The Pharma Innovation Journal 2021; SP-10(7): 638-643

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(7): 638-643

www.thepharmajournal.com
Received: 10-05-2021
Accepted: 16-06-2021

Sandeep Kumar Bangarwa
ICAR-Central Arid Zone Research Institute-Jodhpur, Rajasthan, India

Balwan
ICAR-Central Arid Zone Research Institute-Jodhpur, Rajasthan, India

Kanti Lal Solanki
ICAR-Central Arid Zone Research Institute-Jodhpur, Rajasthan, India

Manoj Choudhary
ICAR-Central Arid Zone Research Institute-Jodhpur, Rajasthan, India

Mahendra Kumar Choudhary
ICAR-Central Institute for Arid Horticulture-Bikaner, Rajasthan, India

Corresponding Author:
Sandeep Kumar Bangarwa
ICAR-Central Arid Zone Research Institute-Jodhpur, Rajasthan, India

An introduction to DNA-Markers and their role in crop improvement

Sandeep Kumar Bangarwa, Balwan, Kanti Lal Solanki, Manoj Choudhary and Mahendra Kumar Choudhary

Abstract
Genetic markers are useful in identification of various genetic variations. Genetic markers are one of the advances which have occured in the genomics era. Molecular markers are used to increase the efficiency and effectiveness of breeding programs. Among genetic markers, molecular markers mainly because of their abundance, are the most widely used them. The development of DNA-based genetic markers has had a revolutionary impact on genetic studies. Some studies which were conducted during the last decade of the 20th century reported numerous DNA markers that have been utilized in plant breeding programs. With DNA markers, it is theoretically possible to observe and exploit genetic variation in the entire genome. These markers can be used to study the evolutionary relationships among individuals, construction of linkage maps, assessing the genetic variations within cultivars and germplasms. Popular genetic markers include allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP, and EST markers. This review focuses on different types of markers and their importance and uses of genetic markers with advent of modern technologies.

Keywords: Genetic markers, genetic polymorphism, gene pyramiding, marker-assisted selection, mapping

Introduction
Conventional breeding is a dynamic area of applied science. It relies on genetic variation and uses selection to gradually improve plants for traits and characteristics that are of interest for the grower and the consumer. Another important way of improvement is the introduction of new genetic material (e.g., genes for biotic and abiotic stress resistance) from other sources, such as gene bank accessions and related plant species. Modern biotechnology provides new tools that can facilitate development of improved plant breeding methods and augment our knowledge of plant genetics. The discovery of restriction enzymes by Smith and Wilcox, and the polymerase chain reaction (PCR) by Karry Mullis and his group has created opportunity to understand the composition of organisms at the DNA level, and obtain a so called genetic fingerprint.

DNA marker is a small region of DNA sequence showing polymorphism (base deletion, insertion and substitution) between different individuals. There are two basic methods to detect the polymorphism: Southern blotting, a nuclear acid hybridization technique (Southern, 1975) [32], and PCR, a polymerase chain reaction technique (Mullis, 1990) [19]. Different studies shown that in case of complex diseases, it is more difficult to identify genetic markers because complex diseases are polygenic i.e caused by defect in multiple genes selective digestion of DNA with enzymes or from a selective amplification of DNA using PCR. DNA fragments that result in different gel patterns between samples or individuals are called polymorphic markers. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene ‘tags’ Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or ‘linked’ to genes controlling the trait (Rabiei, 2010) [25]. There are three major types of genetic markers: (a) Morphological markers (also called "classical" or "visible" markers) which are phenotypic traits, (b) Biochemical markers, those are known as isozymes, including allelic variants of enzymes, and (c) DNA markers (or Molecular markers), which reveal sites of variation in DNA (Jones *et al*., 1997 and Winter and Kahl, 1995) [15, 37].

Morphological Markers
A morphological marker is expressed as a specific and distinct morphological trait.

Morphological marker may be affected by environment. Generally it is incompletely linked with the gene of interest. Its phenotypic expression may be dependent on growth stage. These markers are rare in a natural population and show extremely low level of polymorphism.

Biochemical/protein markers: Protein markers may also be categorized into molecular markers though the latter are more referred to DNA markers. Isozymes are alternative forms or structural variants of an enzyme that have different molecular weights and electrophoretic mobility but have the same catalytic activity or function. Isozymes reflect the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution (Xu, 2010) [39]. Isozyme markers can be genetically mapped onto chromosomes and then used as genetic markers to map other genes. They are also used in seed purity test and occasionally in plant breeding. The major disadvantages of morphological and he major disadvantages of morphological and biochemical markers are that they may be limited in numbers and are influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995) [37]. However, despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders (Eagles *et al*., 2001 and Weeden *et al*., 1994) [7, 34].

DNA markers: DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) (Collard *et al*., 2005) [4] such as RFLP, AFLP, RAPD, SSR, SNP etc. DNA markers are practically unlimited in number and are not affected by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995) [37]. DNA markers are the most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996) [21]. DNA markers are selectively neutral because they are usually located in non-coding regions of DNA. New concepts, such as quantitative trait loci (QTL) mapping followed by the development of statistical tools, have emerged in quantitative genetics to identify the genes involved in the genetic variability of complex traits. The complexity of these traits is influenced by the segregation of alleles at many loci, environmental factors and their interactions (Rahimi *et al*., 2012 [26] and Yamamoto *et al*., 2009) [40]. The choice of markers is thus objective dependent. For example, if differentiation were to be made or example, if differentiation were to be made between two similar accessions of a species, molecular markers like RAPD, RFLP, or AFLP would do the job (Farooq *et al*., 1996) [10]. However, if closely related varieties differing only in few environmentally induced physiological characters are to be discriminated, the non-neutral marker system such as DNA microarray or the isozyme markers would be a better choice. In order to assess the qualitative and quantitative differences produced in a crop before and after the attack of pests or diseases, isozyme would again be preferred (Farooq and Sayyed, 1999 and Farooq and Sayyed, 1999) [11, 12].

Since Botstein *et al*. (1980) first used DNA restriction fragment length polymorphism (RFLP) in human linkage mapping, substantial progress has been made in development and improvement of molecular techniques that help to easily find markers of interest on a largescale, resulting in extensive and successful uses of DNA markers in human genetics, animal genetics and breeding, plant genetics and breeding and germplasm characterization and management. Among the techniques that have been extensively used and are particularly promising for application to plant breeding, are the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP).

Techniques for detection of genetic polymorphism

These markers come from different classes of DNA mutations such as substitution mutations (point mutations), re-assortments (insertions and deletions), replication errors and DNA tandem repeats (Paterson, 1996 Falque and Santoni, 2007 and Zhu *et al*., 2008) [21, 9, 41]. DNA markers involve several sets of markers and divide into two main categories: PCR-based molecular markers and hybridization-based molecular markers. Restriction Fragment Length Polymorphism (RFLP) is a hybridization-based molecular marker. Moreover, Random Amplified Polymorphic DNAs (RAPD), Amplification Length Polymorphism (ALP), Simple Sequence Repeat (SSRs), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplified Regions (SCARs), Sequence Tagged Sites (STS), Sequence Tagged Sites (STS), Single Polymorphic Amplification Test (SPLAT), Variable Number of Tandem Repeats (VNTRs), DNA Amplification Fingerprinting (DAF), Single Nucleotide Polymorphism (SNPs), Micro-satellites or Short Tandem Repeats (STRs) and Single Strand Conformation Polymorphism (SSCP) are PCR-based molecular markers (Paterson, 1996) [21]. The most common methods used in various applications are the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP). The genetic markers can also be classified into SNPs (due to sequence variation, e.g. RFLP) and non-SNPs (due to length variation, e.g. SSR). The marker techniques help in selection of multiple desired characters simultaneously using F2 and back-cross populations, near isogenic lines, doubled haploids and recombinant inbred lines. Single nucleotide polymorphisms (SNPs) have received much attention as potential genetic markers. They have the advantage of a high frequency in the human genome (1 occurs every 1000 nucleotides, on average) and are relatively easy to genotype using current technologies.

RFLP markers: A Restriction Fragment Length Polymorphism is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA sample into pieces with restriction enzymes and then analyzing the size of the restricted fragments by gel electrophoresis. It is the sequence that makes DNA from different sources different, and RFLP analysis is a technique that can identify some differences in sequence (when they occur in a restriction site). RFLP, as a molecular marker, is specific to a single clone/restriction enzyme combination. RFLP markers that are used for high density genomic mapping (Botstein *et al*., 1980) [2] provided a

new technique which overcame some of the problems associated with isozymes and proteins. RFLPs, being co-dominant markers, can detect the coupling phase of DNA molecules, as DNA fragments from all homologous chromosomes are detected. RFLPs, being codominant markers, can detect the coupling phase of DNA molecules, as DNA fragments from all homologous chromosomes are detected. RFLP markers are powerful tools for comparative and synteny mapping. Most RFLP markers are co-dominant and locus-specific. RFLP genotyping is highly reproducible, and the methodology is simple and no special equipment is required. By using an improved RFLP technique, i.e., cleaved amplified polymorphism sequence (CAPS), also known as PCRRFLP, high-throughput markers can be developed from RFLP probe sequences. Very few CAPS are developed from probe sequences, which are complex to interpret. Most CAPS are developed from SNPs found in other sequences followed by PCR and detection of restriction sites. CAPS technique consists of digesting a PCR-amplified fragment and detecting the polymorphism by the presence/absence of restriction sites (Konieczny and Ausubel, 1993) [16].

AFLP markers: Amplified Fragment Length Polymorphism is a highly sensitive method for fingerprinting genomic DNA within any organism (Vos *et al*. 1995) [33]. Variations at many loci can be arrayed simultaneously to detect single nucleotide variations of unknown genomic regions, in which a given mutation may be frequently present in undetermined functional genes. AFLPs are PCR-based markers, simply RFLPs visualized by selective PCR amplification of DNA restriction fragments. Technically, AFLP is based on the selective PCR amplification of restriction fragments from a total double-digest of genomic DNA under high stringency conditions, i.e., the combination of polymorphism at restriction sites and hybridization of arbitrary primers. Because of this AFLP is also called selective restriction fragment amplification (SRFA). An AFLP primer (17-21 nucleotides in length) consists of a synthetic adaptor sequence, the restriction endonuclease recognition sequence and an arbitrary, non-degenerate 'selective' sequence (1-3 nucleotides). The primers used in this technique are capable of annealing perfectly to their target sequences (the adapter and restriction sites) as well as a small number of nucleotides adjacent to the restriction sites. AFLP analysis depicts unique fingerprints regardless of the origin and complexity of the genome. Most AFLP fragments correspond to unique positions on the genome and hence can be exploited as landmarks in genetic and physical mapping. Applications of this technique reach far beyond agricultural applications, ranging from agronomic trait analysis, diagnostics, pedigree analysis, forensics, parental heritage and may be used as a universal fingerprinting system (Pereira *et al*. 2010) [23].

RAPD markers: RAPD is a PCR-based marker system. In this system, the total genomic DNA of an individual is amplified by PCR using a single, short (usually about ten nucleotides/ bases) and random primer. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary (10 mers) oligonucleotide primers (Welsh and Mcclelland 1990; Williams *et al*. 1990) [35, 36]. The primer which binds to many different loci is used to amplify random sequences from a complex DNA template that is complementary to it (maybe including a limited number of mismatches). Amplification can take place during the PCR, if two hybridization sites are similar to one another (at least 3000 bp) and in opposite directions. The amplified fragments generated by PCR depend on the length and size of both the primer and the target genome. The PCR products (up to 3 kb) are separated by agarose gel electrophoresis and imaged by ethidium bromide (EB) staining. Polymorphisms resulted from mutations or rearrangements either at or between the primer-binding sites are visible in the electrophoresis as the presence or absence of a particular RAPD band. RAPD predominantly provides dominant markers. This system yields high levels of polymorphism and is simple and easy to be conducted. First, neither DNA probes nor sequence information is required for the design of specific primers. Second, the procedure does not involve blotting or hybridization steps, and thus it is a quick, simple and efficient technique. An additional drawback is that of poor reliability and reproducibility, and their sensitivity to experimental conditions (Devey *et al*., 1995) [5].

SSR markers: Microsatellite or Simple sequence repeats (SSRs) provide fairly comprehensive genomic coverage. They are amenable to automation, they have locus identity and they are multi-allelic. SSR markers are characterized by their hyper-variability, reproducibility, co-dominant nature, locus-specificity, and random genome-wide distribution in most cases. The advantages of SSR markers include that they can be readily analyzed by PCR and easily detected by PAGE or AGE. SSR markers can be multiplexed, have high throughput genotyping and can be automated. Many agronomic and quality traits show quantitative inheritance and the genes determining these traits have been quantified using Quantitative trait locus (QTL) tools. SSR markers have wide applicability for genetic analysis in crop improvement strategies. They are widely used in plants because of their abundance, hyper-variability, and suitability for high throughput analysis. To develop the locus-specific SSR markers, the isolation and characterization of individual loci and the construction and screening of a DNA library with microsatellite-specific probes, followed by DNA sequencing of positive clones are required (Roder *et al*. 1998). Because these regions are hypervariable, RFLP analysis with probes for micro- and minisatellites gives multilocus patterns which have resolved variation at the levels of populations and individuals (Marquardt and Echt 1995; Butcher *et al*., 1999) [17, 3]. In many species, plenty of breeder-friendly SSR markers have been developed and are available for breeders. For instance, there are over 35,000 SSR markers developed and mapped onto all 20 linkage groups in soybean, and this information is available for the public (Song *et al*., 2010) [31].

SNP markers: An SNP is a single nucleotide base difference between two DNA sequences or individuals. SNPs can be categorized according to nucleotide substitutions either as transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G). In practice, single base variants in cDNA (mRNA) are considered to be SNPs as are single base insertions and deletions (in- dels) in the genome. SNPs provide the ultimate/simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance, and thus they can provide maximum markers. SNPs occur very commonly in animals and plants. Typically, SNP frequencies are in a range of one SNP every 100-300 bp in plants (Edwards *et al.*, 2007; Xu, 2010) [8, 39]. SNPs may present within coding sequences of genes, non-coding regions of

genes or in the intergenic regions between genes at different frequencies in different chromosome regions.

There are several SNP genotyping assays, such as allele-specific hybridization, primer extension, oligonucleotide ligation and invasive cleavage based on the molecular mechanisms (Sobrino *et al*., 2005) [30], and different detection methods to analyze the products of each type of allelic discrimination reaction, such as gel electrophoresis, mass spectrophotome- try, chromatography, fluorescence polarization, arrays or chips, etc. At the present, SNPs are also widely detected by sequencing. Detailed procedures are described in the review by Gupta *et al*. (2001) [14] and the book Molecular Plant Breeding by Xu (2010) [39].

Applications of molecular markers in crop improvement

1. **Marker assisted selection (MAS):** Marker-assisted selection (MAS), sometimes also called marker-aided selection, is a relatively new tool for plant breeding which is primarily based on the phenotypic selection of superior individuals among segregating progenies resulting from hybridization. QTL detection using DNA markers is considered as one of the major advances in characterization of quantitative traits (Paterson *et al*., 1988) [22]. DNA markers which are tightly linked to agronomically important genes, can be used as molecular tools for MAS in plant breeding (Rahimi *et al*., 2013; Rafalski and Tingey, 1993) [27, 28].
2. **Marker assisted pyramiding:** Pyramiding is the simultaneous integration of multiple genes/ QTLs into a single genotype. It's possible through traditional breeding, but in the early generations this may be extremely difficult or impossible. In conventional phenotypic selection, individual plants should be screened for all phenotypic traits. Therefore, evaluation of certain types of plants, populations (e.g. F2), or traits (with destructive bioassays) are more difficult. DNA markers may facilitate selection, because they don't need destructive tests and can examine particular genes/ QTLs using a single DNA sample without phenotyping.
3. **Microarray-based mapping**: DNA microarray technology has given rise to the study of functional genomics. The entire set of genes of an organism can be microarrayed on an area as small as a fingernail and the expression levels of thousands of genes are simultaneously studied in a single experiment (Gupta *et al*. 1999) [13]. Microarrays have demonstrated significant power for genomewide analyses of gene expression, and recently have also revolutionized the genetic analysis of segregating populations by genotyping thousands of loci in a single assay (Drost *et al*., 2009) [6]. DNA microarray technology allows comparisons of gene expression levels on a genomic scale in all kinds of combinations of samples derived from normal and diseased tissues, treated and non-treated time courses, and different stages of differentiation or development.
4. **Construction of linkage maps and QTL mapping:** One of the main applications of DNA markers in agricultural research is the construction of linkage maps for different types of crops. Linkage map can be considered as a "road map" of chromosomes from two different parents (Paterson, 1996; Wu *et al*., 2007) [21, 38]. Linkage maps are used to identify chromosomal regions that contain single gene traits (controlled by a single gene) and quantitative traits using QTL analysis (Mohan *et al*., 1997) [18]. QTL mapping is based on this fact that genes and markers are segregating through chromosomal recombination (chromosomal crossover) during meiosis (sexual reproduction), which permit to analyze them in the progenies (Bernardo, 2008) [1].

The structure of genetic linkage maps using molecular markers is based on certain principles

- Selecting the molecular markers and genotyping system,
- Selecting the parental lines from germplasm collections which are highly polymorphic in the marker loci,
- Creating populations or lines (derived from these populations) using a large number of molecular markers segregating in the population
- Genotyping of each individual/ line using molecular markers and making linkage maps using markers information (Price *et al*., 1997) [24].

Conclusion

Molecular markers make it possible to accelerate the plant breeding process because it is possible to generate high density linkage maps of traits and markers and use them in many genetic backgrounds as required in a breeding program. Having access to thousands of markers linked to traits makes it possible to select the genotype of interest based on markers and so to limit field and breeding activities to plants with the genotype of interest.

Molecular markers and marker mapping are a part of the intrusive new genetics that is pushing its way into all areas of modern biology, from genomics to breeding, from transgenics to developmental biology, from systematics to ecology, and even, perhaps especially, into plant and crop physiology. However, because genes do not function as single entities, it is necessary to know how numerous genes function together. This, in turn, requires the knowledge of the potential and constraints of biological functions of plants. The understanding of the interaction between genes, organs and environmental factors, which include other organisms, is a major challenge for plant biologists. To obtain this information, it is important to exploit the tools of classical and molecular genetics.

Future perspectives

Plant breeding plays a key role in increasing crop production, productivity and food security (Ni *et al*., 2002) [20]. However, plant breeders are faced with major challenges in increased production because of global warming, creating new biotypes of diseases and insects and several abiotic stresses which often reduce crop yield. Integration of desired genes from different backgrounds of elite cultivars led to the development of crops gene pool and the development of improved crop varieties suitable for different agro-climatic conditions.

References

1. Bernardo R. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Science 2008;48(5):1649-64.
2. Botstein D, White RL, Skolnick M, Davies RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 1980;32:314-331.
3. Butcher PA, Glaubitz JC, Moran GF. Applications for microsatellite markers in the domestication and conservation of forest trees. Forest Genetic Resources

Information 1999;27:34-42.
4. Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 2005;142:169-196.
5. Devey ME, Delfino-Mix A, Kinloch BB, Neale DB. Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white pine blister rust in sugar pine. Proceedings of the National Academy of Sciences USA 1995;92:2066-2070.
6. Drost DR, Novaes E, Novaes CB, Benedict C, Brown RS, Yin T *et al*. A microarray based genotyping and genetic mapping approach for highly heterozygous outcrossing species enables localization of a large fraction of the unassembled *Populus trichocarpa* genome sequence. Plant Journal 2009;58:1054-1067.
7. Eagles HA, Bariana HS, Ogbonnaya FC. Implementation of markers in Australian wheat breeding. Crop and Pasture Science 2001;52(12):1349-56.
8. Edwards D, Forster JW, Chagne D, Batley J. What is SNPs? In: Or- aguzie NC, Rikkerink EHA, Gardiner SE and de Silva HN (eds.), Association Mapping in Plants. Springer, Berlin 2007, 41-52.
9. Falque M, Santoni S. Molecular markers and high-throughput genotyping analysis. In: Morot-Gaudry JF, Lea P, Briat JF, editors. Functional plant genomics. Taylor & Francis Group, LLC 2007, 503-27.
10. Farooq S, Iqbal N, Arif M, editors. Detection of Genetic variability in Basmati and non-Basmati rice varieties and their radiation induced mutants through Random Amplified Polymorphic DNA (RAPD). Application of DNA based marker mutations for improvement of cereals and other sexually reproduced crop plants Proceedings of a final research co-ordination meeting Joint FAO/ IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna (Austria) 1996.
11. Farooq S, Sayyed H. Isozyme markers in cotton breeding-II: Inter and intravarietal variation in the activity of isozymes of the enzyme peroxidase as affected by area of cotton cultivation. Pakistan Journal of Botany 1999;31:347-59.
12. Farooq S, Sayyed H. 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 Journal of Botany 1999;31:361-70.
13. Gupta PK, Roy JK, Prasad M. DNA chips, microarray and genomics. Current Science 1999;77:875-884.
14. Gupta PK, Roy JK, Prasad M. Single nucleotide polymorphisms: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. Current Science 2001;80:524-535.
15. Jones N, Ougham H, Thomas H. Markers and mapping: we are all geneticists now. New Phytologist 1997;137(1):165-77.
16. Konieczny A, Ausubel F. A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR based markers. The Plant Journal 1993;4:403-410.
17. Marquardt PM, Echt C. Simple sequence repeats (SSRs) as genetic markers in gymnosperms. (Poster Presentation) Joint Meeting of IUFRO Molecular Genetics Working Group and Somatic Cell Working Group. Gent September 1995, 26-30.
18. Mohan M, Nair S, Bhagwat A. Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular breeding 1997;3(2):87-103.
19. Mullis K. The unusual origin of the polymerase chain reaction. Scientific American 1990;262(4):56-61, 64-65.
20. Ni J, Colowit PM, Mackill DJ. Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Science 2002;42(2):601-07.
21. Paterson AH. Making genetic maps. In: Paterson AH, editor. Genome mapping in plants RG. Landes Company 1996, 23-39.
22. Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 1988;335(6192):721-26.
23. Pereira MJ, Caballero A, Quesada H. Evaluating the relationship between evolutionary divergence and phylogenetic accuracy in AFLP data sets. Molecular Biology Evolution 2010;27:988-1000.
24. Price A, Tomos A, Virk D. Genetic dissection of root growth in rice (*Oryza sativa* L.): A hydrophonic screen. Theoretical and Applied Genetics 1997;95(1-2):132-42.
25. Rabiei B. Linkage map of SSR markers and QTLs detection for heading date of Iranian rice cultivars. Journal of Agricultural Science and Technology 2010;9:235-42.
26. Rahimi M, Dehghani H, Rabiei B, Tarang A. Multi-trait mapping of QTLs for drought tolerance indices in rice. Cereal Research (in Farsi) 2012;2(2):107-21.
27. Rahimi M, Dehghani H, Rabiei B, Tarang A. Mapping main and epistatic QTLs for drought tolerance indices in F5 population of rice. Modern Genetics Journal (in Farsi) 2013;8(4):435-48.
28. Rafalski JA, Tingey SV. Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends in Genetics 1993;9(8):275-80.
29. Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P *et al*. A microsatellite map of wheat. Genetics 1998;149:2007-2023.
30. Sobrino B, Briona M, Carracedoa A. SNPs in forensic genetics: A review on SNP typing methodologies. Forensic Science International 2005;154:181-194.
31. Song Q, Jia G, Zhu Y, Grant D, Nelson RT, Hwang EY *et al*. Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_1.0) in soybean. Crop Science 2010;50:1950-1960.
32. Southern EM. Detection of specific sequences among DNA fragments separated by gel-electrophoresis. Journal of Molecular Biology 1975;98:503-517.
33. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M *et al*. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 1995;23:4407-1444.
34. Weeden N, Timmerman G, Lu J. Identifying and mapping genes of economic significance. Expanding the Production and Use of Cool Season Food Legumes Springer 1994, 726-37.
35. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 1990;18:7213-7218.

36. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 1990;18:6531-6535.
37. Winter P, Kahl G. Molecular marker technologies for plant improvement. World Journal of Microbiology and Biotechnology 1995;11(4):438-48.
38. Wu R, Ma C, Casella G. Statistical genetics of quantitative traits: linkage, maps and QTL. Springer Science & Business Media 2007.
39. Xu Y. Molecular plant breeding. CAB International 2010.
40. Yamamoto T, Yonemaru J, Yano M. Towards the understanding of complex traits in rice: substantially or superficially? DNA research 2009;16(3):141-54.
41. Zhu C, Gore M, Buckler ES, Yu J. Status and prospects of association mapping in plants. The plant genome 2008;1(1):5-20.