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Estimation of Botanical Diversity by Molecular Marker Methods

Zahraa A.N. Al-Yassiry [a*], Basheer Al-Alwani[b]

[a,b] *Department of Biology, College of Science, University of Babylon - Iraq*

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ABSTRACT

Various molecular methods could be utilized in order to investigate botanical diversity. Arbitrary primed DNA, variable number of tandem repeats (VNTR), Restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR) sequencing, amplified fragment length polymorphism (AFLP), and sequence-tagged simple sequence repeats (SSRs) are all briefly reviewed. DNA-based approaches have recently been proved to be useful for crucial tasks, like specimen identification and targeted screening for expected or known invaders, according to a recent study. Prior to more ambitious applications, as extensive surveys of complex environmental samples and propagule pressure prediction, could be conducted, considerable technological obstacles should be solved. The aim of the current review was to estimate the molecular techniques used for assessing the genetic diversity of plants. The degree of variation among the plant species based on genetics is described as the genetic diversity of plants, evaluating the possible value regarding the current invasive species monitoring methods.

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1. Introduction

The "botanical diversity" term throws up some ideas. A few individuals link it with complex natural ecosystems like grasslands or rain forests, which are made up of different hybrids and species. Others might come across herbaria with dried specimens, living botanic gardens, or gene banks with endless rows of seed pots. Molecular geneticists are confronted with a number of diversity assessment difficulties that could be examined utilizing many DNA methods (Karp et al., 1996; Bhandari et al., 2017).

For being conserved and applied in sustainable way, plant genetic resources should be appropriately detected. Using DNA-based markers has revolutionized the process of identifying species (Botstein et al., 1980; Foster et al., 2010). Latest breakthroughs in molecular genetics have offered practitioners working in plant genetic resource conservation with some distinctive techniques for identifying plant species quickly and accurately. Many of such methods were applied to examine the extent and the distribution of diversity in species gene pools, along with solving typical taxonomic and evolutionary problems (Karp, 1997). Codominant inheritance (specifying heterozygous and homozygous states in diploid species), high polymorphism, chosen neutral behavior (DNA sequences of any of the organisms are neutral to the management methods and environmental conditions), and frequent occurrence in the genome, fast and easy assay, ideal DNA markers must have each of data exchange between laboratories as well as high reproducibility (Joshi et al., 1999; Foster et al., 2010).

Taxonomic competence might be non-existent or limited based on the species under discussion (Williams et al., 1990; Fu, 2015). Biodiversity estimations are frequently based on "family-level" or "morphospecies" identifications due to these problems (Caesar et al. 2006). The precision of the morphological identifications has been significantly limited by requirements for invasive monitoring of the species: It is widely established that utilizing morphological criteria for identifying stages of the early life history (larvae and eggs) is challenging (Besansky et al. 2003); however,

*__Corresponding author:__ Zahraa A.N. Al-Yassiry, Department of Biology, College of Science, University of Babylon - Iraq

E-mail: **yaziabdalla2014@gmail.com**

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the recognition of such stages is crucial for tracking invasions.

This study discusses the essential ideas, benefits, requirements, and limitations of the most regularly utilized molecular markers for the genetic diversity research, marker-trait association researches, genetic mapping, and marker-aided selection programs.

2. Molecular Genetic Screening Techniques

2.1. Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA represents an approach that uses arbitrary nucleotide sequence primers to amplify genomic DNA (Williams et al., 1990). Those primers discover polymorphisms without exact information of the nucleotide sequence, and the polymorphisms operate as genetic markers and could be applied for generating the genetic maps. There is no possibility for detecting if an amplified DNA segment is considered to be homozygous (2 similar copies) or heterozygous (2 separate copies) at a certain locus due to the fact that the majority of RAPD markers are dominating. In seldom of the circumstances, the co-dominant markers of RAPD (DNA segments of various sizes amplified from same locus) might be identified (William *et al*, 1990). a) extracting extremely pure DNA, (b) adding a single arbitrary primer, (c) PCR, and (d) fragment separation by the electrophoresis of the gel, (e) RAPD-PCR fragment visualization following the staining of the EtBr under the ultra-violet light, and (f) fragment size determinations with the use of gel analysis software are the basic RAPD steps (Karp et al., 1996; Morris & Shaw, 2018).

In spite of such limitations, the fact that primers are created without the use of DNA probes or sequence information makes this approach particularly intriguing. This procedure does not include any hybridization and blotting steps. All that is needed is the purchase of an agarose gel apparatus, a thermocycling machine, and the essential materials, which are offered as commercial kits (Ready-To-Go RAPD analysis beads; GE Health-care, Buckinghamshire, U.K.). A further benefit is that just a little DNA amount is needed (10ng-100ng per reaction (Karp et al., 1996; Bhandari et al., 2017).

2.2. Restriction Fragment Length Polymorphism (RFLP)

RFLP approach examines the unique pattern formed via digested DNA fragments to distinguish across species. Since the two alleles are identified in a heterozygous sample, the RFLP markers are inherited as simple Mendelian codominant alleles (Agarwal et al., 2008). At the same time, their DNA reorganization is produced through an evolutionary procedure, point mutations inside region of the restriction enzyme recognition, or unequal crossing-over (Mishra et al., 2014; Kumar et al., 2009). Patterns that have been created via band separation with the use of agarose or poly-acrylamide gel electrophoresis from diverse samples are evaluated to distinguish between plant species from which samples will be derived (Agrawal et al., 2008).

They applied 4 RFLP restriction enzymes, which led to a higher level of polymorphism (about 88.70%) compared to when enzymes have not been applied throughout SDS-PAGE (just 80%). RFLP markers were also utilized by Al-Mahmoud et al., (2012) to distinguish sexes in date palm (Phoenix dactylifera) during earliest development stages. The gender-specific primers generated for the present investigation resulted in a superior (90%) accuracy in the characterization of the gender of the date palm across many variety types.

2.3. Amplified Fragment Length Polymorphism (AFLP)

AFLP combines RFLP's strength with flexibility that is related to the PCR-based approach through ligating primer-recognition sequences (i.e. adaptors) to limited DNA (Lynch & Walsh, 1998). AFLP's defining feature is its capability to do "representation of genome," or simultaneous screening regarding representative sections of the DNA that are scattered in a random manner throughout the genome. With no initial investments in development of primers/probes and sequence analysis, AFLP markers might be produced for any organism's DNA. For digestion, both partially degraded and high-quality DNA could be applied; yet, PCR inhibitors and restriction enzyme should be removed from the DNA. AFLP has been thoroughly examined by many authors (Ridout et al., 1999). The initial step in the analysis of the AFLP is to digest 500 ng of the genomic DNA with combination of infrequent cutter restriction enzymes (PstI or EcoRI) and common cutter restriction enzymes (TaqI or MseI).

Following ligation with double-stranded oligonucleotide adaptors, the original restriction site is not restored. For providing the known sequences for the amplification of the PCR, such adaptor has been ligated to the two ends of a fragment. PCR amplification happens only in the case when the primers could anneal to the fragments containing the adaptor sequence and the complementary base pairs to additional nucleotides referred to as selective nucleotides, as stated by Vos et al., (1995). An aliquot is subsequently subjected to 2 further amplifications of the PCR utilizing adaptor-specific primers and 3' selective nucleotides under strict conditions. Primer pairs with a single bp extension are used in the first (preamplification) PCR, while primer pairs with up to a 3bp extension are used in the final (selective) PCR. Because of their extreme selectivity, primers which differ in the AFLP extension by just one nucleotide amplify a single gene. The amount of amplified fragments is reduced by 4, 16, and 64 times, respectively, with a one-, two-, or three-base primer extension. The optimal primer extension length will vary based on the genome size regarding species and will lead to the most bands possible. Throughout electrophoresis, there are not too various bands to generate smears or excessive band comigration, yet there are enough to offer enough polymorphism (Vos et al., 1995; Bhandari et al., 2017).

2.4. Sequence Characterized Amplified Region (SCAR)

SCAR can be defined as one of the polymorphic DNA segments of a known sequence. SCAR markers are repeatable and reliable, making them excellent for a variety of uses (Schuster, 2008). SCAR test represents a PCR-base assay that recognizes DNA fragment through the amplification with a set of distinctive oligonucleotides (15bp – 30bp) primers made from nucleotide sequences of cloned RAPD (or other marker) segments corresponding to characteristics of interest (Bhagyawant, 2016). In our review of literature, it has been only discovered that there is one joint work between Qatar and India that used SCAR markers cloned from RAPD markers to give genetic identification of Knema andamanica. In addition, K. andamanica was successfully distinguished from the majority of genetically varied species using this approach. The SCAR method was described as a reliable approach by authors, who recommended that it be used as DNA bar-code

marker in the authentication of the species (Sheeja et al., 2013).

2.5. Next Generation Sequencing Techniques

A new non-Sanger-based sequencing generation techniques had arisen to fulfill the promise of sequencing DNA at unheard-of speeds, allowing for significant scientific advancements and innovative biological applications. Large-scale population-genetic investigations may now be carried out utilizing complete genomes instead of only small sequences of a single gene thanks to these approaches. Quick breakthroughs in next-generation sequencing regarding various plant species' genomes will aid our understanding of the way genotypic variation translates to phenotypic traits. Next-generation sequencing technology help in understanding the plant evolution by using a comparative genomic method to find functional loci related with geographical, morphological, and physiological diversity. The next generation of platforms does not depend on Sanger chemistry (Sanger et al., 1977), as did machines of the 1st-generation for previous 3 decades (Schuster, 2008).

With landmark publication of 454 Life Sciences' pyrosequencing-based sequencing-by-synthesis method in 2005 (Margulies et al., 2005; Ronaghi et al., 2006), the first of this sort of the second-generation approach of sequencing has been published. Commercial sequencing second-generation technologies are distinguished through using the PCR in the library creation. There are four key platforms, all of which are built on the principle of amplification. The Roche 454GS FLX, the Illumina Genome Analyzer II-x, the ABI SOLiD 3 Plus System, and the Polonator G007 were all used (Lerner & Fleischer, 2010). Depending on technology that has been pioneered by Braslavsky et al., (2003), Helicos Genetic Analysis System created this sequencing method. Other 3rd-generation sequencing technologies, including Life Technologies' and Pacific Biosciences' SMRT technology, are in development and can be available within a year or two. Oxford Nanopore Technology (www.nanoporetech.com) is developing a really groundbreaking electrical, label-free, single-molecule DNA sequencing technique. This approach avoids the requirement for amplification or labeling through identifying a direct electrical pulse (Clarke et al., 2009). Yet, such approach remains in its early phases. Helicos 3rd-generation direct single molecule RNA sequencing technology, which does not need prior RNA to cDNA conversion, has paved the way for bias-free and comprehensive transcriptome knowledge (Ozsolak, et al., 2009).

2.6. Inter Simple Sequence Repeat (ISSR)

ISSR can be defined as one of the DNA segments (<100bp–300bp long) sandwiched between two similar micro-satellite repeat segments (typically 16bp–25bp long) orientated in opposite directions (Reddy et al., 2002). ISSR fingerprinting provides a benefit of establishing SSR marker specificity without needing primer sequence information. ISSR markers have been found throughout genome randomly and have various degrees of polymorphism. In addition, ISSR was utilized to undertake a molecular analysis and specify the fingerprints of the ber cultivars. The authors detected and characterized the 5 ber genotypes. ISSR markers were employed in another Jordanian research to look at the genetic stability regarding micro-propagated Moringa peregrina plants. There were no polymorphisms found, implying that the in-vitro plants are genetically intact (Al Khateeb et al., 2012). A Saudi Arabian research published lately revealed the utility of the ISSR marker to generate DNA fingerprints, conservation support, and early sex identification (Sabir *et al.,* 2014).

To describe and assess the genetic diversity regarding 10 cultivars of date palms (Phoenix dactylifera) and others, the authors utilized ISSR molecular markers to construct DNA fingerprints. Polymorphism levels among cultivars ranged between (20 and 100) %, with an average of 85%, based on their ISSR data (Sabir et al., 2014). Sabir et al., (2014) looked at genetic variations in the endangered Breonadia salicina across populations and geographical areas (Rubiaceae). The authors have employed ISSR markers for showing that certain groups had minimal genetic diversity while others had a lot. ISSR markers were suggested in another work for detecting phenotypic diversity in 15 Sorghum landrace genetic variants produced in Yemen and Saudi Arabia. A total of 8 genotypes of Sorghum bicolor have been efficiently sorted into 2 clusters, one with white ones and the other with dark grains, in this case (Basahi, 2015).

3. Advanced Techniques Related to Plant Diversity Studies

Molecular markers have revolutionized plant science research in domains like transcriptomics, genomics, metabolomics, proteomics, and so on over the previous 20 years, emphasizing this method as "omics" science of plants (Mosa et al., 2017).

3.1. Transcriptomics

The study of the transcripts that are made up of a full collection of RNA synthesized via genome under particular conditions or in a specific tissue, is known as transcriptomics. Those transcripts could be detected using high-throughput approaches like RNA sequencing and DNA microarray. Comparing transcriptomes might aid in identifying genes which express themselves differently in various cell types or as a response to a variety of the treatments (Mosa et al., 2017). In plants, functional genetic diversity might therefore be explored across stress events, as evidenced by a study of the salt stress response in Rhazya stricta, a Saudi Arabian evergreen shrub. Under salt stress, the relevant genes [pentatricopeptide repeat (PPR) proteins] regulated a high number of the transcripts, according to their results (Hajrah et al., 2017). Transcriptomics might show as well the rate of the genomic change. In the date palms (Phoenix dactylifera) cultivars (Fahal, Khalas, and Sukry), for instance, nucleotide substitution has been reported among intra-varietal SNPs, with rates of transversion that are somewhat greater than transitions. Apart from replacement, plastid DNA implantation into mitochondrial genome, as found in several plants, could result in a size increase (Phoenix dactylifera) (Fang et al., 2012).

3.2. Proteomics

Proteomics can be defined as the study of an organism's full protein complement in a biological system or under established, specific conditions. There was substantial technological improvement in detecting individual proteins over the previous few decades, with method of separation now representing the most widely applied approach in the

proteomics (Yu et al., 2010). Those advancements include developments in protein fractionation approaches, mass spectrometry (MS) technology, and tools of bio-informatics for assembling as well as evaluating MS data. The specified protein patterns regarding all Heliotropium dignyum cultivars revealed that proteins from various plant races differed with regard to genetic diversity (Alwhibi, 2017). In addition, seed storage protein was identified in shrub H. dignyum samples from all around Saudi Arabia. In spite of coming from the same geographical place, the protein amounts vary, based on the research results. The proteome related to the date palm (Phoenix dactylifera) leaf has been investigated in order to find the proteins that are involved in the resistance to salt and drought stress. In a case when protein abundance was low or high, the authors discovered genes that were differently expressed (El Rabey et al., 2014).

3.3. Metabolomics

Studying all metabolites is known as metabolomics. Small molecules can be defined as the metabolic products, which are created via the metabolic process in each one of the tissues and cells, and they are indicated as a biological system's metabolome. The spectroscopy-based metabolic profiling approaches NMR and MS could be applied for examining the metabolic variations among various plant species and cultivars. Plants have enormous genetic flexibility, leading to a staggering number of metabolically varied and genetically distinct cultivars for a certain species (Schauer et al., 2005).

The metabolic diversity that is related to non-domesticated species of Solanum lycopersicum was studied. The objective has been to find the bio-chemical markers linked to a required characteristic and use them in crossbreeding with domesticated animals for direct progeny selection. The authors have been capable of building profiles for a range of the secondary metabolite types with the use of GC-MS technology, indicating that increasing quantities of nutritionally-relevant metabolites increased the likelihood of success. Wild species have high quantities of secondary metabolites, specifying that they are a vital source of color and flavor. Stress tolerance studies in seedlings and roots of maize were connected to proline accumulation. Throughout the growing seasons, drought increased the amount of glycine betaine in maize leaf (Yang et al., 1995; Bhandari et al., 2017).

4. Plant DNA Barcoding

According to the findings of our study, DNA barcoding is utilized more frequently for evaluating plant biodiversity compared to any other genomic or molecular technology. Many studies were reported on the authenticity of therapeutic plants which are derived from Saudi herbal medicine markets. Ruta graveolens morphologically resembles the adulterant Euphorbia dracunculoides. rpoC1, rpoB, and nrDNA-ITS were used to determine their taxonomic status (Al-Qurainy et al., 2011b&c). In another research, the authors uncovered a molecular signature regarding the commercially significant date palm (Phoenix dactylifera). Using the psbA-trnH and rpoB genes, Al-Qurainy et al., (2011a) were capable to tell the difference between cultivars. psbA-trnH had more polymorphism sites compared to locus and ropB, according to the researchers. Geographical variance was investigated in the plants from Saudi Arabia's dry climate using rbcL and matK universal primers, along with taxonomy analyses. They came to the conclusion that specific amplification attempts didn't show success because of primer mismatches at the annealing site. Whereas the quest for other primers which cover a wider variety of plant species continues, matK and rbcL can still be utilized for plant barcoding (Bafeel et al., 2011).

The experts argue that because of regional and morphological variances, along with reticulate evolution, developing a universal bar-code for identification of all species of the plants is very challenging. In Arabian Gulf region, plants are more resistant to harsh and extreme circumstances including drought, salt, sun radiation, and high temperatures compared to plants in other parts worldwide (Bafeel et al., 2012).

5. Conclusions

Molecular markers aid in the study of species genomic information. Plant evolution is caused by new discoveries and technical advances based on molecular techniques and evaluate the plant's reaction to environmental changes. A successful molecular technique must be cost-effective, have technical and operational facilities, be convenient, have data available, and be automated. Rather than being used to assess genetic diversity, they are important for assessing plant conservation, developing new breeds and hybrids, and assessing plant response to environmental changes. Genetics is a rapidly evolving field, with new techniques being developed on a daily basis. The goal of most genetic engineers is to create a molecular technique that is less limited and more advantageous.

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