case of domesticated and cultivated species, in the surroundings where they have developed their distinctive properties. Alternatively, such resources may be located *ex situ*, i.e. outside their natural habitats in seed banks or gene banks for example. Plant breeders have introduced germplasm to achieve various breeding objectives such as induction of early maturity, resistance to biotic and abiotic stresses, cytoplasmic male sterility source and food quality [49 - 53]. Over the past few decades, development of new capacities in the field of molecular biology, functional genomics, expression studies and bioinformatics tools have shown great potential for the development of resistant cultivars and abiotic stress tolerant genes in agriculture. Adugna [52] reviewed the possible utilization of introduced germplasm and indicated that germplasm was used in the improvement of disease tolerance, induction of early maturity and uncovering the high yield potential of Sorghum. DNA-based markers have the obvious advantage of sampling the genome directly. RFLP analysis has been widely used for assessing variation in plants [54, 55]. RFLP analysis has been used to distinguish between species of *Oryza* and particularly between *indica* and *japonica* types of *O. sativa* [24, 56].

## DIVERSITY ANALYSES

The ability to identify genetic variation is indispensable to effective management and use of genetic resources [57]. Traditionally, diversity is assessed by measuring variation in phenotypic traits such as flower colour, growth habit or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. This approach has certain limitations: genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence. In the 1960s, biochemical methods based on seed protein and enzyme electrophoresis were introduced, which proved particularly useful in analysis of genetic diversity as they reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes). Use of biochemical methods eliminates the environmental influence;

however, their usefulness is limited due to their inability to detect low levels of variation. DNA-based techniques introduced over the past two decades have potential to identify polymorphisms represented by differences in DNA sequences. These methods are being used as complementary strategies to traditional approaches for assessment of genetic diversity, the major advantage being that they analyze the variation at the DNA level itself, excluding all environmental influences. The analysis can be performed at any growth stage using any plant part and it requires only small amounts of material. Following the advances in molecular biology in the last decade, a variety of different methods have been developed for analysis of genetic diversity [57]. These methods differ with respect to technical requirements, level of polymorphism detected, reproducibility and cost [58, 59].

### 1.1. Diversity at Phenotypic (Morphological) Level.

The rice plant (*Oryza sativa*) shows great morphological variation, especially in vegetative traits such as plant height and leaf length. Plant breeders are interested to evaluate genetic diversity based on morphological traits because they are inexpensive, rapid, and simple to score. Genetic diversity in plants has been traditionally assessed using morphological or physiological traits. Morphological traits have a number of limitations including low polymorphism, low heritability, late expression and vulnerability to environmental influences [60]. However, morphological characterization is the first step in the classification and evaluation of the germplasm [60, 61]. Qualitative characters are important for plant description [62] and are influenced by consumer preference, socio economic scenario and natural selection.

The study of these traits needs neither sophisticated methods nor complicated equipments, and also these traits can be inherited without specific biochemical or molecular techniques. Until now scientific classification of plant was based on morphological traits [63, 64]. Genetic diversity is the basis of plant breeding for the study of molecular assessment and physiological pathways are important for crop management and crop improvement. Morphological markers generally match to the qualitative traits that can be scored visually. They have been found in nature or as the result of mutagenesis experiments. Chang [17] and Takahashi [65] had recognized three eco-species in *Oryza sativa* i.e., *japonica*, *javanica* and *indica*. Matsuo [66] did the genetic differentiation among these three eco species by morphological and phenological analysis. Kinoshita [67] constructed the first genetic linkage map of rice with morphological markers. These markers are highly influenced by environmental factors. The assessment of phenotype may not be a reliable measure of genetic differences as gene expressions were influenced by environment

Genetic diversity based on genome size among and within plant species has been well documented in the literature [68 - 70]. The variation was pronounced in Chinese germplasm collected from diverse geographic locations. It was attributed to the environmental factors [71], cell size, minimum generation time, cell division rate, growth rate [72, 73], polyploid species, species with large seeds and habits type [74, 75].

In rice, Kuwada [76] first time reported the basic chromosome number ( $n=12$ ) for the study of micro-sporogenesis, mega-sporogenesis and mitosis. Since then many workers have confirmed this chromosome number. The somatic chromosomes of rice, however, are very small and difficult to distinguish from each other. The individual chromosomes are easy to identify at pachytene stage of meiosis. Shashtry et al. [77] described the pachytene chromosome complement for the first time. They numbered the chromosomes in decreasing order of length, with longest as 1 and the shortest as 12. Comparative genomics has revealed a level of conservation in gene content and order within the grasses that surprised even the most experienced geneticists.

### 1.2. Diversity at Biochemical Level.

The genetic markers have made possible a more accurate evaluation of the genetic and environmental components of variation. The biochemical markers are one of the interesting measures of genetic diversity. They include protein techniques and isozymes. Biochemical markers are proteins produced by gene expression. These proteins can be isolated and identified by electrophoresis and staining [78]. Isozyme, a type of biochemical marker, is a protein having different molecular form of the same enzyme that catalyzes the same reaction. They are revealed on electrophoregram through a coloured reaction, associated with enzymatic activity. They are the products of various alleles of one or several genes. The first molecular markers that were initially used extensively to study systematic of plants, animals and insects were isozymes (or isoenzyme). Glaszmann [21] examined 1688 rice cultivars from different Asian countries for allelic frequencies at 15 isozymes loci and analyzed the data by a multivariate analysis. According to Obara-Okeyo et al. [79] and Kannenberg and Grons [80], isozymes, as biochemical markers, have some advantages over the other markers for

- (i) they are still the most cost effective means,
- (ii) interpretation of results is easier due to less noise, and
- (iii) the technique is simpler and still being used effectively.

Because isozymes are post transcriptional markers, their expression is influenced by environmental changes leading to polymorphisms that might not reflect real differences at the molecular level.

### **1.3. Diversity at Molecular/Genetic Level.**

Genetic biodiversity finds its natural resources in wild species for which it is important to find out the amount of genetic variability by morphological, biochemical and molecular markers. Characterization of diversity has long been based on morphological traits mainly. However, morphological variability is often restricted; characters may not be obvious at all stages of the plant development and appearance affected by environment. DNA polymorphisms have become the markers of choice for molecular-based surveys of genetic variation.

Nowadays; a variety of different genetic markers has been proposed to assess genetic variability as a complementary strategy to more traditional approaches in genetic resource management. Recent publications examining the available technologies and their application in the analysis of wild plant populations, germplasm collections and plant breeding [81 - 83]. Two new trends for the germplasm management are facilitating the characterization process. One is the development of core subsets, which are subsets of the germplasm collection at large. These subsets have been established to represent the genetic diversity found within the entire collection [42, 84 - 86]. Larger-scale evaluations can then be applied to the reserve collections with more precision and efficiency. The second new trend involves the manner in which germplasm characterizations are being done. Historically, characterizations of germplasm collections have been carried out at several levels, from descriptions of taxonomy [87], biogeography [88 - 91], morphology and agronomic characters [92] to biochemical analysis [93, 94] and the study of molecular traits and markers [95, 94].

The emergence of new PCR-based molecular markers, such as randomly amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs), has created the opportunity for fine-scale genetic characterizations of germplasm collections that was previously impossible. Since the PCR-based markers are highly polymorphic [96] and simple to process, they result in the generation of relatively large amounts of data per unit of time [97]. The increased level of variation detected with these molecular markers has allowed germplasm managers, plant breeders, and geneticists to pursue with new vigor the important questions related to crops and their genetic resources [98]. Recently, a range of DNA-based markers have been employed for the study of plant diversity [99] and each method has its own benefits and constraints. DNA markers are useful in both basic (e.g. phylogenetic analysis and search for useful genes) and applied research (e.g. marker assisted selection, paternity testing and food traceability). A number of markers are now available to detect polymorphisms.

Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. DNA fingerprinting/profiling is used to describe the combined use of several single locus detection systems and is being used as a versatile tool for investigating various aspects of plant genomes including characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy and plant breeding.

#### **1.3.1. Trends of Marker Technologies.**

Molecular tools provide valuable data on diverse studies in plant biotechnology and breeding program through ability to detect variation at the DNA level. The polymerase chain reaction (PCR) based on molecular markers such as microsatellite (SSR) and inter-simple sequence repeats (ISSR) are being extensively used to study the genetic diversity in a number of plant species at the species as well as cultivar level. DNA markers have also been used extensively to characterize germplasm, a process popularly known as fingerprinting, to evaluate the genetic relationships among accessions (genetic diversity) and provide important information in the areas of ecology, population genetics, and evolution.

In plant biotechnology and their genetical studies on various crops molecular markers, are revealing the polymorphism at the DNA level, has been playing in vital role. There are different types of markers viz. morphological, biochemical and DNA based molecular markers. Morphological, isozyme and molecular markers, such as RFLPs, RAPDs, AFLPs and microsatellites, have been used to determine genetic diversity and phylogenetic relationships in *Oryza* [37, 56, 100 - 103]. The DNA based markers are differentiates in two types: first is non-PCR based (e.g. RFLP) and second is PCR based markers (e.g. RAPD, AFLP, SSR, STS, SNP, etc.). DNA markers are widely used, due to its easy use by simple PCR, followed by a denaturing gel electrophoresis for allele size determination, and the high degree of information provided by its large number of alleles per locus.

Molecular markers provide a powerful tool for the study of genetic diversity and evolutionary relationship detection. The extent and nature of genetic diversity of plants from all around the world has been investigated by DNA markers in a set of individuals belonging to several breeds. With the development of modern molecular biology, molecular markers analysis such as amplified fragment length polymorphism (AFLP), random amplified

polymorphic DNA (RAPD), simple sequence repeat (ISSR), and especially full genome sequencing technique are widely used in the study of biological diversity, genetic transformation and the conservation of genetic resources. Molecular markers present numerous advantages over conventional phenotype based alternatives. The choice and selection of an adequate marker system depends upon the type of study to be undertaken and whether it will be able to fulfill at least a few of the mentioned criteria: (a) highly polymorphic between two organisms, inherited co dominantly, (b) evenly distributed throughout the genome and easily visualized, (c) occurs frequently in the genomes, (d) stable over generations, (e) simple, quick and inexpensive, (f) small amounts of DNA samples required, and (g) no prior information about the sample's genome [104, 105]. Microsatellite data are also commonly used to assess genetic relationships between populations and individuals through the estimation of genetic distances [106 - 110].

Molecular markers such as SSRs form useful tools in assessment of genetic diversity and the use of markers from genic regions may be functionally more informative than the 'anonymous' SSRs that are more widely used. The advent of different molecular techniques led breeders to estimate genetic diversity on the basis of data generated by different molecular markers, which provided a means of rapid analysis of germplasm and estimates of genetic diversity. The markers are now being widely used for evaluating genetic relationships of crop germplasm (Table 1). Since these markers differ in their properties they may result in different estimates of genetic diversity. Comparing results of different molecular genetic diversity estimation methods will be helpful for identifying their utility as parental selection tools for plant breeders [111].

Table 1: Estimate the genetic diversity in several crops with different molecular markers.

Sl. No.	Mol. Markers	Crop species	Genetic diversity	References
1.	RAPD vs. SSR	<i>Zea mays</i> (Maize)	Both techniques were efficient	[112]
2.	RAPD vs. SSR	<i>Glycine max</i> (Soybean)	Combined used of both marker was useful and more reliable for estimating genetic diversity	[113]
3.	RAPD vs. AFLP	<i>Oryza sativa</i> L. (Rice)	RAPD was found superior than AFLP	[114]
4.	RAMP vs. SSR	<i>Hordeum vulgare</i> ssp. Spontaneum (Wild Barley)	Estimates given by two marker system were different; RAMP gave slightly better estimates; Genetic variation was revealed by different mechanism	[115]
5.	Phenotypic vs. AFLP	<i>Saccharum</i> spp. (Sugarcane)	AFLP-based genetic diversity was more useful	[116]
6.	Isozyme vs. RFLP	<i>Zea mays</i> (Maize)	RFLP's were superior to identify the origin of an individual on	[117, 118]
7.	Phenotypic vs. AFLP	<i>Triticum aestivum</i> (Wheat)	AFLP-based genetic diversity was more useful clus	[111]
8.	PCR based marker vs. RFLP	<i>Pisum sativum</i> (Pea)	All PCR-based markers were better than RFLP	[119]
9.	RFLP vs. RAPD	Cruciferous species	Similar to show intraspecific relationship RFLP were better while depicting inter specific variation	[120]

Several molecular marker techniques are employed to assess genetic diversity in various crops. In rice, restriction fragment length polymorphism (RFLP) [121], amplified fragment length polymorphism (AFLP) [122], random amplified polymorphic DNA (RAPD) [123], simple sequence repeats (SSR) [124, 125], and single nucleotide polymorphism (SNP) [126] have all been used on a large scale. There are different marker systems for studying genetic diversity in different contexts [127]. Genetic diversity and the relationships among different varieties have great importance for the conservation of genetic resources, breeding, national and international exchange of germplasm [128]. The diversity of genetic material is contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. Genetic diversity provides farmers and plant breeders an options to develop, through selection and breeding, new and more productive cultivars, that are resistant to virulent pests and diseases and adapted to changing environments.

The analysis of diversity data is based on population genetic theory and mainly requires allele frequency data. Initially, diversity studies were based on morphological and agronomical traits. The increasing availability of molecular marker systems opened up new possibilities for the diversity assessment of PGR intended to be used for crop improvement [58, 129]. For an efficient diversity assessment, molecular markers ideally need to be selectively neutral, highly polymorphic, codominant, well-dispersed throughout the genome, and cost- and labour-efficient [129, 130]. The different methods of molecular assessment differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and cost. Depending on the need, modifications in the techniques have been made, leading to a second generation of advanced molecular markers.

Recent decades indicated that, applications in molecular genetics methods for assessing the conservation and use of plant genetic resources have increased. Molecular techniques have well developed, for the analysis of specific genes and variations in allelic levels, as well as to increase understanding of gene action, generate genetic maps and assist in the development of gene transfer technologies. The most useful techniques as micro-satellites, RFLP, RAPD, SSR, AFLP, SCAR and ISSR have been utilized for the study of genetic diversity analysis. Restriction fragment length polymorphism (RFLP) has been intensively used for the investigation of genetic diversity and relationships of crop species and their wild relatives. DNA-based markers have the obvious advantage of sampling the genome directly and RFLP analysis has been widely used for assessing variation in plants [53, 54]. RFLP analysis has been used to distinguish between species of *Oryza* and particularly between *indica* and *japonica* types of *O. Sativa* [24, 55]. Genetic diversity and evolutionary patterns have been illustrated in many crops, such as wheat, barley and rice [121, 131]. Identification, uses and conservation for the genetic diversity within crop germplasm resources are important for their sustainable use in plant breeding. The contribution of some workers regarding genetic divergence in rice is summarized as below in Table 2.

Table 2: Molecular marker based study of genetic divergence in rice varieties/germplasm.

Sl. No.	Utilizing Germplasm	Markers analysis tools	References
1.	<i>Oryza glaberrima</i> germplasm from Mali	SSR markers	[132]
2.	Rice varieties rich in iron and zinc in its endosperm	Microsatellite	[133]
3.	Pakistani rice landraces	Microsatellite	[134]
4.	Sarawak Bario rice	SSR	[135]
5.	Venezuelan rice cultivars	SSR	[136]
6.	Traditional lowland rice of Assam	RAPD/ ISSR	[137]
7.	Kalanamak of Uttar Pradesh	RAPD	[138]
8.	Temperate japonica and Tropical indica rice genotypes	AFLP/ISSR/RAPD	[139]
9.	Long grain Indian aromatic rices	SSR	[140]
10.	Wild rice of China	SSR	[141]
11.	Indian land races of rice	RAPD	[142]
12.	Cultivated rice	Fluorescent labeled Microsatellites	[143]
13.	Aromatic rices	RAPD	[144]
14.	Indian scented rice germplasm	RAPD	[145]

### 1.3.2. Marker-Assisted Breeding/Selection (Molecular Breeding).

Molecular markers may be used in several ways to make the plant breeding process more efficient. The adoption of a marker-assisted selection (MAS) or marker-aided selection (MAS) in a breeding program hinges on the availability of useful molecular marker. Fortunately, this resource is becoming increasingly available to many species, thanks to the advances in biotechnology. This breeding approach is applicable to improving both simple and complex trait, as a means of evaluation of a trait that is difficult or expensive to evaluate by conventional methods. The basic requirement is to identify a marker that co-segregates with a major gene of the target trait. MAS are much beneficial to breeding quantitative traits with low heritability. By MAS, molecular breeding novel technologies must be developed to hasten breeding program through the improved genotyping and phenotyping facilities for the analysis of genetic diversity in core germplasm and mapping populations [146, 147]. The key steps in the implementation of MAS in breeding quantitative trait loci (QTL) are:

- i. Creation of a dense genetic map of molecular markers.
- ii. Detection of QTLs based on statistical association between markers and phenotypic variability.
- iii. Definition of a set of desirable marker alleles based on the results of the QTL analysis.
- iv. The use of and/or extrapolation of this information to the current set of breeding germplasm to enable marker-based selection decisions to be made.

However, as these researchers noted, MAS for more complex traits is still challenging, partly because of the difficulty of effective detection, estimation, and utility of QTLs and their effects. The problem is more significant with more complex traits (such as grain yield) that are controlled by many genes under the influence of epistasis (gene-by-gene-interaction) and gene-by-environment (G×E) interaction effects. On the contrary, most researchers engaged in evaluations of mapping and MAS tend to assume that QTLs act independently (i.e. no interaction with other genes and/or environment). To overcome this problem, Podlich and colleagues proposed a new approach to MAS from the conventional one, which assumes that desirable QTL alleles, one identified, will remain relevant throughout many cycles of selection during plant breeding. In the conventional breeding, researchers tend to estimate QTL effects at the beginning of the project and continue to apply the estimates to new germplasm created during the breeding process. The assumption of fixed QTL values is appropriate if the traits are controlled by additive genes. This will allow MAS to be conducted by independently assembling or stacking desirable alleles. This assumption is not applicable to situations in which context dependencies occur. On such occasions, the value of QTL alleles can change depending on the genetic structure of the current set of germplasm in the breeding program. These progressive changes in genetic structure may make the initial combinations of alleles no longer the best target or no longer significant in increasing the trait performance in future breeding cycles.

The development and use of molecular markers for the detection and exploitation of DNA diversity is one of the significant achievements in the field of molecular genetics which accelerate breeding by establishment of molecular fingerprints for distinct and most divergent genotypes using diversity analysis. DNA based markers could use to assist breeding through Marker Assisted Selection (MAS) to select prospective genotypes in a variety of plant species such as *Jatropha curcas* [148], *Citrus* [149], *Fritillaria imperialis* [150], *Ricinus communis* [151], *Hordeum vulgare* [152] and *Pongamia pinnata* [153]. Besides these, the DNA based markers have also been widely used as a tool for assessing genetic diversity in number of insect and pest species [154].

The advances in phenomics approaches, including instrumentation, robotics and computational software, have made high-throughput phenotyping feasible. Equally important is the possibility of collecting and analyzing various environmental factors that affect the field trials. Now whole-genome strategies facilitate effective design and implementation of molecular marker-assisted plant breeding by bringing together all the relevant information about genotypes, phenotypes and the environments. Although molecular breeding through gene transfer and marker-assisted selection (MAS) has been successful in the private sector (especially in the multinational corporations), its wider use, particularly in the public sector institutions in the developing world, is still limited by several bottlenecks [155 - 157]. The constraints include limited availability of cost-effective and high-throughput genotyping systems, less understanding of genetic architecture of complex traits, unsuitable molecular techniques. The current rapid development of bioinformatics, genomics, and molecular biology as well as conventional breeding methods provides useful means to mine the desirable genes in the resources. Many of the agronomical important regions, biotic and abiotic stress tolerance genes have been further characterized (e.g. by map based cloning and genome sequencing) and even manipulated (via genetic engineering and marker-assisted selection) in breeding programs [158]. These molecular and genomic efforts will continue and will even be enhanced by newer molecular techniques such as High throughput sequence technology, DNA arrays and transcriptome analysis [159]. The potential uses of modern biotechnological and in-silico analysis (bioinformatics tools) genome information will lead to greater impact in plant breeding development. A schematic representation for utilization of plant genetic resources in crop improvement through marker assisted selection (MAS) and advance genomic technologies is presented in Figure 1.

However, the development of a second generation of molecular markers based on the sequence information is more amenable to application in plant breeding programs [160]. On the other hand, breeding by MAS was carried out with success in the case of several legume crops [161], transgenic *Bt* plants [158], etc.

#### 1.3.2.1. QTLs selection for MAS.

Although, MAS has proven success in the case of simple trait controlled by major genes, the availability of molecular markers and genetic maps make it feasible also for QTL [161]. Theoretically, all markers that are tightly linked to QTLs could be used for MAS. However, due to the cost of utilizing several QTLs, only markers that are tightly linked to no more than three QTLs are typically used [162], although there have been reports of up to 5 QTLs being introgressed into tomato via MAS [163]. Even selecting for a single QTL via MAS can be beneficial for crop

improvement strategies; such a QTL should account for the largest proportion of phenotypic variance for the specific trait. Furthermore, all QTLs selected for MAS should be stable across environments [164].

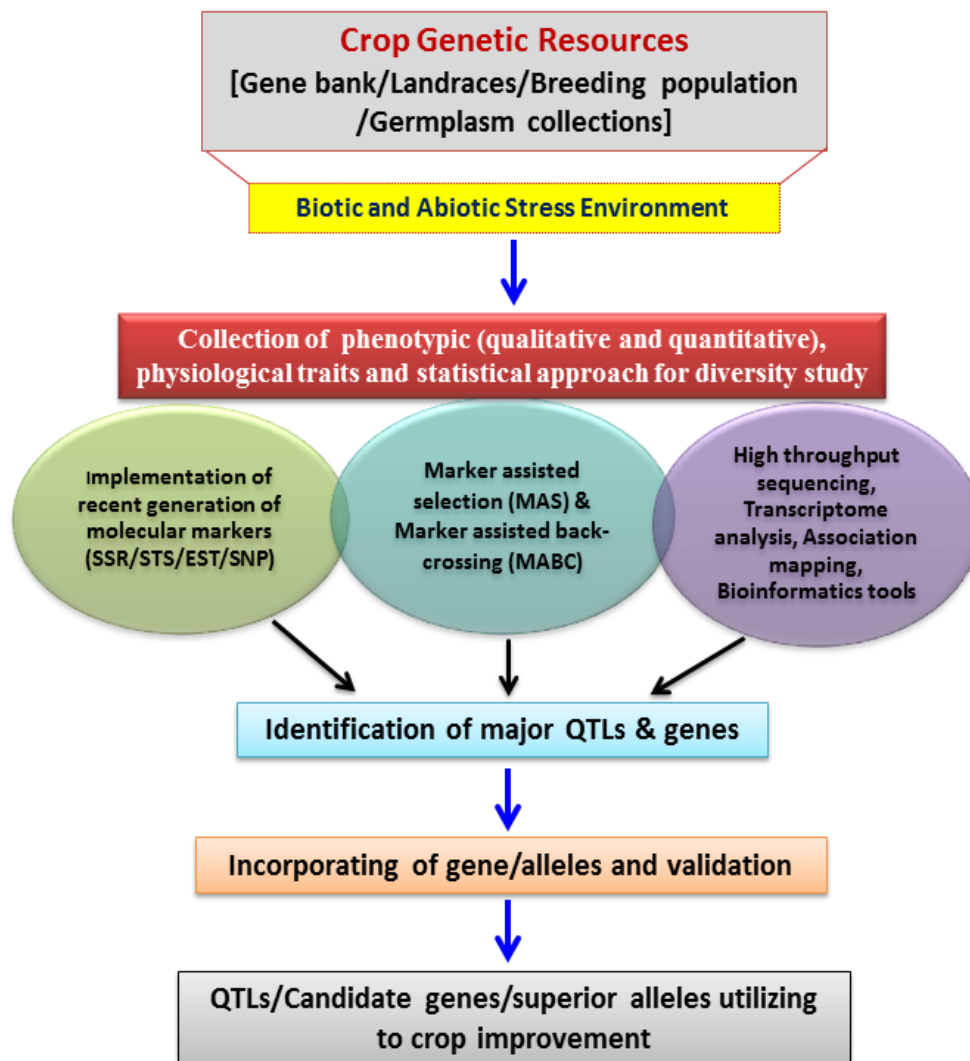


Figure 1: A schematic representation for utilization of plant genetic resources in crop improvement through marker assisted selection (MAS) and advance genomic technologies.

#### 1.3.2.2. Effective Marker–Trait Association (MTA) and Marker Validation.

The identification of the new QTL has been increasing tremendously and this was very much clear from the past two decades' publication scenario in Pubmed. Now this involves almost all crop plants and all types of agronomic traits [165]. However, reports of QTL mapping to date have tended to be based on individual small to moderately sized mapping populations screened with a relatively small number of markers, providing relatively low resolution of marker–trait association or MTA [166]. Very few of the QTLs reported have been used for MAS. Most MTA reports to date have been based on segregating populations generated, in most cases, from two inbred lines. Genetic variation detected in the mapping population (particularly recombination patterns in the region of the target gene) may not be shared by other genetic and breeding populations because of allelic diversity. Thus, QTL markers identified using a single mapping population may not be automatically used directly in unrelated populations without marker validation and/or fine mapping [167]. The MTA must be validated in representative parental lines, breeding populations, and phenotypic extremes before it can be used for routine MAS, although this process may be incorporated into genetic mapping programs. In a portion of cases, markers will lose their selective power during

this validation step. In these cases, the most suitable approach is to identify new markers (through fine mapping or candidate gene analysis) in the genomic region around the target locus to find MTAs that are shared across different breeding populations. By developing several markers within or around a single gene, it is much more likely that the parents of any breeding population will be polymorphic for at least one of them. This will then allow breeders to track the alleles donated from each parent throughout the breeding process, speeding up MAS and marker-assisted breeding (MAB) in any cross.

#### 1.3.2.3. Multi Locational High-Throughput Phenotyping.

The quantity and quality of phenotyping is becoming the most significant factor affecting the accuracy of genetic mapping and thus the power of the resultant MAS, particularly for complex traits. However, precision and global phenotyping of a large number of plant samples is very expensive and time-consuming. The level of heritability of measured traits depends in part on whether the phenotyping can be repeated across different seasons, locations, and environments. Clustering target locations into mega-environments and comparing these with the success of selection at different locations has been used to understand how breeding programs can optimize their selection processes to generate germplasm with the best yield and other agronomic characters for specific target environments [168]. Cross-population and environment comparison of phenotyping will determine how the MTAs identified under one environment can be used for selection under another. In this case, well-characterized environments and well-established selection criteria are essential prerequisites for the development of a reliable precision phenotyping system. Precision and high-throughput multilocational phenotyping, together with effective sampling and data acquisition systems being developed for many traits, provide the potential to develop a phenomics-based protocol for trait-specific improvement programmes. This will not only help our understanding of the phenotypic profile that a plant possesses, but also it can improve the precision of genetic mapping and thus MAS for the target phenotype.

#### 1.3.2.4. Marker-Assisted Backcrossing (MAB).

Using conventional methods, it typically takes 6–8 backcrosses to fully recover the recurrent parent genome. The theoretical proportion of the recurrent parent genome after  $n$  generations of backcrossing is given by:  $(2n+1-1)/2n+1$  (where  $n$  = number of backcrosses; assuming an infinite population size). The percentages are usually lower in smaller population sizes that are typically used in actual plant improvement programs. Although the average percentage of the recurrent parent genome is 75% (for the entire  $BC_1$  population), some individuals possess more of the recurrent parent genome than others. Therefore, if tightly-linked markers flanking QTLs and evenly spaced markers from other chromosomes (i.e. unlinked to QTLs) of the recurrent parent are used for selection, the introgression of QTLs and recovery of the recurrent parent may be accelerated. This process is called marker-assisted backcrossing. The use of additional markers to accelerate cultivar development is sometimes referred to as 'full MAS' or 'complete line conversion' [169]. Simulation studies using PLABSIM—a computer program that simulates recombination during meiosis—indicate that efficiency of recurrent parent recovery using markers is far greater compared to conventional backcrossing. This is an important consideration for researchers because the accelerated release of an improved variety may translate into more rapid profits by the release of new cultivars in the medium to long-term.

#### 1.3.2.5. Selective Genotyping and Pooled DNA Analysis.

There have been two broad types of approaches to identifying MTA. The first is based on genotyping an entire segregating population with markers densely covering the entire genome, and then testing for associations between phenotypic differences and marker genotypes. The genotyping for this approach is extensive, time-consuming, and expensive, while generating precision phenotype data at this scale may be logistically difficult or even impossible. The second approach is based on genotyping only that part of the population exhibiting extreme phenotypes for the target trait (selective genotyping), association is then inferred by finding allelic frequency differences between the groups of plants with contrasting phenotypes. Combining DNA pool analysis with selective genotyping (so-called bulked segregant analysis) is then the simplest approach to identifying markers for major genes, because it requires analysis of only two DNA pools representing the two phenotypic extremes. Pooled DNA analysis has been very successful in genetic mapping in plants using RFLP and SSR markers with numerous reports for single major genes [170, 171] and even two to three major QTLs. Developments in single nucleotide polymorphism (SNP) genotyping technologies and methodologies offer a vision of future possibilities for molecular plant breeding. It is possible to carry out genome-wide association mapping by using an integrated technology package including selective genotyping, pooled DNA analysis, and microarray-based SNP genotyping with numerous markers. This system has the power to estimate allele frequencies and identify unique alleles from a pooled DNA sample of several

individuals. When this approach can be successfully implied, it will resolve many of the constraints of bulked segregant analysis described above [172]. Allele frequencies can be estimated either by collating individual genotypic scores when genotyping is based on individuals or by signal strength comparison when genotyping is based on pooled DNA samples. As high density genome-wide SNP markers will soon be available in many crop species, it is now possible to narrow down the target locus to less than 1 cM in one step using selective genotyping alone. However, optimizing SNP genotyping systems for pooled DNA analysis is considerably more complicated than for SSR markers and suffers a much higher level of redundancy. The high density of SNP markers will soon be available in rice and maize (amongst cereals). Pooled DNA analysis will also be very useful for large-scale analysis of landraces that are highly heterogeneous and thus to date not well characterized.

With recent advances in genomics, the MTA analysis is increasingly the phenotyping and not the genotyping. Thus, it may be more efficient to genotype the whole population first to identify the most informative subset of individuals in terms of minimum level of relatedness between individuals plus optimum subpopulation structure and allele representativeness; then, carry out precision phenotyping of this subset, particularly for the traits that are difficult or expensive to evaluate. A final refinement to achieve maximum efficiency would be to combine selective phenotyping with selective genotyping thereby focusing on a very small proportion of the total number of genotypes for the full analysis but ensuring that these are the most informative plants or families. In this approach, the total number of individuals to be phenotyped and genotyped may not change, but the power of the analysis will be dramatically increased. This would be most effective for traits where phenotypic extremes can be easily identified using a simple screening method. For example of abiotic stress tolerance, where a large number of plants can be eliminated easily under stress conditions through visual symptoms scoring. Although the level of stress and the selection threshold need to be carefully optimized to maximize the probability of finding genes that confer economic tolerance (with little or no yield penalty) as opposed to survival traits (with a substantial negative effect on yield). Following selective genotyping of these individuals with extreme phenotypes, precision phenotyping of the resultant subset of individuals can be performed using physiological component and surrogate traits. High-density planting and selection at early stages of plant development, combined with selective phenotyping and genotyping should also be investigated as a potential option for some traits to allow one to work with more plants or families. Where the target trait is influenced by planting density or strong selection pressure this will clearly confound the ability to make genetic gain. However, many major gene-controlled traits can be investigated in this way without much disturbance. It can be inferred that phenotypic extremes or extremely stress-tolerant plants are those with an accumulation of favorable alleles from multiple loci, each with small to large effects, so that genetic mapping, particularly with relatively small populations, will identify the genetic regions with relatively large accumulative effect on the target trait. This is supported to some extent by results on drought tolerance in rice [173]. When allele frequencies can be estimated from the selected individuals or the DNA pools on which genotyping is based, the putative gene locations can be identified based on the comparison of allele frequencies, even if markers have distorted segregation ratios. However, when only one extreme (only survivors or most resistant individuals) is available, which might be true in the case that no susceptible individuals survive, or reliable estimation of allele frequencies is not possible, a control population is needed to distinguish selection effects from segregation distortion. Genome-wide linkage disequilibrium (LD)-based association mapping may provide a shortcut to discovering functional alleles and allelic variations that contribute to agronomic traits of interest. The Generation Maize is being screened via resequencing and precision phenotyping to test the feasibility of the whole "population" based approach for simply inherited traits. Thus, the selective genotyping and pooled DNA analysis discussed here can be extended to using extremes of inbreeds selected. For association mapping of quantitative traits governed by a large number of minor genes which interact with each other and the environment, through either whole population analysis or selective genotyping, we will face the same challenges as experienced with linkage-based QTL mapping. Trait-specific genetic and breeding materials, with novel properties including phenotype extremes, eternal or fixed segregating populations (e.g., recombinant inbred lines, doubled haploids, near isogenic lines (NILs), introgression lines), genetic stocks (e.g., single segment substitution lines), and mutant libraries, have been developed and maintained by many groups across the world spanning most crops. These are valuable directly for the purpose they were developed but also offer a powerful opportunity when used collectively. In many cases, they have been phenotyped in multiple environments by taking advantage of their permanent property and fixed segregation pattern. By collecting phenotypic extremes from currently available genetic and breeding materials, and using selective genotyping and pooled DNA analysis, one 384 plate could be used for genetic mapping of almost all important major gene- or QTL-controlled traits. This assumes that each trait needs only two wells and that in one plate it is possible to carry out 192 pairwise DNA pool-based comparisons. When such existing materials are used collectively and combined with LD mapping, they will also provide a shortcut toward one-step genome-wide association mapping for all target traits [174].

### **1.3.3. Allele Mining in Germplasm.**

The diversity of crop genetic resources has greatly contributed to the genetic improvement for many crops and given important effects on continuing production of crops. Present challenges that modern plant breeders face are, to develop higher yielding, nutritious and environmentally friendly varieties that improve our quality of life without harnessing additional natural habitats to agricultural production. The improvement of cultivated plants considerably depends on the extent of genetic variability available within the species. It is very important to set up a collection, conservation, utilization and research system of crop genetic resource.

Recent trends in technological developments in the area of genome sequencing, expression analysis and genotyping serve to redefine the scope of germplasm classification. Further, precise phenotyping of germplasm accessions belonging to natural population will facilitate marker-trait association mapping, and also for mining rare alleles of genes and controlling important traits through allele mining approach.

Allele mining has focused on the identification of SNPs/InDels at coding sequences or exons, Non coding sequence or intron of the gene. The variation of the exon region was expected to affect the encoded protein structure and/or function. Kumar et al. [175] demonstrate the sequence variations in genic regions to altering the phenotyping. However, recent reports indicate that the nucleotide changes in non-coding regions (5' UTR including promoter, introns and 3' UTR) also have significant effects on transcript synthesis and accumulation which in turn alter the trait expression.

The key developmental challenges for molecular markers now lies in developing new breeding strategies where the objectives will be increasing the germplasm base and increasing the number of traits that can be effectively selected simultaneously. The new marker technologies that offer greatly reduced costs in marker screening and high multiplexing capabilities will be central to these developments. However, it will be better to pay attention to the development of vast amount of genomic resources in every crop. This will help to develop the breeder-friendly molecular markers like SSR, STS, and SNP and improve genetic understanding of complex traits, relations among traits, and between target trait and environment.

Exploitation of gene banks for efficient utilization depends on the knowledge of genetic diversity, in general, and allelic diversity at candidate gene(s) of interest, in particular. Hence, allele mining seems to be a promising in characterization of genetic diversity or allelic/genic diversity among the accessions of the collection in terms of its utility for improving a target trait [176]. Mutations create new alleles or cause variations in the existing allele and allelic combinations. They take place in coding and non-coding regions of the genome either as single nucleotide polymorphism (SNP) or as insertion and deletion (InDel). There are two major approaches for the identification of sequence polymorphisms for a given gene in the naturally occurring populations: (1) modified Targeting Induced Local Lesions in Genomes (TILLING) procedure and (2) sequencing based allele mining. The availability of sequence and sequence variation that affects the plant phenotype is of greatest importance for the utilization of genetic resources in crop improvement [177]. The existing allelic diversity in any crop species is caused by mutations, the evolutionary driving force [175]. The high-through genotyping platforms with new generation sequencing technologies are rapidly evolving. This will certainly provide cheap and large scale screening of segregating individuals for selecting desirable genotypes.

### **1.3.4. Gene Identification in Crop Species.**

The sequencing and assembly of large complex crop genome remains a valuable goal, but at the moment, a significant amount of knowledge can be gained from low coverage shotgun sequencing of these genomes. In this contest, the second generation sequencing technologies are particularly suitable to know genes and gene promoters in crop plants that are homologous to related species and genera. Therefore, designing polymerase chain reaction (PCR) primers to the read pairs enables the amplification and sequencing of the gene and corresponding genomic region in the target species. This approach to gene discovery offers the potential to identify genes, gene promoters and polymorphisms in a wide range of agronomically important crop species [178]. Microarray represents functional genomic approaches that have revolutionized global gene expression profiling. In fact they allow studying the entire gene complement of the genome in a single experiment [179, 180]. At the moment, cDNA and oligonucleotide microarrays have been widely used in plants, such as *Arabidopsis*, rice, maize, strawberry, petunia, ice plants and lima bean, to study and compare global gene expression levels in specific organs and/or tissues under controlled physiological conditions.

### **1.3.5. Bioprospecting and Xenogenomics Diversity.**

Genetic material comprises any material of plant, animal, microbial or other origin containing functional units of heredity. It provides valuable leads for novel genes/product development and many research institutes look for new

applications of biological species that have not been studied earlier. Bioprospecting plays a dominant role in discovering leads for useful target traits development [181]. Nature can provide with original novelty and complexity that can be modified in the laboratory.

The term 'xenogenomics' refers to the discovery and functional analysis of novel genes and alleles from indigenous and exotic species, where as bioprospecting of biodiversity using high-throughput genomics experimental approaches. Such a program has been initiated for the determination of molecular genetics, physiology and biochemical pathways against to abiotic stress tolerance. Ulrik et al. [182] reported utilizing the indigenous and exotic germplasm for prospecting of novel genes/allele mining for stress tolerance. Biodiversity prospecting can be divided into three phases as collecting information about species/samples, utilization of molecular markers for genome analysis and validation with primary requirement food crops/commercialization. Highly efficiency and sensitive techniques developed to improved analysis, making the screening of natural cultivars. These are now being applied to existing collections, and as a result, novel and naturally occurring useful genes as greater nutrient efficiency, tolerance to abiotic stresses and improved quality.

## CONCLUSIONS

Rice is an excellent system for plant genomics as it represents a modest genome size of 430 Mb. It feeds more than half the population of the world. Utilizing the genetic resources is one of the best approaches for the identification of gene/alleles against to biotic and abiotic stresses. In addition, the efficient use of genetic resources in all plant-breeding programs requires knowledge about genetic diversity. Within the last twenty years, molecular biology has revolutionized conventional breeding techniques in all areas. Morphological, cellular, biochemical (proteins and isozymes) and molecular markers have been used on the wide scale for the study of the genetic diversity of the cultivated and wild relatives of rice.

Molecular marker technology has well developed over the last few decades and is beginning to be incorporated various genes into different cultivars through the breeding programs in worldwide. The markers have been utilized in various applications including examination of characterization genetic relationships between individuals, identification of biotic, abiotic stress tolerance genes, and allelic variation among the genotypes. Exploitation of plant germplasm for efficient utilization depends on the knowledge of genetic diversity, in general, and allelic diversity at candidate gene(s) of interest, in particular. The beneficial alleles from vast rice genetic resources existing worldwide were derived from cultivated germplasm. However, a significant portion of these beneficial alleles still reside in the wild rice germplasm. Nowadays, considerable attention has been focused on allele mining (gene polymorphisms) and their potential use to alter protein function in ways that might prove biologically important. But increasing numbers of polymorphisms are also being identified in the regulatory and non coding regions of genes. Therefore, allele mining is a promising approach to dissect naturally occurring allelic variation at candidate genes controlling key agronomic traits which has potential applications in crop improvement programs. Allele mining can be effectively used for discovery of superior alleles, through 'mining' the gene of interest from various plant germplasm. It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles. In addition, the rate of evolution of alleles; allelic similarity/dissimilarity at a candidate gene and allelic synteny with other members of the family can also be studied. Awareness of the level of genetic diversity and the proper management of genetic resources are important issues in present scenario. Molecular characterization can play an important role in uncovering the history, and estimating the diversity, distinctiveness and population structure. Recent advances in DNA sequencing technology are radically changing biological and biomedical research and will have a major impact on crop improvement. The new information on genome sequence will be very useful to identify genes involved in agronomical traits that could be used to improve the nutritional characteristics and the productivity of this crop.

New markers deriving from DNA technologies are valuable tools to study genetic variability for conservation purposes. The knowledge of genome nucleotide sequences also could be useful to identify new sequence polymorphisms, which will be very useful in the development of new variety-specific molecular markers and in the implementation of more efficient protocols for tracking useful genes from the genetic resources.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this review paper.

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