Review Article Open Access

Molecular Markers and Marker Assisted Plant Breeding: Current Status and their Applications in Agricultural Development

Fiaz Ahmad[1,*], Ayesha Akram[1], Kiran Farman[1], Tanveer Abbas[1], Asma Bibi[1], Sobia Khalid[1] and Muhammad Waseem[2]

[1]Institute of Pure and Applied Biology, Botany Division, Bahauddin Zakariya University, Multan, Pakistan
[2]Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing, P. R China

Edited by:
Shabir Hussain Wani, Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, J. & K., India
Reviewed by:
Wayne Smith, CSIRO Plant Industry, Qld, Australia
Rashid Mehmood Rana, PMAS Arid Agriculture University, Rawalpindi, Pakistan
Muhammad Naeem, The Islamia University, Bahawalpur, Pakistan

Received
May 11, 2017

Accepted
June 17, 2017

Published Online
June 30, 2017

Abstract: The availability of molecular markers during the last few decades has played a developmental role in the agricultural sector. Plant breeding through marker assisted selection (MAS) has massive potential to improve the efficiency of conventional methods. Molecular markers are also useful to identify the economically important traits in the breeding population for the further manipulation in short time. Different marker techniques are available that facilitate us from complex genomic research to genic and finally at proteomic level for useful breeding purpose in agricultural field. Here, we have tried to explore the advantages of different available forms of molecular markers and their applications at the global as well as at domestic level.

Keywords: Molecular markers, markers assisted selection, polymorphism, next generation markers, plant breeding.

Corresponding author: Fiaz Ahmad, E-mail: fiazbiotechnologist@gmail.com

Cite this article as: Ahmad, F., A. Akram, K. Farman, T. Abbas, A. Bibi, S. Khalid and M. Waseem. 2017. **Molecular markers and marker assisted plant breeding: current status and their applications in agricultural development.** Journal of Environmental and Agricultural Sciences. 11: 35-50.

1 Introduction

Integration of recent advances in genome research, biotechnology and applications of molecular markers in combination with conventional practices of plant breeding has created new directions for plant breeding as interdisciplinary science for crop improvement (Moose and Humm, 2008). Molecular markers have widely been used as new era of plant molecular research. It involves DNA fingerprinting to detect polymorphism among different individuals. It has become a basic tool in crop improvement via plant breeding methods (Ahmad et al., 2010). Discovery of molecular markers in late 70s and their usage in plant breeding resulted in yield improvement of important crops for the first time proposed the probability to use molecular markers for agricultural development (Beckmann and Soller, 1986). Recently, on behalf of genomic research many comprehensive and promising molecular markers techniques have been developed in plant genetics (Poczai et al., 2013).

Applications of molecular markers and strategies for their development are based on polymorphism among organism genomes and polymorphism occurs commonly in nature between the individuals. Davey et al. (2011) assayed 30 % loci of *Drosophila pseudo obscura* and found it to encode electrophoretically distinguishable proteins, hence denied the concept of little polymorphism for the first time. Designing efficient molecular markers depends upon the nature and type of polymorphism. Many plant genomes have been fully sequenced recently, e.g., Arabidopsis, rice, mustard etc (Li et al., 2015).

A large variety of molecular markers are available presently, but choice of suitable markers to attain objectives is necessary. Ideal markers must show high level of polymorphism, co-dominant, ease of allele detection, distributed thoroughly throughout the genome, economical, neutral, reproducibility and ease to use. DNA marker polymorphism should be expressed in all cells and tissues of the plant

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

irrespective of developmental stage (Smulders and De Klerk, 2011).

Plant breeding in combination with use and development of molecular markers has boost up the crop yield and better productivity over a long period. Through marker technique the identification of targeted loci and its amplification in crop plants is used commonly for further utilization. Many disease resistance genes, tolerances to abiotic stresses and quality traits like nutrient and water use efficacy are major foci of plant breeding efforts as predicted population growth has put a pressure on environment for increased food production. Thus, plant breeding will play a key role in coordination with the molecular markers for increased yield of crop.

2. Description and types of genetic markers

A gene, referring to the heritable DNA segment responsible for a functional trait in the population and loci, associated with the collective transmission of homologous genes. The traits are considered to be of genetic origin only if same gene appears to be responsible in two related individuals giving same phenotype; that can be confirmed by inheritance analysis. In case of plants, most of the loci are polyploid (Doyle and Egan, 2010). Hence, the trait which depicts some phenotype reliably and unambiguously is used as genetic marker which includes all the related genes of that particular phenotype. In order to understand molecular markers and purpose of invention, it is necessary to know all of genetic markers employed in past (Farooq and Azam, 2002).

Genetic markers constitute three major categories; morphological markers, biochemical markers and molecular markers. Morphological and biochemical markers are also termed as pre-DNA markers (Manzo-Sanchez et al., 2015). Morphological markers are the classical markers which involves visual identification. Before the development of DNA markers, isozymes were one of the most popular and frequently used markers. These are the proteins/ enzymes encoded by one or more loci, and separated using electrophoresis technique (Jinek et al., 2012). Primary gene products are proteins/ enzymes that play important role in metabolism processes of the organisms. The protein structure constitutes amino acids which are placed in sequential way as the nucleotides in DNA. Determination of differences in electrostatic charges in amino acids represents differences in nucleotide sequence of the coding genes. Only one change per amino acid usually does not disturb confirmation of enzyme molecule, but the electrostatic charge and hence provides a useful way to evaluate the differences among individual plants. This technique can be used at any stage of plant's life cycle. It revolutionized population genetic studies in the past as the inheritance pattern followed by allozymes is of Mendelian genetics. The traits transmitted are represented by one gene loci or if many, accompanied by co-dominant gene action and ensures identification of both genes at the locus. As the isozymes markers are indirectly based on genetics of the organism, they were used in almost every field of plant breeding studies (Silvertown and Charlesworth, 2009). Also, there is a proven assumption that such biochemical markers are selectively neutral (Gregorius and Bergmann, 1995). However, differences of quantitative and qualitative aspects regarding isozymes markers were observed in cotton (*Gossipium hirsutum*) grown under normal and stress conditions. Hence isozymes believed to be influenced by environmental factors (Farooq and Sayyed, 1999). Due to non-neutral behavior, limited number of markers and lack of universal protocol, isozymes markers were replaced by molecular markers.

Cytological markers were also used by some scientists based on the chromosome karyotype and band. The patterns of bands differ in width, color, order and position, distributions of heterochromatin and euchromatin. However, the direct use of cytological markers has been very limited in genetic mapping and plant breeding. With the aid of molecular techniques, physiological and the physical maps of morphological markers laid the foundation of genetic linkage.

2.1. DNA-Markers

Polymorphisms among small DNA sequences of different individuals that are usually located in non-coding regions are used as DNA markers. Any target gene is not taken as genetic marker generally, but they act as signs or indicator of some particular gene presence due to its linkage with it and placement in close proximity (Rabiei, 2010).

In the early 1980s, a molecular marker restriction fragment length polymorphism (RFLP) was developed (Botstein et al., 1980). Having fragment size of 1000 nucleotide base pairs and co-dominant nature, these are available in unlimited number. These can be used for any genome that may constitute billions or more nucleotide base pairs arranged linearly in chromosomes. Development of polymerase chain reaction (PCR) was another breakthrough in biotechnology (Williams et al., 1990).

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

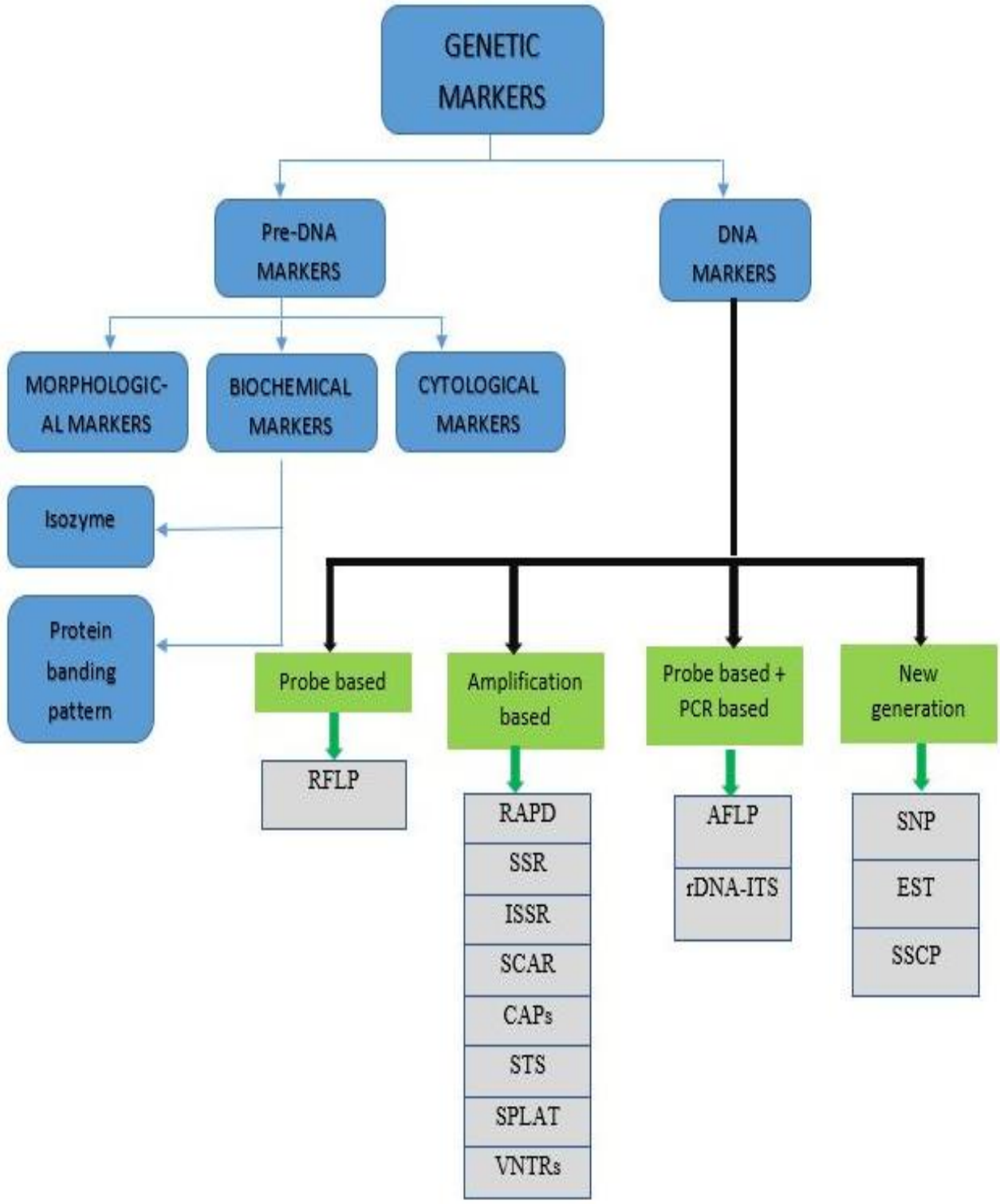

Fig. 1. Flow chart categorizing different genetic markers

Due to introduction of PCR, a new era of molecular markers began. Many molecular markers including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), short tandem repeat (STR), sequence-tagged sites (STS), single-nucleotide polymorphism (SNP), and expressed sequence tag (EST) etc. were introduced in plant breeding research. The molecular markers are usually neutral, most are of co-dominant in nature, mobile, easy to use, unlimited in number,

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

quick, and many are present in chloroplast and mitochondrial genome too (Rahimi et al., 2014). Hence these found frequent applications in MAS and crop breeding. It is important to have clear concepts and deep knowledge about these markers before availing their applications practically.

Inheritance patterns in plants can be bi-parental i.e. nuclear genes inheritance will be independent; or uni-parental i.e. only maternal nuclear and organelle gene or paternal organelle gene will be inherited independently. Also, the transmitted genes mode can be haploid or diploid; dominant or co-dominant. Ideal properties that a DNA marker should have: high degree of polymorphism, neutral, unaffected by pleiotropic and epistatic interactions, ease of access, fast, highly reproducible, frequent presence in genome, and follow principles of Mendelian inheritance. DNA markers are very common today and widely used due to ease of detection at any stage in lifecycle and high prevalence. DNA markers based on principle of detection are of two types: PCR based and hybridization based. Restriction fragment length polymorphism (RFLP) is hybridization based molecular marker while most of the other molecular markers are PCR based (Mondini et al., 2009).

2.2. Polymorphism: Nature and occurrence

Polymorphism in DNA represents the difference in the nucleotide sequence between individuals and it can be single base pair change or even change in the number of copies of concerned DNA sequence. So, how can polymorphic DNA be used for genetic mapping? Infect genetic mapping deals with the amount of recombination that exist between two markers during meiosis. Initially, the markers used by the plant breeders and geneticists for genetic mapping experiments included alleles of genes that produced different traits or physical appearance. Now, it is very important to have an understanding of polymorphism and employ markers according to suitability. The DNA markers come as result of mutations in organisms (point mutations, re-assortments, insertions, deletions, replication errors) and tandem repeats in DNA (Falque and Santoni, 2007; Zhu et al., 2008).

2.3 Types of molecular markers

In plant breeding, various markers have been used extensively i.e. (RAPD), AFLP, STSs, SNP, RFLP, diversity array technology (DArT), sequence characterized regions (SCARs), simple sequence repeats (Microsatellites/ SSR), inter simple sequence repeats (ISSR), expressed sequence tag (EST), cleaved amplified polymorphic DNA (CAPS), and many more. A flow chart and comparison between some important molecular markers of plant breeding is given in the following Fig. 1.

2.4 Why chose molecular markers for Breeding?

Before the advent of molecular era, only few plant species were under genetics consideration which could be crossed in controlled conditions and whose data could be monitored easily. After development of molecular approaches, scientists became able to analyze all life forms and understand the genetics of the biological world. Genetics is a vast science and the information is limitless. To distinguish analogous and homologous features of organisms, DNA hybridization techniques were used. By comparing relative levels of genetic differentiation of organisms, events of natural selection and divergence during evolution were appraised.

In conventional plant breeding, DNA polymorphism was determined through observational and visual selection. But with the development of molecular markers it is now determined at molecular level. After extraction of DNA, it is subjected to PCR or hybridization and gel electrophoresis for final diagnosis immediately (Bernardo, 2008; Jena and Mackill, 2008). Genetic markers can be used to tag and track individuals, cells, tissue, nucleus, genes or chromosomes. Labelling and tracking of genetic variations in DNA samples is done using genetic markers. Polymorphism is usually taken as a relative entity, i.e. variation in genetic locus of relative genome. Ultimate use of markers is the study of genetics and heredity (Jombart et al., 2009).

Molecular markers are used frequently in plant breeding laboratories due to their promising and reliable results. One edge over classical and conventional markers is that, as molecular data are genetic and never influenced by environment. In phylogenetic studies, molecular markers have a great significance because it is associated with heredity. In contrast, morphological traits bear unreliable and misleading data, as many characters appear due to environmental influence. Plants show great plasticity towards such characters more than animals (Avise, 2004). Some of the major advantages of molecular markers are discussed below.

3. QTL mapping and constructing linkage maps

In the field of plant/crop science, one of the biggest roles of molecular markers is constructing linkage maps. These maps are useful to locate single gene traits and QTL (quantitative trait loci) on chromosomes (Mohan et al., 1997). Use of genetic information generated by molecular markers depends

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

on knowing their relative location in plant genome. In other words, linkage maps can be considered as track maps which show distance between markers on chromosomes (Wu et al., 2007). All these findings lead to the identification of QTLs and other genes of interest which find basis in belief that Mendelian law of segregation and genetic recombination principles during meiosis takes place (Tondelli et al., 2014). Ultimately all of these can be analyzed in the subsequent progenies, as in most known cases, markers tightly linked to the genes are inherited in the progenies together (Bernardo, 2008). Using molecular markers genetic linkage constructed, their structure depends on the selection of genotyping system and type of molecular markers. The parental lines having highly polymorphic germplasm for the marker loci are selected to create progeny with all those polymorphic markers loci segregating in the population. Each individual line is then processed to create linkage map utilizing the information of markers collection in their genome (Price et al., 1997). Map units or genetic distance units can be calculated as recombination frequency which corresponds to ratio of physical distance to genetic distance (Xu, 1997). The regions with active recombination events are termed as recombination hotspots (Wang et al., 2009).

QTLs were actually identified in biparental populations for first time. The QTLs not transferrable to other populations, for segregating multiple populations researchers discovered meta-QTL and joint QTL populations analysis. Markers for polyploid plants and to sequence whole crop genome are in use (Varshney et al., 2009). After the advent of molecular markers, scientists thought that there was a need to identify functional markers linked with the QTLs for marker assisted selection (MAS) (Rahimi et al., 2014).

3.1 Marker assisted selection (MAS)

Characterization of traits via QTL determination was a major achievement. It leads to the MAS, which is not less than a revolution for the plant breeders (MacKill, 2006). After QTL and linkage mapping discovery, it was believed that these markers can be directly utilized for MAS however further advancements such as QTL validation, QTL effects and fine mapping with high resolution were needed to confirm tightly linked markers that can be used reliably to predict phenotypic traits (MacKill, 2006).

The genes should be mapped with high accuracy and without any negative effect on other traits (Rabiei, 2010). In the past, using small populations, a single gene with low resolution mapping was done but tightly linked markers are the prerequisite of MAS. Mapping done in populations of four hundred individuals, resolution of less than 1 cM was obtained for gene location. After the identification of the gene of interest, the markers linked to it can be developed (Avise, 2004).

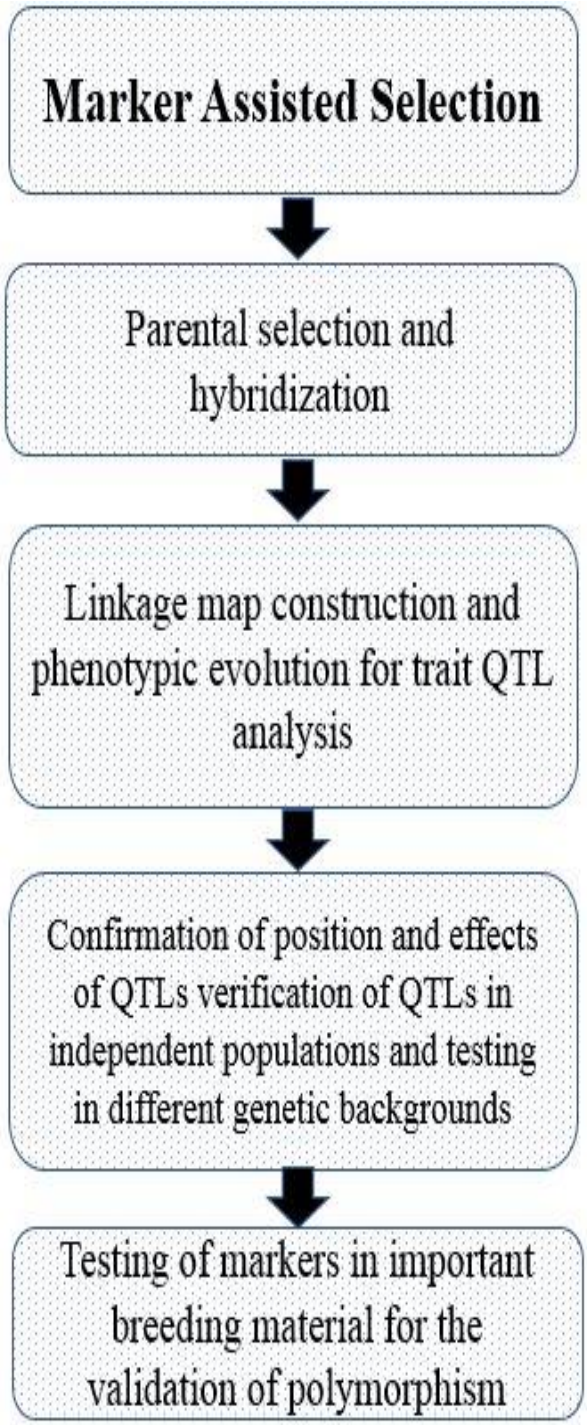

Fig. 2. Flow chart of Marker Assisted Selection in Crops

Markers are not involved in recombination and genes shuffling events and are able to distinguish favorable alleles in the germplasm (Avise, 2004). Hence such markers are reliable for studying trait genes in the population. Markers which are co-dominant i.e. able to distinguish between homo and heterozygote are generally preferred to track the target gene after back crossings (Collard et al., 2005). A comprehensive flow chart that depicting the way of marker assisted selection is shown in Fig. 2.

3.2 Marker assisted pyramiding

Marker assisted pyramiding is possible through conventional breeding methods which involves integration of multiple QTL genes simultaneously in single individual germplasm. The individual plants are then assayed for all the phenotypic traits which can be destructive. However, the use of molecular markers is a safe, reliable and robust way of identifying the desired F_2 progeny. Using a single DNA sample is enough for analysis and no phenotyping is required. Integrating a single gene in an individual is sometimes useless, as pathogens overcome single gene resistance soon. Marker assisted pyramiding is a useful technique as scientists are able to integrate multiple stress related and disease resistance genes into the single genotype and obtain broad spectrum resistance (Eathington et al., 2007).

Previously, pyramiding of resistance related multiple genes was not possible as all show the similar phenotype. But now, by the application of linked DNA markers, many resistance genes in a plant are detectable easily. The insertion of resistance related traits (under QTL control) is another way of creating disease resistance in crops.

4. Genotype identification and genetic diversity

Sufficient genetic diversity is required for plant breeding programs to assist production of new improved cultivars against various stresses and increase of yield. Attempts of developing superior genotypes and evaluations made on the basis of genetic similarity or genetic distance between parents have also been made. Genetic distance can be used as probable genetic variances in different sets of segregating progenies made from different crosses (Abrams et al., 2007).

Generally, major advantages of molecular markers promise are:

a. Using any plant part, genomic DNA can be isolated and subjected to fast and rapid molecular markers analysis at any stage of plants life and plant related information be obtained before pollination. Hence allowing breeders to carry out more accurate and informed crosses.

b. Various disease resistance diagnostic tests are conducted using DNA markers which are tightly linked to gene without resorting to pathogen inoculation in the field or greenhouse.

c. Molecular markers are neutral i.e. unaffected by environmental conditions while morphological and biochemical markers are influenced by environmental conditions, making experiments inaccurate and unreliable.

d. Molecular markers save time and resources. Markers allow breeders to select breeding lines early in experiments and exclude other progenies from program.

e. Complex traits can be precisely selected which were difficult to select in past. Markers linked to QTL are used to keep track of gene of interest in crosses.

f. Analyzing characters of interest is one advantage of molecular markers. Besides this wild species having some potential traits can also be analyzed.

5. Selecting reasonable marker

Selection of ideal marker depends on work objectives e.g. if in a species, some environmentally influenced traits are to be differentiated, non-neutral markers such as isozymes or DNA-microarray should be chosen. The DNA-microarray is relatively an expensive technique and requires a skillful worker. Hence many developing countries especially choose to utilize isozymes markers in such cases. In crops where there is an epidemic of disease or attack of pests, to differentiate between plants, again the isozymes would be considered preferable (Farooq and Sayyed, 1999). To differentiate between species of similar accessions (intra-specific), RFLP, RAPD and AFLP provide the required results accurately (Farooq et al., 1998). Once there was a problem of discriminating commercial cultivars of Basmati 370 and its two dwarf mutants which was finally solved using RAPD analysis (Farooq, 2001). It's not possible to rely on a single technique for all purposes, different methods differ in their level and power of genetic resolution (M'Ribu and Hilu, 1996).

5.1 Genetic marker and genotyping using next generation sequencing

Genomics and transcriptomics have been revolutionized by the applications of next generation sequencing, which can also be used to advance and validate genetic markers. In this regard, few practices by scientists has been done. These molecular markers

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

can be applied to the organisms with sequenced genome, as well as to the species with unavailable genomic data (Davey et al., 2011). Using conventional techniques, scientists were unable to perform complete genome studies in single step but using NGS several techniques are available that facilitate them to some extent, thousands of markers across whole genome can be assessed in a one step process.

Development of conventional markers is usually costly as it involves various steps (cloning, primer designing) that can't be parallelized but this problem was solved through the development of SNPs; but there are limitations i.e. population specific (serious problem for studying wild type) and requires ample resources. However, hundreds of individuals can be studied for sequencing, genotyping and discovery of thousands of markers by NGS based techniques. Genomic DNA is utilized and involve a single step processing (Davey et al., 2011). Genotyping by sequencing is another approach. Originally, it used a single restriction enzyme in processing and detecting the sequence between restriction sites. Hence, selection of restriction enzyme (able to detect repetitive sequences) is the crucial factor which determines GBS efficiency. It is applicable to the species with no reference genome data available, and sequence tags are used for analysis (Mir et al., 2013). GBS is a great potential technique that can locate thousands of SNPS in short time during one experiment that are very suitable for breeding experiments, characterization of genome, genome mapping and also for population study (Huang et al., 2010).

6. Success stories

Molecular markers have been used for improvement of various crop traits. The successful application of the markers in different crops is mentioned as bellow.

6.1 Wheat

The usage of molecular markers for the improvement of wheat crop is very authentic and valuable (Devos et al., 1995). In case of wheat a new marker system introduced by Zhang et al. (2011) that allelic specific markers of γ-gliadin genes (*Gli-1*) were used for the estimation and detection of specific alleles linked to the *Glu-A3*, *Glu-B3* and for *Glu-D3* that was authentic and useful in the identification of LMW-GS genes in bread wheat. The system proved very authentic and beneficial for high throughput analysis of the concerned genes in improving and making good quality wheat.

Similarly, Polyphenol oxidase (PPO) is responsible for discoloration and darkening of wheat and the marker related with PPO activity play a major role to rush the selection efficiency for low PPO activity in order to improve the wheat programs. In this respect, the markers PPO16, PPO18, STA01 and F-8 for the loci *Ppo-D1* on the chromosome 2DL, *Ppo-B1* on the chromosome 2B and *Ppo-A1* on the chromosome 2AL have been developed. Wheat flour color is another factor in the taxation of flour quality. A low LOX activity and bright yellow color of flour in bread wheat have very advantageous aspects (Geng et al.,2012). Two markers LOX16 and LOX18 located on 4BS were introduced to differentiate between low and high activity of LOX in different cultivars of wheat (Geng et al., 2012).

6.2 Rice

Rice is another major crop that is feeding half of the world population and situation relating to rice improvement through molecular markers, transgenic approaches and through genomic evaluation is not different to other crops. The cultivars with improved yield have always been the theme of the breeders and in case of rice, super rice is an ideal plant architecture using the molecular concept and design (Rao et al., 2014). Different markers like SSRs and RFLP that are co-dominant and their position on rice genome for better crop is well known and the RAPD and AFLP are largely co-dominant and they produce random amplification. RFLPs and SSRs markers are very efficient in rice and can detect a high degree of polymorphism and are very suitable for the estimation of genetic diversity among highly similar cultivars of rice (Paux et al., 2012)

Molecular breeding in rice through the discovery of new genes, markers and QTLs in rice for biotic and abiotic resistance is vital and very important (Wang et al., 2009). Discovery of RILs through germination rate under low temperature that was set as a stress caused the synthesis of seven new QTLs that were highly effective against temperature stress in rice and most of the QTLs that are resistant to cold are located on chromosome 4, 6 and 9 in rice (Rao et al., 2014). Further in rice hybrid the markers SCAR co-segregated with Sty-bi and Wx-mg were used to find out the targeted loci in segregation generation and at the same time CAPS associated to Wx-mg transferred simultaneously into high yielding rice varieties and newly developed rice line (Ning, 9108) with great potential of yield, improved quality, disease resistant and agronomic traits.

High-photosynthetic efficiency is an important agronomic trait for the enhancement of rice biomass

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

and grain yield and the targeted markers and genes have already been identified for this trait in rice. The targeted genes and markers have been used for high photosynthetic efficiency as a new tool for breeding. The major target and focus of rice breeding for high photosynthetic efficiency is to determine potential of single leaf photosynthesis rate. Physiological analysis reveals that Phosphoenolpyruvate carboxylase (PEPCase) is a key gene/enzyme for CO_2fixation in C_4 plants and the result of a transferred PEPCase gene from maize to rice variety caused a great improvement at different growth stages of plant as well as also played role in the improvement of yield and quality (Wanget al., 2009).

6.3 Maize

Maize is another most important crop in the world. One of the first published experiments associating marker genotypes documented phenotypic responses in maize. High level of genetic variability in maize and speculated on the possible value of RFPs in many breeding applications has been reported. With the development of PCR technology, SSR markers emerged (Xia et al., 2005). These molecular tools had an additional advantage of detecting higher levels of polymorphism than RFPs. As automated molecular technology improved, genotyping has moved to SNP markers (Eathington et al., 2007).

Current platforms for genotyping are highly reliable and quick and able to produce more than 100,000 SNPs in one day with least error about less than 1% (Jenkins and Gibson, 2002). In short, the breeding programs in maize have been doubled on behalf of molecular markers and markers related techniques than the conventional breeding programs that were without markers. Further, through molecular markers/marker techniques it is greatly facilitated the introgression of transgenic traits into commercial maize germplasm for better adaptation and yield development.

6.4 Cotton

Cotton (*Gossypium* spp.) is an important and cash crop that is grown in more than 80 countries and has great value of adoptability in hot and dry environment (de Sousa et al., 2015). The genome mapping of it for targeted traits plays a vital role in breeding programs. Microsatellites, also known as SSR and SNPs markers are applied widely in genetic analysis, in addition to marker-assisted selection (MAS). Thus, through the availability of polymorphic markers we can identify the targeted quantitative trait loci (QTL). The genetic map of cotton is still incomplete for cotton breeding programs because of additional reliable technological requirements. However, in 1994 its genetic map has been published with low level of efficiency to be considered for its application in breeding systems. ESTs markers are widely used in fiber development of *G. barbadense* similarly, 79SSR loci were used in the development of hybrid. SSR markers have widely been used to evaluate the genetic diversity, for the identification and phylogenetic analysis of major cotton cultivars. In case of fusarium, a common disease in cotton throughout the world cause yellowing, wilting, damage of various vascular tissue, defoliation etc. Identification of markers for targeted genes against the disease is vital and very important. Genotyping by sequencing (GBS) based on sequencing at genome-wide level for the detection and isolation of useful markers and loci against various traits and characters (Elshire et al., 2011). Overall, the selection assisted by markers and the deveolpment of transgenic will also be the valuable techniques in the improvement of cotton crop.

7. Molecular markers used at domestic level for crops improvement

Distinguishing of different genotypes of crops is necessary when a variety of interests is to be selected from complex germplasm, newly formed lines need to be registered and maintain purity. Molecular markers have widely been accepted and used as valuable tools in plant breeding in the whole world including Pakistan as well. Use of DNA markers has opened a new gateway towards agriculture which is now termed as molecular breeding. In the world, they have been used in breeding of various crops i.e. wheat and forage species (Jahufer et al., 2003). There is long history of molecular profiling of cultivated varieties and cereal species. The technology first became popular was RFLP, followed by the introduction of RAPDS, then AFLP and most recently popular being the SSR (Zhu et al., 2008). The SSR markers have few unique advantages such as most of the SSRs are mono-locus, highly informative, easy, automated, economical, and availability of high number of public SSR primer pairs. Few success stories of how the molecular markers were used at domestic level (in Pakistan) are given below.

Major cereal crops of the world included wheat (*Triticum aestivum*) that is a staple food for a big population of the world (Peng et al., 2011). To fulfill the requirement of flour it is very important that improvement in yield and quality must be enhanced. And according to an estimate with the increasing of world population there must be 2% increase in the quantity of flour annually. This is reality, the wheat cultivars resulted through high level ranging selection

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

of breeders showed high in yield and quality traits (Goutam et al., 2015). Marker breeding is another quick and authentic tool for wheat breeders that can boost up the traits to fulfill the requirement with increasing population (Gupta et al., 2010).

Different form of markers has already been applied for improvement of various crops in Pakistan and especially the PCR based markers have largely been reported (Ahmad, 2000). Other factors such as chemicals and enzymes present in wheat also play important role. One of the enzymes class is PPOs (poly phenol oxidases). These enzymes are responsible for browning of the wheat products. Association of molecular markers with the enzymes was successfully found. The three pairs of molecular markers (primers) i.e. PP043, PPO30 and WP2-2 generated unique pattern to distinguish between different PPOs level (Abbas et al., 2016). Alleles of several loci associated with yield, quality and adaptability were investigated in Pakistani wheat landraces and improved historical wheat cultivars. In wheat landraces, alleles *Psy-A1b, TaCKX6-D1b, TaGW2-6A-A, Vrn-A1a and Wx-D1b were not found however alleles Ppo-A1a, Psy-D1a, Psy-B1b, Psy-A1a, Pinb-D1a, Pina-D1b, Glu-D1d, Glu-A1b, TaCKX6-D1a, TaGW2-6A-G, TaCwi-A1a, Rht-D1b, Ppd-D1a, TaZds-D1b, TaLox-B1b and Wx-D1a* were found. All the collections of alleles in wheat varieties will prove to be useful in future breeding programs. (Rasheed et al., 2016).

In Pakistan, a major threat to wheat and cause of limited bread wheat production is rust disease. Resistant varieties can be made to control rust in wheat for which identification of responsible gene is the prerequisite. Different molecular approaches were used to evaluate the stripe rust and leaf rust resistant gene *Lr34/Yr18* and the stem resistant gene Sr2 in perfect way. Through the introduction of resistant gene thirteen very important cultivars are generated in Pakistan as NR 337, NR 339, NR347, NR 350, Manthar, Margalla 99, Saleem 2000, Iqbal 2000, Marwat 2001, pirsabak 2004, 03FJ26, Wafaq 2001 and Fareed 2006. And all the cultivars were evaluated through the existance of DNA marker *csLV-34* through the PCR amplification of DNA fragment. Similarly, the 36 Pakistan's spring wheat varieties were reported with *Sr2* a stem rusts resistant gene using stm560.3tgag marker (Qamar et al., 2014)

It provides resistance against above mentioned rusts and powdery mildew. These markers are valuable tools for MAS breeding to develop resistant wheat lines (Mammadov et al., 2012). Stripe rust resistance related genes were investigated in 67 Pakistani wheat varieties using microsatellites and STS. Resistance gene *Yr26* was found associated with *Xgwm11* and STS marker CYS5. Previously reported genes *Yr5, Yr9, Yr10, Yr17* and *Yr18* were also investigated. The studies can be applied successfully to future varieties for rust resistance gene pyramiding and MAS (Ayala et al., 2015). Another rust resistance gene *Lr 26* was investigated using STS marker iag95. It confirmed resistance in five wheat varieties namely Zarlashta 90, Bhakar 2008, Khyber 79, Mehran 89 and Potohar 70 (Ali et al., 2015). In wheat growing countries, barley yellow dwarf is a prevalent disease which has now became a major threat for Pakistani wheat cultivars too. Resistant variety TC14 was crossed with Inqilabi91, the most commonly used wheat variety. Presence of *Bdv2* was detected by SCAR markers. Seven resistant varieties were obtained and low viral titer in others (Kausar et al., 2015).

After wheat, maize (*Zea mays*) is most important cereal crop in Pakistan and 98% of the total maize of country is cultivated in Punjab and KPK area. To improve effectiveness of breeding programs and keep check of purity levels in maize cultivars, RAPD markers were applied for DNA fingerprinting and hybrid identification (Asif et al., 2009). Seventeen Pakistani maize genotypes have been improved by using 10 simple sequence repeats (SSR) primer sets by detection and utilization of genetic diversity in germplasm. SSRs and SNP markers have emerged an important source of ubiquitous markers for genomes of many eukaryotic organisms and also useful to facilitate the dissection of complex traits in maize (Shah et al.,2009).

World's leading fiber crop cotton is produced on a large scale in Pakistan. Pakistan reserves the 5th position in producing world's cotton and 3rd in exporting raw cotton. Cotton leaf curl resistant varieties were evaluated using RAPD markers. About 20 were found to be extremely resistant and DNA fingerprinting was used for estimation of genetic divergence among elite cotton varieties (Rahman et al., 2002). Another RAPD analysis done on 30 genotypes of *G. arboretum* was performed and close relationship between most of the cultivated varieties was found. Genotypes were clustered into two major and three smaller categories (Yasmin et al., 2008). Similarly, other markers such as RFLP, AFLP, SSR, and EST were also employed by various researchers.

Table 1. Year wise data of molecular markers usage and application in Pakistan

Year	Molecular marker	Crop /Species	Purpose	Reference
1998	RAPD	Mustard (*Brassica juncea)*	Genetic diversity	(Rabbani et al., 1998)
1999	RFLP	Cotton	Leaf curl viral disease resistance	(Aslam et al., 1999)
2001	SRAP	*Brassica napus*	Genetic diversity	(Riaz et al., 2001)
2005	RAPD	Rice (*Oryza sativa*)	Genetic diversity –Pakistan's germplasm	(Arif et al., 2005)
2008	RAPD	Lentil (*Lens culinaris*)	Genetic diversity	(Sultana and Ghafoor, 2008)
2008	RAPD	Sorghum (*Sorghum bicolor*)	Molecular characterization	(Mehmood et al., 2008)
2009	Microsatellites	Wheat (*Triticum aestivum)*	Molecular characterization	(Ijaz and Khan, 2009)
2009	RAPD	Wheat	Genetic diversity	(Bibi et al., 2009)
2009	RAPD, SSR	Cotton	Parentage confirmation of hybrids	(Yasmin et al., 2008)
2009	RAPD, SSR	Cotton	Genetic linkage map of leaf hairiness	(Zafar et al., 2009)
2009	RAPD	Fennel (*Foeniculum vulgare*)	Genetic diversity, assessment of indigenous germplasm	(Zahid et al., 2009)
2010	RAPD	Wheat	Genetic diversity in germplasm from Baluchistan area	(Khatab et al., 2016)
2010	RAPD	*Caralluma tuberculata* and *Caralluma edulis*	Molecular and morphological characterization	(Mahmood et al., 2010)
2010	RAPD	Rice	Genetic variability assessment	(Pervaiz et al., 2010)
2010	RAPD	Cotton	Genetic diversity and germination pattern	(Mumtaz et al., 2010)
2010	RAPD	Sorghum (*Sorghum bicolor*)	Biodiversity in Pakistan's germplasm	(Iqbal et al., 2010)
2011	RAPD	Sesame (*Sesamum indicum*)	Genetic diversity –Pakistan's germplasm	(Akbar et al., 2011)
2011	RAPD	*Tragus roxburgii*, *Eragrostis poaeoides*, *Brachiaria distachya*, *Dactyloctenium aegyptium*, *Setaria glauca*, *Setaria verticillata*, *Chrysopogon aucheri*, *Heteropogon contortus*, *Saccharum spontaneum Themeda anathera*.	Molecular characterization	(Zeb et al., 2011)
2011	RAPD	Turmeric (*Curcuma longa*)	Genetic diversity assessment	(Jan et al., 2011)
2012	SSR	Wheat	Salinity tolerance	(Shahzad et al., 2012)
2015	SCAR	Mungbean	Screening of mung bean yellow mosaic disease resistant cultivars	(Binyamin et al., 2015)
2015	RAPD	Linseed	Genetic diversity analysis	(Bibi et al., 2015)
2016	Microsatellite	Snapmelon (*Cucumis melo* var *momordica*)	Genetic variability and phylogenetic relationship	(Rasool et al., 2016)
2016	SSR	Wheat	Drought tolerance	(Iqbal et al., 2016)
2016	SSR	Wheat	Grain quality	(Shahzad et al., 2016)

A genetic linkage map of leaf hairiness was developed using RAPD and SSR markers. The hairiness is inheritable trait which can be employed to enhance insect resistance in cotton (Zafar et al., 2009).

The SNP markers were used successfully to identify minute genetic variations in cotton. *G. hirsutum* cv. TM-1 and *G. barbadense* cv. Hai7124 were evaluated for the FIF1 genes and three basic substitutions were detected, the first in non-coding region while other two were found in coding regions. It resulted in change of amino acids Arginine to tryptophan and proline to leucine (Ahmad et al., 2007). One of the most important crop that is a source of food for 3/4th of world's population, no more a luxurious crop but major calorie source for rural and urban both people is rice (*Oryza sativa* L.) (Sasaki and Burr, 2000). Pakistan is one of the leading exporters of rice due to its good quality aromatic rice varieties and old landraces. Many varieties have been commercialized with improved traits against environmental stresses and to improve the quality. Aromatic varieties have narrowed the germplasm because breeding lines have been repeatedly chosen from selected basmati varieties. And most of the scientists focused on grain quality while resistance to stresses was relatively neglected. About seven basmati varieties are cultivated in Pakistan currently of which five have the same cultivar (Basmati-370) as one parent (Arif et al., 2005). Besides this, several molecular markers for evaluation of diversity and variability at the molecular level are available e.g. restriction fragment length polymorphism (RFLP) (Arif et al., 2005), random amplified polymorphic DNA (RAPD) (Williams et al., 1990), simple sequence repeats (SSR) (Levinson and Gutman, 1987), inter simple sequence repeats (ISSRs) (Albani and Wilkinson, 1998), amplified fragment length polymorphism (AFLP) (Mackill et al., 1999) and SNPs (Vieux et al., 2002). About 23 percent of total foreign exchange of Pakistan is shared by rice, hence called as "Golden grain" (Shah et al., 1999).

Lentil (*Lens culinaris*) is consumed as food on a large scale in Pakistan and is one of the oldest cultivated legumes. Sultana and Ghafoor (2008), carried out study on lentil germplasm from Pakistan for isozymes and RAPD markers. High heterogeneity was observed for Baluchistan germplasm and prevalence of indigenous landraces over time was suggested. Validity of the diversity was explored and found irrespective of sample size and geographic pattern. Isozymes and seed proteins analysis proved to be a poor source for diversity analysis while RAPD has proven to be the best source for observing inter and intra-specific differences in lentil. Hence further investigation must be continued and is required to extend germplasms and primers to continue the research (Sultana and Ghafoor, 2008). Using SCAR markers, mung bean yellow mosaic virus disease (MYMVD) resistant varieties were screened. None of the genotypes were found to be highly resistant. SCAR markers proved to be valuable tool for development of MYMVD resistant cultivars due to highly specific band determination (Binyamin et al., 2015). A whole story of different markers used for different crops improvement in Pakistan is explained (Table 1).

Apart from all of the advantages described above, cost is usually high making it economically unfeasible. However, there is no need to sequence whole genome for assessment of some species. Markers if used wisely, can be used to address specific required research areas. Furthermore, considerable training and technical skill is required.

5. Conclusion

Overall, plant breeding has played a remarkable role in crop improvement. It is clear that current breeding programmes continue to make progress through commonly used breeding approaches. Day by day different DNA data are being generated and the trend is toward cross-referencing genes and genomes using sequence and map-based tools. Polymorphism is a major limitation factor for many species so, SNP based markers will be valuable tools for plant geneticists and breeders. Different markers and specially SNP markers have become tremendously popular in plant molecular genetics due to their genome-wide abundance and acquiescence for high to ultra-high throughput detection platforms. New marker technology can potentially reduce the cost of marker assisted selection considerably. If the effectiveness of the new methods is validated and the equipment can be easily obtained, this will allow marker based breeding to become more widely applicable. Latest markers are now being used for improvements in plant breeding and specially to enhance yield of major crops like rice, wheat, maize and cotton.

List of abbreviations: AFLP: Amplified Fragment Length Polymorphism; CAPS: Cleaved Amplified Polymorphic DNA; DArT: Diversity Array Technology; DNA: Deoxyribonucleic acid; EST: Expressed Sequence Tag; EST: Expressed Sequence Tag (EST); GBS: Genotyping by sequencing; ISSR: Inter Simple Sequence Repeats; MAS: Plant breeding through marker assisted selection; PCR: Polymerase

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11

chain reaction; PPO: Polyphenol oxidase; QTL: Quantitative trait loci; RAPD: Random amplified polymorphic DNA; RFLP: Restriction fragment length polymorphism; SCARs: Sequence Characterized Regions; SNP Single-Nucleotide Polymorphism; SSR: Simple Sequence Repeats; STR: Short Tandem Repeat; STS: Sequence-Tagged Sites.

Acknowledgements: Authors are thankful to Dr. Seema Mahmood Professor of botany, Institute of Pure and Applied Biology (IP&AB) for guidance and in review of article. The authors also thankful to Dr. Asif Saleem, Assistant professor of Plant Breeding and Genetics (PBG), Bahauddin Zakariya University, Multan for his support and discussion during the writing of article.

Conflict of Interest: It is declared that, the authors have no competing interests.

Author Contribution: FA planned the theme, AK, KF, TA, AB and SK coordinated and wrote the manuscript; FA and MW finalized the article.

References

Abbas, Z., B. Javaid, N. Majeed, A. Rehman and S.S. Naqvi. 2016. Association of molecular markers with polyphenol oxidase activity in selected wheat genotypes. Pakistan J. Bot. 48: 689-692.

Abrams, D. I., C. Jay, S. Shade, H. Vizoso, H. Reda, S. Press, M. Kelly, M. Rowbotham, and K. Petersen. 2007. Cannabis in painful HIV-associated sensory neuropathy A randomized placebo-controlled trial. Neurology. 68(7): 515-521.

Ahmad, F., A. Khan, F. Awan, B. Sadia, H. Sadaqat, and S. Bahadur. 2010. Genetic diversity of chickpea (*Cicer arietinum* L.) germplasm in Pakistan as revealed by RAPD analysis. Gene. Mol. Res. 9(3): 1414-1420.

Ahmad, M. 2000. Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor. Appl. Genet. 101(5): 892-896.

Ahmad, M.S.A., F. Javed and M. Ashraf. 2007. Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa* L.) genotypes. Plant Growth Regul. 53(1): 53-63.

Akbar, F., M.A. Rabbani, M.S. Masood and Z.K. Shinwari. 2011. Genetic diversity of sesame (*Sesamum indicum* L.) germplasm from Pakistan using RAPD markers. Pakistan J. Bot. 43: 2153-2160.

Alhani, M.C. and M.J. Wilkinson, M. J. 1998. Inter simple sequence repeat polymerase chain reaction for the detection of somaclonal variation. Plant Breeding. 117(6): 573-575.

Ali, L., I. Ullah, S. ud Din, W.M. Khan, M.S. Khan, I. Ahmad, M. Anwar and W. Ali. 2015. Marker assisted selection for rust resistant gene LR26 in wheat (*Triticum aestivum* L.). Pure Appl. Biol. 4(3): 302-305.

Arif, M., S. Kousar, M.A. Bajwa, A. Arif and Y. Zafar. 2005. Genetic diversity among rice genotypes of Pakistan through random amplified polymorphic DNA (RAPD) analysis. Pakistan J. Bot. 37: 585-592.

Asif, M., M. Rahman, J.I. Mirza and Y. Zafar. 2009. Parentage confirmation of cotton hybrids using molecular markers. Pakistan J. Bot. 41: 695-701.

Aslam, M., C. Jiang, R. Wright and A. Paterson. 1999. Identification of molecular markers linked to leaf curl virus disease resistance in cotton. Pakistan J. Biol. Sci. 2: 124-126.

Avise, J.C. 2004. Molecular markers, natural history and evolution. Springer Science & Business Media.

Ayala, M., J.B. Alvarez, M. Yamamori and C. Guzmán. 2015. Molecular characterization of waxy alleles in three subspecies of hexaploid wheat and identification of two novel Wx-B1 alleles. Theor. Appl. Genet. 128: 2427-2435.

Beckmann, J.S. and M. Soller. 1986. Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica. 35(1): 111-124.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Sci. 48: 1649-1664.

Bibi, S., M.U. Dahot, I.A. Khan, A. Khatri and M. Naqvi. 2009. Study of genetic diversity in wheat (*Triticum aestivum* L.) using random amplified polymorphic DNA (RAPD) markers. Pakistan J. Bot. 41: 1023-1027.

Bibi, T., H.S.B. Mustafa, E. Hasan, S. Rauf, T. Mahmood and Q. Ali. 2015. Analysis of genetic diversity in linseed using molecular markers. Life Sci. J. 12: 28-37.

Binyamin, R., M. Khan, N. Khan and A. Khan. 2015. Application of SCAR markers linked with mungbean yellow mosaic virus disease-resistance gene in Pakistan mungbean germplasm. Genet. Mol. Res. 14: 2825-2830.

Botstein, D., R. White, M. Skolnick and R. Davis. 1980. Construction of a genetic linkage map in

man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer and E.C.K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica. 142: 169-196.

Davey, J.W., P.A. Hohenlohe, P.D. Etter, J.Q. Boone, J.M. Catchen and L.M. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat. Rev. Genet. 12(7): 499-510.

de Sousa, C.C., L.C. de Figueirêdo, M.D.G.M. Arrais and S.E. dos Santos Valente. 2015. Genetic map of cotton with molecular markers. Am. J. Plant Sci. 6(2): 289-297.

Devos, K.M., G.J. Bryan, A.J. Collins, P. Stephenson and M.D. Gale. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor. Appl. Genet. 90(2): 247-252.

Doyle, J. J., and A. N. Egan. 2010. Dating the origins of polyploidy events. New Phytologist. 186 (1): 73-85

Eathington, S.R., T.M. Crosbie, M.D. Edwards, R.S. Reiter and J.K. Bull. 2007. Molecular markers in a commercial breeding program. Crop Sci. 47: 154-163.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One. 6 (5): e19379.

Falque, M. and S. Santoni. 2007. Molecular markers and high-throughput genotyping analysis. Functional Plant Genomics. Science Publishers, Enfield, New Hampshire. p 503-527.

Farooq, S. 2001. Marker-assisted plant breeding in wheat, rice and cotton. New horizon in DNA technology. Center for Agriculture Biotechnology and Biochemistry (CABB), University of Agriculture, Faisalabad. p. 73-88.

Farooq, S. and F. Azam. 2002. Molecular markers in plant breeding-I: Concepts and characterization. Pakistan J. Biol. Sci. 5: 1135-1140.

Farooq, S. and H. Sayyed.1999. Isozyme markers in cotton breeding-III. Variation of isozyme peroxidase in different loci of different varieties and their relationship with cotton leaf curl virus disease. Pakistan J. Bot. 31: 361-370.

Farooq, S., N. Iqbal and M. Arif. 1998. Detection of genetic variability in Basmati and non-Basmati rice varieties and their radiation induced mutants through random amplified polymorphic DNA (RAPD). Technical Document N. p. 85-96

Geng, H., X. Xia, L. Zhang, Y. Qu and Z. He. 2012. Development of functional markers for a lipoxygenase gene on chromosome 4BS in common wheat. Crop Sci. 52(2): 568-576.

Goutam, U., S. Kukreja, R. Yadav, N. Salaria, K. Thakur and A.K. Goyal. 2015. Recent trends and perspectives of molecular markers against fungal diseases in wheat. Front. Microbiol. 6:861.

Gregorius, H.R. and F. Bergmann. 1995. Analysis of isoenzyme genetic profiles observed in forest tree populations. Population Genetics and Genetic Conservation of Forest Trees. SPB Academic Publishing, Amsterdam. p 79-96.

Gupta, P.K., P. Langridge and R.R. Mir. 2010. Marker-assisted wheat breeding: present status and future possibilities. Mol. Breed. 26(2): 145-161.

Huang, X., X. Wei, T. Sang, Q. Zhao, Q. Feng, Y. Zhao, C. Li, C. Zhu, T. Lu and Z. Zhang. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat. Genet. 42: 961-967.

Ijaz, S. and I. Khan. 2009. Molecular characterization of wheat germplasm using microsatellite markers. Genet. Mol. Res. 8: 809-815.

Iqbal, A., B. Sadia, A. Khan, F. Awan, R. Kainth and H. Sadaqat. 2010. Biodiversity in the sorghum (Sorghum bicolor L. Moench) germplasm of Pakistan. Genet. Mol. Res. 9: 756-764.

Iqbal, M.J., Y. Maqsood, Z.U. Abdin, A. Manzoor, M. Hassan and A. Jamil. 2016. SSR markers associated with proline in drought tolerant wheat germplasm. Appl. Biochem. Biotechnol. 178: 1042-1052.

Jahufer, M., B. Barret, A. Griffiths and D. Woodfield. 2003. DNA fingerprinting and genetic relationships among white clover cultivars. Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association. p. 163-169.

Jan, H.U., M.A. Rabbani and Z.K. Shinwari. 2011. Assessment of genetic diversity of indigenous turmeric (*Curcuma longa* L.) germplasm from Pakistan using RAPD markers. J. Med. Plants Res. 5: 823-830.

Jena, K. and D. Mackill. 2008. Molecular markers and their use in marker-assisted selection in rice. Crop Sci. 48: 1266-1276.

Jenkins, S. and N. Gibson. 2002. High-throughput SNP genotyping. Comp. Funct. Genomics. 3(1): 57-66.

Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, and E. Charpentier. 2012. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science. 337: 816-821.

Jombart, T., D. Pontier, and A. Dufour. 2009. Genetic markers in the playground of multivariate analysis. Heredity. 102(4): 330.

Kausar, S., S. Hameed, K. Saleem, I. ul-Haque, M. Zamurrad and M. Ashfaq. 2015. Molecular confirmation of Bdv2 gene in wheat germplasm and its field based assessment for resistance against barely yellow dwarf viruses. Adv. Life Sci. 3: 16-22.

Khatab, I. A., M. A. Farid, and T. Kumamaru. 2016. Genetic diversity associated with heading date in some rice (*Oryza sativa* L.) genotypes using microsatellite markers. J. Environ. Agric. Sci. 6: 58-63.

Levinson, G. and G.A. Gutman. 1987. High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in Escherichia coli K-12. Nucleic Acids Res. 15(13): 5323-5338.

Li, C., C. K. Y. Ng, and L. M. Fan. 2015. MYB transcription factors, active players in abiotic stress signaling. Environ. Exp. Bot. 114: 80-91

Mackill, D., H. Nguyen and J. Zhang. 1999. Use of molecular markers in plant improvement programs for rainfed lowland rice. Field Crop. Res. 64: 177-185.

MacKill, D.J. 2006. Breeding for resistance to abiotic stresses in rice: the value of quantitative trait loci. Plant breeding: The Arnel R. Hallauer International Symposium. Wiley Online Library. p. 201-212.

Mahmood, T., S. Muhammad and Z.K. Shinwari. 2010. Molecular and morphological characterization of Caralluma species. Pakistan J. Bot. 42: 1163-1171.

Mammadov, J., R. Aggarwal, R. Buyyarapu and S. Kumpatla. 2012. SNP markers and their impact on plant breeding. Int. J. Plant Genomics. 2012: 1-11.

Manzo-Sánchez, G., M. Buenrostro-Nava, S. Guzmán-González, M. Orozco-Santos, M. Youssef, and R. M. E. G. Medrano. 2015. Genetic diversity in bananas and plantains (*Musa* spp.). In: Molecular Approaches to Genetic Diversity: InTech.

Mehmood, S., A. Bashir, A. Ahmad, Z. Akram, N. Jabeen and M. Gulfraz. 2008. Molecular characterization of regional *Sorghum bicolor* varieties from Pakistan. Pakistan J. Bot. 40(5): 2015-2021.

Mir, R.R., P.J. Hiremath, O. Riera-Lizarazu and R.K. Varshney. 2013. Evolving molecular marker technologies in plants: from RFLPs to GBS. Diagnostics in Plant Breeding. Springer. p. 229-247.

Mohan, M., S. Nair, A. Bhagwat, T. Krishna, M. Yano, C. Bhatia and T. Sasaki. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed. 3: 87-103.

Mondini, L., A. Noorani, and M. A. Pagnotta. 2009. Assessing plant genetic diversity by molecular tools. Diversity. 1(1): 19-35.

Moose, S.P. and R.H. Mumm. 2008. Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol. 147(3): 969-977.

M'Ribu, H.K. and K.W. Hilu. 1996. Application of random amplified polymorphic DNA to study genetic diversity in *Paspalum scrobiculatum* L. (Kodo millet, Poaceae). Genet. Resour. Crop Evol. 43: 203-210.

Mumtaz, A.S., M. Naveed and Z.K. Shinwari. 2010. Assessment of genetic diversity and germination pattern in selected cotton genotypes of Pakistan. Pakistan J. Bot. 42: 3949-3956.

Paux, E., P. Sourdille, I. Mackay, and C. Feuillet. 2012. Sequence-based marker development in wheat: advances and applications to breeding. Biotechnol. Adv. 30(5): 1071-1088.

Peng, J.H., D. Sun and E. Nevo. 2011. Domestication evolution, genetics and genomics in wheat. Mol. Breeding. 28(3): 281-301.

Pervaiz, Z.H., M.A. Rabbani, Z.K. Shinwari, M.S. Masood and S.A. Malik SA. 2010. Assessment of genetic variability in rice (*Oryza sativa* L.) germplasm from Pakistan using RAPD markers. Pakistan J. Bot. 42: 3369-3376.

Poczai, P., I. Varga, M. Laos, A. Cseh, N. Bell, J.P. Valkonen and J. Hyvönen. 2013. Advances in plant gene-targeted and functional markers: a review. Plant Methods. 9: 1-31.

Price, A., A. Tomos and D. Virk. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.) I: a hydrophonic screen. Theor. Appl. Genet. 95: 132-142.

Qamar, M., S.D. Ahmad, M.A. Rabbani, Z.K. Shinwari and M. Iqbal. 2014. Determination of rust resistance genes in Pakistani bread wheats. Pakistan J. Bot. 46(2): 613-617.

Rabbani, M.A., A. Iwabuchi, Y. Murakami, T. Suzuki and K. Takayanagi. 1998. Genetic diversity in mustard (*Brassica juncea* L.) germplasm from

Pakistan as determined by RAPDs. Euphytica. 103: 235-242.

Rabiei, B. 2010. Linkage map of SSR markers and QTLs detection for heading date of Iranian rice cultivars. J. Agric. Sci. Technol. 9: 235-242.

Rahimi, M., B. Rabiei, H. Dehghani and A. Tarang. 2014. Mapping main and epistatic QTLs for drought tolerance indices in F5 population of rice. Mod.Genet. 8(4): 435-448.

Rahman, M., D. Hussain and Y. Zafar. 2002. Estimation of genetic divergence among elite cotton cultivars–genotypes by DNA fingerprinting technology. Crop Sci. 42: 2137-2144.

Rao, Y., Y. Li and Q. Qian. 2014. Recent progress on molecular breeding of rice in China. Plant cell Rep. 33(4): 551-564.

Rasheed, A., X. Xia, T. Mahmood, U.M. Quraishi, A. Aziz, H. Bux, Z. Mahmood, J.I. Mirza, A. Mujeeb-Kazi and Z. He. 2016. Comparison of economically important loci in landraces and improved wheat cultivars from Pakistan. Crop Sci. 56: 287-301.

Rasool, G., M.J. Jaskani, A. Ullakh and R. Ahmad. 2016. Genetic variability and phylogenetic relationship of Pakistani Snapmelon (*Cucumis melo* var. Momordica) by using microsatellite markers. J. Agr. Sci. 8(7): 163-172.

Riaz, A., G. Li, Z. Quresh, M. Swati and C. Quiros. 2001. Genetic diversity of oilseed Brassica napus inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breeding. 120: 411-415.

Sasaki, T. and B. Burr. 2000. International Rice Genome Sequencing Project: the effort to completely sequence the rice genome. Curr. Opin. Plant Boil. 3: 138-142.

Shah, R., M.Z. Sulemani, M.S. Baloch and G. Hassan. 1999. Performance of coarse rice genotypes in the plains of D.I. Khan, Pakistan. Pakistan J. Biol. Sci. 2(2): 507-509.

Shah, Z., I. Munir, S. Ali, A. Iqbal, S. Mumtaz, R. Nwaz and Z.A. Swati. 2009. Genetic diversity of Pakistani maize genotypes using chromosome specific simple sequence repeat (SSR) primer sets. Afr. J. Biotechnol. 8(3): 375-379.

Shahzad, A., M. Ahmad, M. Iqbal, I. Ahmed and G. Ali. 2012. Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. Genet. Mol. Res. 11: 679-692.

Shahzad, M., S. Khan, A. Khan, M. Sajjad, A. Rehman and A. Khan. 2016. Identification of QTLs on chromosome 1B for grain quality traits in bread wheat (*Triticum aestivum* L.). Cytol. Genet. 50: 89-95.

Silvertown, J., and D. Charlesworth. 2009. Introduction to plant population biology: John Wiley and Sons.

Smulders, M., and G. De Klerk. 2011. Epigenetics in plant tissue culture. Plant Growth Regul. 63 (2): 137-146.

Sultana, T. and A. Ghafoor. 2008. Genetic diversity in ex-situ conserved *Lens culinaris* for botanical descriptors, biochemical and molecular markers and identification of landraces from indigenous genetic resources of Pakistan. J. Integ. Plant Biol. 50: 484-490.

Tester, M., and P. Langridge. 2010. Breeding technologies to increase crop production in a changing world. Science. 327 (5967): 818-822.

Tondelli, A., D. Pagani, I.N. Ghafoori, M. Rahimi, R. Ataei, F. Rizza, A.J. Flavell and L. Cattivelli. 2014. Allelic variation at Fr-H1/Vrn-H1 and Fr-H2 loci is the main determinant of frost tolerance in spring barley. Environ. Exp. Bot. 106: 148-155.

Varshney, R.K., S.N. Nayak, G.D. May and S.A. Jackson. 2009. Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends Biotechnol. 27: 522-530.

Vieux, E.F., P.Y. Kwok and R.D. Miller. 2002. Primer design for PCR and sequencing in high-throughput analysis of SNPs. Bio Tech. 32: 28-30.

Wang, R.X., L. Hai, X.Y. Zhang, G.X. You, C.S. Yan and S.H. Xiao. 2009. QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai Yu8679. Theor. Appl. Genet. 118(2): 313-325.

Williams J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Wu, R., C. Ma and G. Casella. 2007. Statistical genetics of quantitative traits: linkage, maps and QTL. Springer Science & Business Media.

Xia, X.C., J.C. Reif, A.E. Melchinger, M. Frisch, D.A. Hoisington, D. Beck, K. Pixley and M.L. Warburton. 2005. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers. Crop Sci. 45(6): 2573-2582.

Xu, Y. 1997. Quantitative trait loci: separating, pyramiding, and cloning. Plant Breed. Rev. 15: 85-139.

Yasmin, T., N. Tabbasam, I. Ullah, M. Asif and Y. Zafar. 2008. Studying the extent of genetic

diversity among *Gossypium arboreum* L. genotypes/cultivars using DNA fingerprinting. Genet. Resour. Crop Evol. 55: 331-339.

Zafar, Y., M. Asif, M. Ashraf, S. Riaz, S. Zafar, A. Wahid, S. Maqsood, M. Niaz and S.Q. Abbas. 2009. Development of genetic linkage map of leaf hairiness in *Gossypium hirsutum* (cotton) using molecular markers. Pakistan J. Bot. 41: 1627-1635.

Zahid, N., N. Abbasi, I. Hafiz and Z. Ahmad. 2009. Genetic diversity of indigenous fennel (*Foeniculum vulgare* Mill) germplasm in Pakistan assessed by RAPD markers. Pakistan J. Bot. 41: 1759-1767.

Zeb, A., Z.K. Shinwari and T. Mahmood. 2011. Molecular markers assisted genetic characterization of some selected wild Poaceae species. Pakistan J. Bot. 43: 2285-2288.

Zhu, C., M. Gore, E.S. Buckler and J. Yu. 2008. Status and prospects of association mapping in plants. Plant Genome. 1(1): 5-20.

Journal of Environmental & Agricultural Sciences (JEAS). Volume 11