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Full Length Review Article

Overview: Morphological and Molecular Markers role in Crop Improvement Programs

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The knowledge of existing plant genetic diversity is crucial for effective management of crop genetic resources. The variability obtained in the genome of a species can be grouped in to visible and non visible characteristics. Ethino-botanical classification, morphological, biochemical and molecular characterization are schemes used for measurement of genetic diversity. Morphological characterization is highly recommended at the beginning prior to biochemical and molecular studies. Morphological markers allow assessment of genetic variability based on individual phenotypic difference yet there are limitations associated to these markers. These limitations led to the development of molecular markers. Molecular marker techniques are based on naturally occurring polymorphisms in DNA sequences. Thus, these markers had several advantages over conventional phenotypic characterization.

Key words: Genetic diversity, Crop improvement, Characterization, Morphological and Molecular markers

INTRODUCTION

Awareness of existing genetic diversity in plant population is fundamental for basic science and applied aspects like the efficient management of crop genetic resources. The improvement of crop genetic resources is dependent on continuous infusions of wild relatives, traditional varieties and the use of modern breeding techniques. These processes all require an assessment of diversity in order to select resistant, highly productive varieties. Genetic diversity can be measured using morphological, biochemical characterization and evaluation (Mondini *et al*, 2009). The first category which refers to characterizing visually detectable variability includes morphology and structure of plants. This is primarily for their botanical and taxonomical classification, Characteristics that affect their agronomic management and production, Reaction to biotic and abiotic environmental condition. This is the most significant steps determining the utilization of a collection (Phundan, 2000). Characterization may be defined as the scoring of characters that can be easily detected and have high heritability based on the form and structure of the organism, especially their external form (Perrino and Monti, 1988 quoted by Woyessa, 2006). These are complementary activities that describe the quantitative and qualitative attributes of the accessions of a given species to differentiate them. These activities use descriptors for characters that are considered to be important and or useful to describe a single population of species (Benjamin *et al*, 2008). The second category characterizes variability that is not detectable by simple visual observation. Here, the assessment of genetic diversity within and between populations is routinely performed at molecular level using various laboratory based techniques (Mondini, *et al*, 2009). Generally there are four methods of measuring genetic diversity namely farmers perception and folk (ethino-botanical) classification, morphological characterization, biochemical characterization and molecular characterization (Hoogendijk & Wiliams, 2001).

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Morphological characterization

As a scientific discipline, morphological characterization is originated by Goethe in 1790 **(**(Donald, 2001). It is carried out on a representative population of an accession using a list of descriptors for the species (Benjamin *et al,* 2008*)*. Principal Component Analysis (PCA) of the characterization results will identify a few key or minimum descriptors that effectively account for the majority of diversity observed. This saves time and effort for future characterization effort. This approach has been used successfully for different types of crops in different countries (Otoo *et al.*, 2009). A character is a feature of an organism that can be measured, counted or assessed (Heywood, 1967). Characters build the central theme of any study concerned with identification and classification of the organisms. Characters may not always be of equal value for the purpose of comparison (Pankhurst, 1991). Characters are chosen on criteria based on their ease for observation, availability and usefulness in classifying and identifying organisms. Some characters are not modified by environmental factors and have a genetic basis such that they are unlikely to change readily (Heywood, 1967; Jeffry, 1996; and Deborah, 1998). These may be referred to as constant characters and are highly heritable. In contrary, other characters are easily modified, to a greater or lesser extent, by the environment. Their phenotypic expression is the product of the combined effect of the environment and the genotype.

According to Donald (2001), there are four major areas of studies in plant morphology and each study overlaps with another field of the biological sciences.

- Morphological characterization examines structures of different plants of the same or different species, then draws comparisons and formulates ideas about similarities. When similar structures in different species are believed to exist and develop as a result of common, inherited genetic pathways, those structures are termed homologous. This aspect of plant morphology overlaps with the study of plant evolution.
- The second area of plant morphology observes both somatic and reproductive structures of plants. The somatic structures of plants include study of shoot system and root system. Plants reproductive structures are more varied and are usually specific to a particular group of plants. The detailed study of reproductive structures in plants led to the discovery of the alternation of generations found in all plants. This area of plant morphology overlaps with the study of biodiversity.
- The third area of plant morphology studies plant structure at a range of scales. Plant cells structural feature only visible with the aid of an electron microscope. At this scale, plant morphology overlaps with plant anatomy.
- The fourth area of plant morphology examines the pattern of plant development starting from its seedling till maturation. Plants constantly produce new tissues and structures throughout their life. Morphological characterization studies this process, its causes and results. This area of plant morphology overlaps with plant physiology and ecology. **g**

It is evident that plant morphology would contribute to plant genetics in the characterization of the phenotype. But, there are limitations that are associated with morphological markers. Its major drawback is high dependency of this marker to environmental factors (Stuber *et al,* 1999). The limitations of phenotype based genetic markers led to the development of molecular markers. These markers may or may not correlate with phenotypic expression of a trait (Sonnante *et al*, 1994; Akkaya *et al*, 1995).

Molecular markers

Molecular marker techniques are based on naturally occurring polymorphisms in DNA sequences (Wetermeier, 1993). The concept of genetic markers is started in the nineteenth century by Gregor Mendel who employed phenotype based genetic markers in his experiments. Later, phenotype based genetic markers for Drosophila melanogaster led to the founding of the theory of genetic linkage (Barcaccia *et al*, 2000; Milee, 2008). Plant breeding has witnessed a revolution due to emergence of molecular breeding. It is a subject which deals with all aspects of plant molecular biology that uses in crop improvement programmes. Molecular breeding consists of two major areas which are the transgenic crops and the molecular marker technology. However, molecular marker technology has been more preferred in plant breeding programmes. Molecular marker technology is user friendly and no bio-safety or bioethics questions raised against transgenic crops. In fact, due to its high cost and non availability of high throughput approaches for handling large segregating populations have limited the use of molecular marker technology for plant breeding. Though, now a day's more efficient molecular marker systems which are inexpensive and involving high throughput detection systems are being developed (Gupta *et al*, 2001). Molecular markers can be considered as constant landmarks in the genome. They cannot be considered as a gene since they do not have any known biological function. They are only identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. They work by either measuring directly or indirectly a specific DNA sequence difference between various genotypes. They rely on DNA assays in contrast to morphological markers and biochemical markers that are based on visible traits and proteins produced by genes respectively (Semagn *et al*, 2006; Marica, 2008).

In General, Molecular markers are well established and their applications as well as limitations have been realized. Molecular markers offer numerous advantages over conventional phenotype characterization. They are stable and detectable in all tissues regardless of growth differentiation, development, pleiotropic effect, epistatic effects and not confounded to environment where they grow (Milee *et al*, 2008). These techniques provide opportunities to obtain high amplification of genetic traits for the development of genetic maps, variety identification and for the analysis of important morphological and agronomic traits (Fatokun *et al*., 1997; Tostain *et al*, 2003; Dumont *et al,* 2005). In addition, these markers reveal a high level of polymorphism on plant materials (Sonnante *et al*, 1994; Akkaya *et al*, 1995). A perfect molecular marker technique should have the following criteria (Semagn *et al*, 2006; Milee *et al*, 2008; Mondini *et al*, 2009) (1) polymorphic and evenly distributed throughout the genome (2) provide adequate resolution of genetic differences (3) generate multiple, independent and reliable markers (4) simple, quick and inexpensive (5) need small amounts of tissue and DNA samples (6) have linkage to distinct phenotypes (7) require no prior information about the genome of an organism (8)Transferability between laboratories

However, no molecular markers are present that perform all these requirements. Techniques differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and cost (Semagn *et al*, 2006; Milee *et al*, 2008). Therefore, it is important to choose a technique for a particular study based on the level of diversity information needed, expected level of variation, accessibility of probes and primers site, the time constraints of a specific project, the level of operational and financial investment available (Karp *et al*., 1997). It is also important to consider whether a single technique can provide all of the required information. Otherwise, optimization will be needed for a combination of different techniques (David & Arvind , 2006).

Types of Molecular markers

Non-PCR based genetic markers

Restriction fragment length polymorphism (RFLP)

RFLP was the first reported molecular marker technique in the detection of DNA polymorphism for the construction of genetic maps (Millee *et al,* 2008). Restriction enzymes are

endonucleases produced by a variety of prokaryotes. Their natural function is to destroy invading, foreign DNA molecules by recognizing and cutting specific DNA sequence motifs. They have mostly four to six bases. Each enzyme has specific, typically palindrome recognition sequence. Bacteria usually protect their own DNA from being cut by methylating the cytosine or adenine residues within this sequence. Digestion of a particular DNA molecule with a particular restriction enzyme results in a reproducible set of fragments of well defined lengths (McClelland *et al*, 1994; Kurt, 2005). Mutations in the plant DNA sequence leads to simple or large base pair changes as a result of inversion, translocation, transpositions or deletion which may occur a loss or gain of a recognition sites and in turn lead to restriction fragment of different lengths (Jonah, 2011). This length difference enables to screen polymorphism between different genotypes. In RFLP, DNA polymorphism is detected by hybridizing a chemically labeled DNA probe to a Southern blot of DNA. These probes are mostly species specific, single locus probes of about 0.5–3.0 kb in size, obtained from a cDNA library or a genomic library (*Kurt et al*, 2005).

Advantages and drawbacks of RFLP

RFLP markers are co-dominance and high reproducible. Multilocus RFLP markers were mostly used for parentage analysis and genotype identification. Nevertheless, this technique is not very widely used as it is time consuming and tedious experimental procedure. It involves expensive toxic radioactive reagents and requires large quantities of high quality genomic DNA. It needs prior sequence information for probe construction which makes it complex. These drawbacks led to the development of new less technically complex methods known as PCR based techniques (Kurt *et al*, 2005; milee *et al*, 2008; Mondini *et al,* 2009).

Polymerase Chain Reaction Based Markers

Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a powerful method for fast in vitro enzymatic amplification of specific DNA sequences (Grunenwald, 2003). The major advance of DNA based molecular markers was driven by the invention of PCR by Mullis and coworker (Saiki, 1985). It was first time; any genomic region could be amplified and analyzed in many individuals without the requirement of cloning or isolating large amounts of ultra pure genomic DNA (Kumar *et al*, 2009). PCR technology has the ability to create large numbers of markers in short period of time (Kurt, 2005). PCR amplifications can be grouped into three different categories. These are standard PCR, long PCR, and multiplex PCR. Standard PCR involves amplification of a single DNA sequence which is less than 5 Kb in length and is useful for a various applications like cycle sequencing, cloning, mutation detection etc. Long PCR is longer than 5kb and up to 40 kb in length. Its application include long range sequencing, amplification of complete genes, PCR based detection, molecular cloning, diagnosis of medically important large gens insertion or deletion. The third type is multiplex PCR. It is used for the amplification of multiple sequences which are less than 5 kb in length. Its application includes forensic studies, pathogen identification, linkage analysis, genetic disease diagnosis and population genetics (Grunenwald, 2003).

Components needed to perform PCR (Kurt *et al*, 2005; Semagn *et al*, 2006**)**

Amplification buffer: It includes KCl, TrisCl and MgCl2. It controls the pH drop when incubated at the extension step.

Thermal stable DNA polymerase: - Earlier conventional E.coli DNA polymerase was used, but it is not stable at 95 degrees and new polymerase had to be added fresh after each denaturation cycle. There were also no thermocyclers which moves the tubes from one temperature bath to another for several hours. Since, this was done manually. Later, it was changed with the discovery of Taq (*Thermus aquaticus*) by Kary Mullis. It is a bacteria that used by the bacterium Thermus auquaticus in hot springs. It was stable at the denaturation temperature and could be used throughout the entire process without having to add any more. The discovery of Taq DNA polymerrase was decisive for vast utility and popularity of PCR based techniques. The original function of this enzyme is to facilitate the in vivo replication of DNA in the thermophilic bacteria. This DNA polymerase is stable at high temperature needed to perform the amplification while other DNA polymerases become denatured.

Four deoxyribonucleotide triphosphates (dNTPs):- dATP, dCTP, dGTP, dTTP

Forward and reverse PCR primers:- A particular DNA sequence, two single stranded oligonucleotide primers which are complementary to motifs on the template DNA are designed, the primer sequences are chosen to allow base specific binding to the two template strands in reverse orientation

Template DNA: - It is a target DNA needed to be amplified.

Basic Principles of Polymerase Chain Cycling Reaction
(Semagn *et al*, 2006; Grunenwald, 2003)

Addition of thermo stable DNA polymerase in a suitable buffer system, cyclic programming of primer annealing, primer extension and denaturation steps result in the exponential amplification of the sequence between the primer binding sites and the primer sequences. A typical PCR assay has three temperature controlled steps which can be repeated in a series of 25 to 50 cycles.

- Double stranded DNA is denatured at high temperature of 92 to 95^{o} C for 2 to 5 minutes to ensure the complete separation of the DNA strands
- Primers bind to the single stranded complementary templates at ends nearby the target sequence at lower annealing temperature 55 to 70 ^{0} C for 30 second to 1 minute
- The temperature is raised usually to 72^{o} C and sometimes 68^{o} C. At this temperature DNA polymerase enzyme catalyze the template directed syntheses of new double stranded DNA molecules that are identical in sequence to the starting material

- It is suggested that a final extension step of 5 to 10 minutes at 72^0 C will ensure that all amplicons are fully extended however there is no clear proof that this step is necessary

- The newly synthesized double stranded DNA target sequences are denatured at high temperature, and the cycle is repeated.

The amplification of target DNA can be exponential means that every cycle has the potential to double the amount of target DNA from the previous cycle, if there is sufficient amount of DNA polymerase, primers, and deoxynucleotide triphosphates (dNTPs) in the reaction solution. Although the basic protocol of PCR is uncomplicated, each application requires optimizing the various parameters for the species to be studied.

Major advantages of PCR techniques compared to hybridization based methods (Semagn *et al*, 2006)

- A small amount of DNA is required
- Elimination of radioisotopes in most techniques
- DNA sequences can be amplified from preserved tissues
- Accessibility of methodology for small labs in terms of equipment, facilities, and cost
- No prior sequence knowledge is required for many applications
- High polymorphism that enables to generate many genetic markers within a short time
- It is able to screen many genes simultaneously either for direct collection of data or as a feasibility study prior to nucleotide sequencing efforts
- It is amenable to automation which is an important requisite for the high throughput assays needed in molecular breeding programs (Kurt, 2005)

Random amplified polymorphic DNA (RAPD)

It is the first PCR based molecular marker (Wiliams *et al*, 1991). The basis of this technique is differential PCR amplification of genomic DNA using short primers. It assumes DNA polymorphisms produced by ‘‘rearrangements or deletions at or between oligonucleotide primer binding sites in the genome’’ (milee *et a*l, 2008; Mondini, 2009)**.**

Advantage of RAPD

The amplicons are analyzed using 1.52.0% agarose gels along with ethidium bromide and visualized under ultraviolet light. The simplicity and low cost of agarose gel electrophoresis has made RAPD popular (semagn, 2006). The key advantage of RAPDs is that they are quick and easy to examine. Only low quantities of template DNA usually 5–50 ng is required per PCR reaction. Random primers are commercially available and no sequence data for primer construction are needed. In addition, RAPDs have a very high genomic abundance and are randomly distributed throughout the genome (Kumar *et al*, 2009).

Draw Back of RAPD

The main drawback of RAPDs is their low reproducibility (Schierwater and Ender, 1993; Kumar *et al*, 2009). The inherent problems of reproducibility make RAPDs unsuitable markers for transference or comparison of results among research teams working in a similar species and subject. Hence, highly standardized experimental procedures are needed because of their sensitivity to the reaction conditions so may vary within two different laboratories. (Kumar *et al*, 2009). This marker is not locus specific; band profiles cannot be interpreted in terms of loci and alleles since it is dominant marker (Spooner *et al*, 2005; Kumar *et al*, 2009). Similarly, if distinct loci in the genome are amplified by each primer, profiles are not able to discriminate heterozygous from homozygous individuals (Bardakci, 2001; Milee, 2008).

Amplified Fragment Length Polymorphism

Amplified fragment length polymorphism (AFLP) technology was developed by the Dutch company, Keygene due to reproducibility limitation associated with RAPD (Vos *et al,* 1995; Milee *et al*, 2008; Mondini *et al*, 2009). It is essentially intermediate between RFLPs and PCR. AFLP refers to molecular markers obtained by selective PCR amplification of restriction fragments. It generates fingerprints of any DNA, regardless of its source, and without prior knowledge of DNA sequence. Scoring AFLP data is easy since polymorphisms are recognized in the form of presence or absence of data rather than determination of sizes at various loci. This technique involves the following three steps. i) restriction enzyme digestion ii) ligation of adapters iii) selective amplification of restriction fragments based on recognition of unique nucleotides flanking the restriction site. By varying the number of these additional nucleotides that extend beyond the restriction sites into the unknown sequences, it is possible to control the proportion of the ligated fragments that could be amplified. In general, 75-150 fragments are amplified with each primer combination. Each fragment represents a unique site (Farooq and Azam, 2002).

Advantage of AFLP

AFLPs strength lie in its high genomic abundance, considerable reproducibility, generation of many informative bands per reaction, their wide range of applications, and no sequence data for primer construction are required. AFLPs can be analyzed on automatic sequencers, but sometimes problems are encountered during scoring (Vos *et al*, 1995; Kumar *et al*, 2009).

Disadvantage of AFLP

This technique is time consuming and costly compared to other PCR based markers. It requires good quality DNA for ensuring complete digestion by enzymes in order to protect in non reproducible variation of DNA profiles (Monhapatra and Chopra, 2000). However, the major disadvantage of AFLP markers is that these are dominant markers (Kumar *et al,* 2009).

Simple sequence repeat or short tandem repeats

Variable Number of Tandem Repeats (VNTR) markers are dispersed throughout the eukaryotic nuclear genome and their polymorphisms are the result of variations in the number of tandem repeats in a short core sequence. VNTR markers have two main classes microsatellites and minisatellites which are

usually characterized by a high degree of length polymorphism Microsatellites are stretches of short DNA sequence in which a motif of one to six bases and can repeat from about five to hundred times at each locus. Minisatellites are tandemly repeating motifs of eight to hundred bases that can repeat from two to several hundred times at each locus (Qiu-Hong, 2004). Microsatellites are not limited to the nuclear genome. They occur in chloroplast as well as in mitochondrial genome (Soranzo *et al*, 1999; Farooq and Azam, 2002) as a repetition of guanine and cytosine (Farooq and Azam, 2002). With the beginning of polymerase chain reaction (PCR) technology this property of microsatellite DNA was converted into a highly versatile genetic marker. Microsatellites can be isolated from almost any target species of interest as about one hundred five microsatellite loci are held in the genome. They are inherited in a codominant Mendelian manner and can reveal heterozygote and homozygote in each individual. The variability of microsatellites is often so high that even with a small number of loci and a large number of individuals. It has therefore potential to deal with issues such as discrimination, relationships, structure and classification both at the population and individual level (Qiu-Hong, 2004).

Advantage of Microsatellites

- These are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes (Farooq and Azam, 2002; Qiu-Hong, 2004; Jonah *et al*, 2011).
- These are heritable, useful to monitor gene flow, excellent for parentage determination and ideally suitable for high throughput analysis through multiplexing by highly reproducible profiles (Qiu-Hong, 2004). The reproducibility of microsatellites is such that, they can be used efficiently by different research laboratories to produce consistent data (Milee *et al*, 2008; Jonah *et al*, 2011).
- They may be used across species and genus boundaries (Qiu-Hong, 2004)

Drawbacks of Microsatellites (Kumar *et al,* 2009; Qiu-Hong, 2004)

- One of the main drawbacks of microsatellites is that high development costs are involved if adequate primer sequences for the species of interest are unavailable, making them difficult to apply to unstudied groups .
- Mutations in the primer annealing sites may result in the occurrence of null which may lead to errors in genotype scoring. Null alleles may result in a biased estimate of the allelic and genotypic frequencies and an underestimation of heterozygosity.
- Homoplasy may occur at microsatellite loci due to different forward and backward mutations, which may cause underestimation of genetic divergence.
- A very common observation in microsatellite analysis is the appearance of stutter bands that are artifacts in the technique that occur by DNA slippage during PCR amplification. These can complicate the interpretation of the band profiles because size determination of the fragments is more difficult and heterozygotes may be confused with homozygotes. However, the interpretation may be clarified by including appropriate reference genotypes of known band sizes in the experiment

Single Nucleotide Polymorphisms (SNPs)

SNPs are naturally occurring variants that affect a single nucleotide. Single nucleotide variations in genome sequence of individuals of a population are known as SNPs. SNPs are the most abundant molecular markers in the genome and most common form of genetic variation between individuals which occurs once every 1,000 bases. Sometimes, two nucleotide changes and small indels up to a few nucleotides considered as SNPs but in this case, simple nucleotide polymorphism more preferred. The SNPs are usually more prevalent in the non coding regions of the genome. Within the coding regions, when a SNP is present, it can generate either non-synonymous mutations that result in an amino acid sequence change or synonymous mutations that not alter the amino acid sequence. However, synonymous changes can modify mRNA splicing that result in phenotypic differences (Angaji, 2009; Mondini, 2009).

Advantages and Draw Backs of SNPS

They are used for a wide range of purposes, including rapid identification of crop cultivars and construction of ultra high density genetic maps (Mondini, 2009). SNPs may be found both in the non repetitive coding and in the repetitive non coding sequences. When present in the coding sequences, they may or may not determine the mutant phenotype though they will show 100% or less association with the trait. In earlier case, they will be extremely useful both for MAS and for gene isolation. Even if, when the association of SNPs with traits less than 100%, the association with the economic traits will have use in MAS and in positional cloning. On other hand, they may be away from any gene and may not prove useful. Consequently, one may need to discover many more SNPs, than the number really needed. At this time, it is difficult to guess the proportion of SNPs that will be of immediate use (Gupta *et al*, 2001). However, generally due to the abundance of SNPs and development of sophisticated high throughput SNP detection systems, SNP markers will have a great influence on future mapping research studies and MAS (Angaji, 2009).

Transposable Elements

These are mobile genetic elements that are able to change their genetic location by transposition. Based on transposition mechanism, eukaryotes transposable elements divided in to the following two classes (Kurt *et al*, 2005).

Class I transposons

This class consists of retrotransposons, which transpose in a replicate manner using an RNA intermediate. This means that each transposition event creates a new copy of the transposon while the original copy remains intact at the donor site (Grzebelus, 2006). They are also termed as retroelements and again subdivided in to Retroviruses, long terminal repeat (LTR) retrotransposons, long interspersed elements (LINES), short interspersed elements (SINES). Retroviruses consists of an *env* gene in their genome. The protein encoded by these gene allows retroviruses to enter and leave their host cell.

These are the only infectious type of retroelement where they spread from cell to cell and organism to organism (Kurt *et al*, 2005).

Class II Transposons

Transposition usually follows a non replicative cut and paste mechanism. This means that they excise themselves from the donor site and reintegrate themselves at the acceptor site (Grzebelus, 2006).

Retrotransposons based Molecular Markers

Retrotransposons consist of long terminal repeats (LTR) with a highly conserved terminus. It is exploited for primer design in the development of retrotransposon based markers. Several variations of retrotransposon based markers exist (Kumar *et al*, 2009). Even though they vary from a technical point of view, in principle, they all provide the same type of information. They can be used to identify transposon insertion sites using PCR amplification or hybridization (Grzebelus, 2006).

Application of retrotransposon based markers (Kumar *et al*, 2009)

- Both within and between species they revealed high degree of heterogeneity and insertional polymorphism.
- Retrotransposon insertions are irreversible and hence considered particularly useful in phylogenetic studies.
- They are wide spread throughout the genome and often observed in regions adjacent to known plant genes which revealed their potential to be used in gene mapping study

Markers Assisted Selection (MAS)

The narrow genetic base of modern crop cultivars is the serious obstacle to maintain and improve crop productivity due to rapid susceptibility of genetically uniform cultivars by potentially new biotic and abiotic stresses. However, plant germplasm resources include wild plant species, modern cultivars, and their crop wild relatives. These are the important reservoir of natural genetic variations. The efficient exploiting of these genetic diversities is vital to overcome future problems associated with narrowness of genetic base of modern cultivars. Many agriculturally important variations like productivity, quality, tolerance to environmental stresses, and some type of disease resistance are controlled by polygenes and greatly depends on genetic × environmental interactions. These complex traits are referred to as quantitative trait loci (QTLs). It is difficult to identify QTLs based on only traditional phenotypic assessment but it needs further QTLs mapping in a genome of crop species using molecular markers (Abdurakhmonov and Abdukarimov, 2008).

A major breakthrough brought to plant breeding is the use of molecular markers to select genotypes not only for qualitative traits but also for complex traits that involve a broad range of genes .It has been estimated that 98% of the important traits in domesticated crops are of quantitative nature (Utomo & Linscombe, 2008). They are commercially important traits in crop plants, domestic animals, as well as in humans from (Karsey and Farquhar, 1997). Even though there are several applications of DNA markers, marker assisted selection is the most promising technique for cultivar development (Collard, and Mackill, 2006). Molecular marker assisted selection, referred to as marker assisted selection (MAS) of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers (Choudhary *et al*, 2008) and further exploitation of these individuals for crosses in breeding program (Kurt, 2005) . A marker can either be located within the gene of interest or be linked to a gene determining a trait of interest (Brumlop and Finckh, 2010). Such markers can be detected too early in the selection procedure thus the breeder can significantly reduce the number of seedlings grown and screened. This help to reduce expenses and to increase efficiency of breeding (Kurt *et al*, 2005). The success of MAS is influenced by the relationship between the markers and the genes of interest. There are three such types of relations as indicated below (Dekkers, 2004; Babu *et al*, 2004; Ruane and Sonnino, 2007; Choudhary *et al*, 2008).

- The first type is when molecular marker is located within the gene of interest. In this situation, one can refer to gene assisted selection (GAS). These kinds of markers are the most preferred one but they are uncommon and difficult to find.
- The second type is linkage disequilibrium (LD). It is the tendency of certain combinations of alleles to be inherited together. Population wide LD can be found when markers and genes of interest are physically very close to each other. Selection using these markers can be called LD-MAS.
- The third type is when molecular marker is not in linkage disequilibrium. Selection using these markers can be called LE-MAS. This is the most difficult situation for applying MAS.

Schemes of marker assisted breeding

Selection is the most important activity in conventional plant breeding programmes. The efficiency of phenotypic selection is largely depends on the extent of genetic variability present in a population and the heritability of the concerned characters. Generally, it is more effective for characters with high heritable than those having low heritability (Singh, 2000). Markers are used for selecting qualitative as well as quantitative traits. MAS can aid selecting for all target alleles that are difficult to assay phenotypically. Especially in early generations, where breeders usually restrict their selection activities to highly heritable traits because visual selection for complex traits like yield is difficult using only few plants per plot (Brumlop and Finckh, 2010). Thus, Marker assisted selection now plays a prominent role in the field of plant breeding and often used the following breeding strategy (Choudhary, *et al*, 2008; Brumlop and Finckh, 2010).

Simple traits or QTLs selection from breeding lines or populations

MAS can assist selecting of all target alleles which are complicated to examine phenotypically particularly in early generations. The prerequisite for successful early generation selection with MAS are large populations and low heritability

of the selected traits however breeders usually limit their selection activities to extremely heritable traits.

Marker assisted Recurrent Selection (MARS)

Recurrent Selection is one of convectional breeding method to select and develop elite hybrids through crossing several improved lines or breeds**.** Quantitative traits can be improved through phenotypic recurrent selection while it needs long selection cycle. But, MARS will accelerate this breeding selection scheme. In continuous nursery programs pre flowering genotypic information is used for marker assisted selection and controlled pollination. Therefore, several selection cycles are possible within a year and can accumulate favourable QTL alleles in the breeding population.

Pyramiding

Several genes can be combined into a single genotype by using MAS. The most frequent strategy of pyramiding is combining multiple resistance genes. Different resistance genes can be combined in order to develop broad spectrum resistance.

Marker assisted backcrossing (MABC):- is a method in plant breeding to transfer favourable traits from a donor plant into an elite genotype. Markers can be used in MABC to either control the target gene or to accelerate the reconstruction of the recurrent parent genotype. Traditional backcross breeding requires more than six generations reconstructing recurrent parent genotype, while MABC may reduce this to only few generations.

Conclusion

Genetic diversity refers to the variety of genes in all organisms from human beings to crops, fungi and viruses (Thijssen *et al*, 2008). It determines the uniqueness of each individual, or population, within the species. There are four methods of measuring genetic diversity namely ethino-botanical classification, morphological, biochemical and molecular characterization (Hoogendijk & Wiliams, 2001). Morphological markers enable the detection of genetic variation based on individual phenotypic variations. However, there are limitations confined to these types of markers. Morphological markers limitation led the assessment of biodiversity from relying on morphological markers to using isozymes and DNA markers that became known as molecular markers. There are different types of molecular markers which are classified based on variation type at the DNA level, mode of gene action and method of analysis (Alain *et al*, 2002; Milee *et al*, 2008). They are key tools in genome analysis which ranges from localization of a gene to improvement of plant varieties through marker assisted selection (Jonah *et al*, 2011). Even though there are several applications of DNA markers, marker assisted selection is the most promising technique for crops cultivar development (Collard, and Mackill, 2006).

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