Journal of Pharmaceutical Research International

33(45B): 464-473, 2021; Article no.JPRI.74964
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Study of Plant Genetic Variation through Molecular Markers: An Overview

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i45B32828
Editor(s):
(1) Prof. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.
Reviewers:
(1) Isna Arofatun Nikmah, Indonesia Defense University, Indonesia.
(2) Kundan K. Mishra, N. V. Patel College of Pure and Applied Sciences, (CVMU), India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74964

Review Article

Received 30 July 2021
Accepted 06 October 2021
Published 09 October 2021

ABSTRACT

This article refers to viewing the role of molecular markers during analyzing the genome of plants and their importance in plant biotechnology. In recent years, we observed the role of molecular techniques in programs for improving plant breeding and preserving genetic resources has been observed, and molecular and biochemical indicators which represent basic material through determining the diversity between genotypes for indicators it is never affected by external surrounding conditions as always in the phenotype features. Molecular markers of DNA have been widely applied to answer a range of questions related to taxonomy, molecular evolution, population genetics, and genetic diversity, as well as monitoring trade in plants and food products , in addition to its having a role in studying gene expression , genetic mapping, and studies of species evolution providing fast and accurate results. In this work, the advantages and limitations of the molecular techniques applied in plant sciences such as: RAPD (Random Amplification Polymorphic DNA Marker); ISSR (Inter Simple Sequence Repeat Marker); SSR (Simple Sequence Repeat Marker); AFLP (Amplified Fragment Length Polymorphic Marker); RFLP (Restriction Fragment Length Polymorphism Marker); SNP (Single Nucleotide Polymorphism) and Real Time PCR.

Keywords: Plant; molecular markers; genetic variation.

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1. INTRODUCTION

Molecular markers are considered one of the very accurate methods as they are used in human studies in the field of forensic genetics applications providing highly controlled information [1]. These techniques are used to amplify the genetic material *in vitro* using the polymerase chain reaction technique because it causes link indicators with genetic material, the tools permit to diagnose polymorphisms in DNA sequences that amplified at various weights according to the type of marker used. The indicators available are clear genetic differentiation between individuals who are similar phenotypically but different genetically. Correlation of molecular indicators with other kinds of genetic indicators is helpful in diagnosing diverse biological materials to generate potential tools for the diagnosis of plant germplasm.

The principles of genetic indicators are well known; since the 19th century, genetic scientist Gregor Mendel used genetic markers which depend on - phenotype in the experience. This led to the creation of a genetic relationship theory that occurs within a specific genetic site.

Disadvantages of genetic markers depending on phenotype results and advancement of genetic markers. Molecular indicators can be defined as a genomic locus, it is diagnosed through a special probe or specific primer to distinguish the traits carried on the chromosome which represent as always extremity regions 3' - 5'[2].

Typically, genetic marker should have features as a following: provide ideal resolution for genetic differences ; polymorphism is distributed over the entire genome ; fast, low cost, and simple techniques ; reliable and independent markers ; require little amount of DNA samples ; linking distinct phenotypes ; no need to have previous knowledgment about the genome sequences of organism . The different methods used for the molecular evaluation described in this review vary from one another to another that related to the main traits: the level of polymorphism and genetic abundance, specificity of loci, technical requirements and costs. Therefore, the molecular markers need to have modifications leading to the second generation of molecular techniques such as: Random Amplified Polymorphic DNA; Inter Simple Sequence Repeats; Simple Sequence Repeats; Restriction Fragment Length Polymorphism; Amplified Fragment Length Polymorphism; Single Nucleotide Polymorphism; Real Time PCR. These markers with their advantages and limitations are documented in this work [3].

2. ROLE OF MOLECULAR MARKERS ASSESSMENT OF GENETIC DIVERSITY

Genetic variation can be assessed within a population by:

1- number and percentage of polymorphic genes.
2- alleles number per gene.
3- ratio sites of heterozygous for each genotype.

This work documented different types of markers using DNA analysis, providing a clear indication on the assessment of molecular diversity in individuals not effected by surrounding environment [4].

2.1 DNA Molecular Markers

Molecular tools can be distinguished between different individuals within the nucleic sequence polymorphism, these differences including insertions , deletion , duplication, point mutation, and translocation. There are two types of techniques , either based on PCR , or based on restriction hybridization of DNA, or both. Table 1 illustrates the features of molecular markers. These tools are also assessing either several locus employed primers, with arbitrary sequences or specific primers, single site markers, or used probes, in addition the types of these markers dominant can distinguish the appearance or nonappearance of DNA fragments in each loci while cannot discernment between homozygote and heterozygotes for the same allele while others markers co-dominant allow for discernment between homozygote and heterozygote locus. See Table 1 showing a comparison between some molecular markers [5] as below:

Table 1. illustrates the features of molecular markers

Molecular Markers	RFLP	RAPD	AFLP	SSR	SNP
Polymorphism %	M	M	M	M	H

Molecular Markers	RFLP	RAPD	AFLP	SSR	SNP
Specificity site	Y	N	N	N	Y
Dominance & co-dominance	C	D	D	C	C
Replication	H	L	H	M	H
Information of sequence	Y	N	N	N	Y
DNA amount	H	L	M	L	L
Assay prices	H	L	M	L/M	L

Key: (H = High, M= Medium, L = Low, Y = Yes, N= No).

3. MOLECULAR PCR TECHNIQUES

By use technique, Polymerase Chain Reaction (PCR) that Discovered by Mullis et al. [6], through amplification, regions of DNA with primers lead to get discrete DNA products. PCR based techniques subdivided two different types : random primed based PCR techniques or nonspecific sequence techniques ; specific primed based PCR techniques or targeted sequence techniques.

3.1 Random Amplification Polymorphic DNA Marker (RAPD)

Can be using in molecular diversity methods [7,8]. This technique utilized a small amount of DNA material associated with random single primer, 10-15 nucleotides long by thermo cyclic amplification after setting a suitable annealing temperature. Then separation of DNA fragment products on agarose gel electrophoresis to detect polymorphism by presence or absence of primer binding sites on the region of DNA template and finally, visualization under UV light [9].

The advantages of this marker now needs prior information about DNA sequence, low costly, easy reaction, simplicity, and can amplify several regions and each code and noncode zone [10,11,12]. Limitation, this marker has dominant behavior because there are variable reproducibility results but limited genetic information per locus , not able distinguish between heterozygotes from homozygote individuals, and reaction conditions which contrast between laboratories [13,14]. In a study of genetic diversity and relationships between nine cultivars of Iraqi *Hordeum vulgare* that using 18 primers produced 106 bands or 60% were polymorphic, and the percentage of polymorphism for each primer ranged 25-100%. Moreover resulted a number of bands that were unique to each variety and that useful as cultivar specific marker in future. The the results arrangement using dendrogram cluster analysis that grouped nine varieties in to three clusters depending on their origin and morphological traits. These data can be used in the future for barley breeding and improvement programs [15].

Another study applied RAPD molecular markers for fingerprinting and genetic similarity between 18 *Mesona chinensis* cultivars and the hybrid using 3 primers that resulted similarity and full discrimination between 19 samples. Then results grouped the cultivars in to five clusters using clustering analysis depending on correlation between parental sources and distribution of regions. These results are useful for the cultivation of species, classification and identification [16]. In the study of RAPD markers described, 9 *Ocimum* individuals are grown in India, genetically, *Ocimum africanum* and *Ocimum basilicum* produced various genotypes. The results scored the study of phenotype-genotype traits represented as supplementary procedures to describe the diversity of species in *Ocimum* for taxonomic classification and exact assessment [17].

3.2 Inter Simple Sequence Repeat Marker (ISSR)

This technique is a modification of SSR based on microsatellite sequences when primers anchored at 5 terminus 3 of DNA that amplify inter SSR sequences or SSR flanking sequences lead to variable genome size, large number bands and high polymorphism percentage. This markers are useful in studies about plant genetic differentiation [18,19]. The features of this marker, simply high reproducibility, depending on high annealing temperature , efficient repeatability , high polymorphism , need, low DNA require , easy handling, and high genomic distribution [20,21,22]. The drawback of this marker, dominant through the presence band observed in homozygote or heterozygote individuals lead to occurring insertions and deletions in the binding sites of the primer

[23,24]. In a study of using ISSR markers to build a phylogenetic of Barley Genotypes between nine Iraqi barley varieties using 9 ISSR primers produced, 28 bands were polymorphic and the remaining monomorphic bands were 13, these bands can be used as a DNA profiling of all studied genotypes. The average polymorphic rate was 70.5% and ranged 25-100% . in this study we generated 5 unique bands, The results showed Genetic distances ranged between 0.0854-0.9897 among barley varieties. Then the results were grouped in to two main clusters by cluster analysis depending on their ancestors and their morphological traits that lead to the possibility of obtaining an appropriate genetic variability in the hybrid population [25].

In a report pointing a quick and accurate diagnosis of *Mentha* L. genotype, the technique of ISSR was employed to determine inter-and intraspecific diversity in *Mentha* species, and characterization correlation among individuals was used to estimate the genome size. 37 primers with amplification 333 loci , ratio 100% polymorphism. Seven clusters resulted using the UPGMA assay with varieties that divided. This techniques are recommended for conservation and germplasm collection and diagnosis of *Mentha* L. genotype/varieties through breeding generation [26]. Another report of thirteen genotypes of *Perovskia abrotanoides* using 9 primer ISSR that resulted 80.7% polymorphism percentage, indicating a high level of genetic variation through genetic distance among individuals pointed (0.07 - 0.79). The available results are important for the quality of the phenotypic, biochemical, and agricultural characteristics of these varieties [27].

3.3Simple Sequence Repeat Marker (SSR)

This Technique became their name for Microsatellite Markers instead of Simple Sequence Repeat or Short Tandem Repeats [28,29]. Found within eukaryotic genomes [30,31] consisting of repeat units with length from two to six bp, nucleotides depending on basic short motifs, polymorphism occurs between specific loci generating variation in microsatellite length [32,33]. Microsatellite assays required small DNA material by using pair specific primers set 20-30 bases in PCR analysis appear multiple shapes of length between species for specific sites. SSR marker have a wide range using in agricultural species with field genetic variation, linkage mapping for comparative, systematic taxonomy and molecular evolution [34,35].

Microsatellites observed codominance and multi alleles with high genetic information, high cost primers, and the need for prior knowledge of DNA sequences [36,37]. Primers made to specific individuals that employed in other individuals within a population resulted SSR locus through individuals [13]. Microsatellites were divided in three types depending on the content sequence repeats such as: perfect repeat without any interruption, twelve GTGTGTGTGTGTGT, non-integral repeats, interactions of bases that do not correspond to the idea of the motif, GTGTGTGTaGTGTGTT, a complex repetition of 2 or more than one repeats of microsatellite GTGTGTGTGTGTCACACACACACA [38]. In our study of detection of genetic polymorphism between nine Iraqi Barley using SSR-PCR Analysis has been differentiated to detect genetic diversity by using six SSR primers for genetic screening of samples, resulting 8 polymorphic bands through PCR method with ratio polymorphism 80% ranging 50-100%.

This study generated one unique band with size 200bp used as a DNA profiling of all studied genotypes and genetic distances between 0.01098-0.99708, then the results arrangement using cluster analysis can be divided nine varieties in two main groups according to their origin and type spike.

Results are useful for the management of barley germplasm in terms of biodiversity protection and breeders rights protection [39].

Another study reported the molecular variation and individual structure of 18 individuals of Rosmarinus officinalis, which was analyzed using SSR markers. Result s with high version of variation and the low individual synthesis. 11sites of microsatellites can be multishapes within each eighteen individuals in R. officinalis, which produced 231 alleles different, all information about the life, plant history, and reproductive characteristics were documented [40].

3.4 Amplified Fragment Length Polymorphic Marker (AFLP)

One of the techniques depends on the combination of PCR analysis via DNA material digestion using endonuclease restriction enzymes due to polymorphism. This marker was considered the intermediary between RAPD and RFLP markers as it mixture strength of RFLP mixing with the flexibility of RAPD. This

technique was applied in the field of plant cultivation through differentiating between individuals subspecies that close relationship between gene and physical maps and fingerprinting. AFLP assay required primers to generate 50-100 bands per assay represented as amplification products, number of selective nucleotides in AFLP primer combination, GC content , specificity , resolution, complexity and genome size [41,42,43]. The advantages of this marker are to need previous information about DNA sequence, AFLP products containing unique site for amplification the genome can be used in gene map diagnosing high polymorphism [44]. Otherwise, the disadvantages did not distinguish between homozygotes from heterozygote individuals dominant nature because their content had few information per locus [13]. Sources of AFLP polymorphism from: mutations either create or delete of the restriction locus ; occurs deletions , insertions , duplications within the amplification sequence ; mutations in the restriction sites on their flanking sequence and complementary of the selective primer extension allowing possible primer annealing. These mutations lead to (increase or decrease) or (presence or absence) as a modification of an amplified restricted product. AFLP assay abbreviated steps by cutting fragment sequence using two restriction enzymes , resulting specific adapters associated to the ends of fragments , amplified of fragments via PCR using specific primers to distinguish the adapters sequence , then checking of amplified fragments by high resolution gel [45].

AFLP techniques represented the study genetic diversity in plants of *mentha* (Lamiaceae) between ten accessions, with seven varieties with one multi-specific hybrid combination with six primers resulted 2049 bands as 1779 polymorphic, 29 monomorphic and 141 unique bands. Estimate genetic similarity can be performed by cluster analysis to build a dendrogram that discovered a degree relationship between hybrid Neerkalka CIMAP/C63 within other one population of (M. rvensis) [46]. Another study reported the same genotype and the same markers, molecular variation, and correlations among *mentha* populations to determine taxonomy multi-specific hybrids as 62 individuals of five species and three hybrids, rate of polymorphism from 50 to 60% despite of only small samples of germplasm used in this study comparison with large samples accessions also observed polymorphism by using AFLP markers [47].

4. MOLECULAR MARKER NON-BASED PCR

4.1 Restriction Fragment Length Polymorphism Marker (RFLP)

Named restriction hybridization technique, By employing *restriction endonuclease* and bacterial enzymes to cut DNA and hybridization method. [48], after enzymatic digestion can be produced restriction fragment polynucleotides with variations in length lead to changes in sequence ex: (point mutations ; deletion ; translocation and alterations in restriction sites).

Polymorphisms will occur by differences in genetic constitution between two individuals there for RFLP marker was discovered [49]. Restriction fragment length polymorphism, variable numbers of tandem repeat markers based on restriction hybridization technique, polymorphisms in DNA fragments diagnosed due to probe chemically with DNA labeled using southern blot method when DNA analyzed by restriction endonuclease enzymes lead to the formation of various DNA fragments corresponding to the goal regions of the enzyme in their size. The advantage of this marker can distinguish between homozygote and heterozygote genotypes, highly polymorphic, co-dominant, highly sample replication, and test numerous samples in the same time. While this disadvantage is not widely used because high cost , required large quantity and high quality of DNA and previous knowledge about the sequence, the required radioactive toxic reagents as a probe with prior information about structure generate danger and hard work [13]. RFLP markers reported in phylogenic studies using results data from the classification *Avicennia* spp, *Verbenaceae*, in to another family *Aveicenniaceae* [50]. Another study documented the toxic present in *Salvia divinorum* samples using the combination between RFLP method and analytical chemistry to support forensic and toxic science [51] through studying this marker on *Salvia* genotypes and the results reviewed the linkage between molecular data and the degree of essential oil products. Therefore, their results required more studies to detect genetic variations about *Salvia* in future [52].

The phenomenon of appearance between the hybrid plants is a natural case that may affect production or the extinction of rare species, The RFLP technique with the morphological testes can distinguish between endangered taxa by

hybridization with exotic plant *Lantana strigocamara* [53].

5. SINGLE NUCLEOTIDE POLYMORPHISM (SNPS)

Phenomena occurring in DNA sequence of individuals of a species through variation in a single nucleotide A, T, G, or C are known as SNPs. Considered abundant molecular marker in genome. SNP distributed in non-coding and coding regions of the sequence, when this marker is present can be observed either nonsynonymous mutations due to alteration in amino acid sequences [54] or synonymous mutations result no alteration in amino acid sequence, the synonymous change will lead to RNA splicing modification produced phenotypic variations [55]. New SNP identification by computational methods, genotyping approaches, and retranscription of genome sequences [56].

SNP genotyping depended on : hybridization of specific alleles, oligonucleotide linkage, primer extension. Methods of genotyping that special has been high accurate data results such as : DNA chips, PCR and primer extension based on SNP using in crop cultivar genetic diversity studies and building high density genetic map [57]. In addition, SNP technique can detection variation between control and disease resist DNA place associated advantage traits.

6. MOLECULAR MARKERS BASED ON RNA

There are some techniques based on RNA such as Real Time PCR using to study amplification of cDNA to detect the mechanism of gene expression that needful for biological response and development programming of individuals.

7. REAL TIME PCR

This technique depends on the polymerase chain reaction amplifying the target DNA fragments [58]. It can be shown by quantification of the number of copies when intercalated with DNA input or intercalated with additional genes of DNA sample sequence. Real time PCR using in studies related to phylogenetic and genetic variation. The protocol of this reaction abbreviates through accumulates amplified quantification DNA after every cycle of reaction. There are two methods of reaction : (i) by fluorescent pigments reacted with DNA double strands, and (ii) the used probes representing DNA oligonucleotide modification through linkage with integral DNA by hybridizing can be fluorescent. Advantage of this molecular marker, it is quickly used in temperature changes, sensitivity, and speed results to the diagnosis of data.

The appearance of genetically modified organisms on the food market a few years ago and the demand for more precise and reliable techniques to detect foreign (transgenic or pathogenic) DNA in edible plants have been the driving force for the introduction of real-time PCR techniques in plant research. This was followed by numerous fundamental research applications aiming to study the expression profiles of endogenous genes and multi-gene families. Since then, the interest in this technique in the plant scientist community has increased exponentially [59,60].

Some studies appear that user qualitative and quantitative polymerase chain reaction (PCR) techniques are increasingly used for the detection of genetically modified (GM) crops in foods. Qualitative PCR and TaqMan real-time quantitative PCR methods to detect and identify three varieties of insect resistant cotton, i.e., Mon531 cotton (Monsanto Co.) and GK19 and SGK321 cottons (Chinese Academy of Agricultural Sciences), which were approved for commercialization in China. Furthermore, a real-time quantitative PCR assay based on TaqMan chemistry for the detection of insect resistant gene, Cry1A(c), was developed. In the quantitative PCR assay, the quantification range was from 0.01 to 100% in 100 ng of the genome DNA template, and in the detection of 1.0, 3.0, and 5.0% levels of three insect-resistant cotton lines, respectively, the results TaqMan real-time PCR assays appeared reliable and practical for GM insect resistant cottons quantification and to detect the three insect resistant cottons qualitatively and quantitatively [61,62].

8. CONCLUSIONS

Molecular marker are useful for assessment molecular variation, building linkage maps. Genetic plans for linkage indicators were employed to connect genetic relationships with physical relationships. Linkage between heredity styles led to the construction of meiotic genetic mapping of individuals in populations via determining the traits of single gene and multigene through the target area of plant

genome sequences. The results data provided from PCR reaction of DNA molecules from old DNA samples can be observed during development processes within the genomes pooled on the broad term and for periods of time. Now available of information considered key in devising of a suitable strategy of conservation. Molecular markers of plant response to climate conditions considered major phenomena at the global level led to the reduce of variable trait loci controlling of physical response and climate. Therefore, we conclude through study and use molecular markers, it is essential to the assessment and maintenance of genetic variability due to the provided adaption container to environment changes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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