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Promising Molecular and Genomic Techniques for Biodiversity Research and DNA Barcoding: A Review

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ABSTRACT

The DNA barcoding technique is considered one of the most successful scientific leaps in the field of genetic engineering, especially in molecular genetics. Where this technique was able to decipher the puzzles and many blades that were not known to human beings in the past. This technology has also provided many positive paths in many fields and different branches of science. This technique was developed mainly to identify the different life species present in a specific ecological area, and therefore it is a very important method in studying the biodiversity of living organisms in general and this is the main objective of this research. Also, one of the most important uses of DNA barcoding is preserving the endangered species of various plants and animals after studying their biodiversity, preserving their sequencing in the gene bank and making decisions according to their optimal use in the future, which brings positive benefits to humans.

Keywords: DNA barcoding, Biodiversity, Biotechnology, Plant Breeding, Molecular Markers.

1. Introduction

There is no doubt that environmental systems and excessive human activities, often without proper regulation, play a vital role in influencing biodiversity, which is at stake and often leads to serious environmental degradation. In general, the continuous deterioration and decline in taxonomic experiences needs to improve a kind of taxonomic and identification tools on molecular markers for the rapid and effective identification and detection of the identity of organisms to know and evaluate their effects on the environment and to understand the evolutionary relationships between organisms and this new strategy is the basis of biology, (Ahmed 2022). Therefore, identification of species is the starting step for knowing and measuring the extent of biological diversity, but this step often faces great difficulties due to the lack of professional knowledge of taxonomy, (Chase *et al.,* 2005). If the process of study and research in the taxonomic field and knowledge of the identity of species and genera is done in a traditional way, this matter takes a long time because it depends primarily on the basic stages of the growth of the organism. Thus, these studies are very stressful and arduous because they also depend on the results of previous classifications, (Costion *et al.,* 2011 & Huang *et al.,* 2015). Taking into account that the taxonomic experience has begun to erode and decline due to modern mechanisms of scientific reduction. DNA barcoding technique is one of the latest techniques used for rapid and effective detection and differentiation between different varieties and types depending on the molecular database and DNA sequences, (Abdel Sattar and El-Mouhamady 2012.). One of the most famous positive benefits and scientific and practical applications of DNA barcoding is maintaining the safety of natural plant and animal products widely used in traditional medicine, studying the biodiversity of unidentified and endangered plant and animal species, forensic analysis, phylogenetic analysis and the safety of food products, (El-Keredy *et al.,* 2003 A, B, C; El-Mouhamady *et al.,* 2011 and 2014 & 2016 & 2017 & 2019). New and rapid improvements in a range of modern sciences such as bioinformatics

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and molecular biology made the database at the molecular level more flexible than the rest of the data in other files, which improved the application and analytical field of DNA barcoding, (El-Mouhamady and El-Metwally 2020 A & Ahmed, 2022). Accurately identifying species and genera is critical for analyzing genetic diversity and studying the extent of biological diversity to conserve and better exploit those species, (Mosa *et al.,* 2019 and El-Mouhamady and Ibrahim, 2020 b). Further, the DNA barcoding technique has opened up important horizons in the fields of ecology, evolution and species conservation, in the context of providing the scientific method or the fastest and most accurate tool for identifying species, (El-Mouhamady *et al.,* 2020 C and Gostel and Kress, 2022). After all that has been listed, it can be briefly mentioned the purpose of that lecture, which sheds light on the most promising molecular genetic parameters and genetic techniques used in studying the biodiversity of all living things, with a focus on the importance of DNA barcoding in this regard.

2. Review Methods

As part of its procedural methodology, this review conducted a larger literature search and synthesis of relevant peer-reviewed journal articles, workshop papers, books, thesis works, and symposia.

3. Main Text

3.1. Biodiversity and its threats

Humanity is suffering from an unprecedented crisis of biodiversity, as the evidence indicates that the special reports of extinctions have greatly exceeded the estimated and natural rate of extinction. The human element has had a large share in mass extinctions, and it alone has the ability to stop that threat. More than 900 extinct species have been documented since 1500, and up to 400 species of birds became extinct in prehistoric times, (Abo-Hamed *et al.,* 2016; El- Demardash *et al.,* 2017; Al-Kordy *et al.,* 2019; Sayol *et al.,* 2021). The efforts of many researchers and scientists have resulted in summarizing the most important global threats that threaten the destruction and loss of biodiversity, (El-Mouhamady 2003 & 2009; Bellard *et al.,* 2022). Among the most dangerous factors threatening biodiversity are overfishing, massive exploitation of species in general, biological invasions, pollution and climate change, (Brook *et al.,* 2008). Also, one of the most famous examples of biodiversity is the Arabian Peninsula, which is rich in plant genetic resources and is genetically diverse. Therefore, analyzing the biodiversity of such genetic resources with the aim of securing and preserving them is a very important step in the rapid and accurate classification and discrimination of the different types of wild plants. Further, knowing their genetic diversity and determining the degree of threats to their biodiversity (Mosa *et al.,* 2019). Also, (Eldessouky *et al.,* 2016) detected the genetic diversity among some rice accessions under drought stress conditions besides, many papers were conducted to study the biodiversity and genetic diversity in wheat crop through studying all mechanisms responsible for water stress tolerance (El-Mouhamady *et al.,* 2023). As the ecosystems in the Arabian Peninsula are threatened by a large number of risks that negatively affect the biodiversity of rare plant species in this region, given that it is an arid region. These risks include overgrazing, poaching, overexploitation of resources, climate change, pollution, unwise driving on such rough roads, as well as uncontrolled and unregulated human activity, (El-Mouhamady *et al.,* 2010 & 2012 A & 2013 A & B and El-Keblawy 2018). One of the most important areas in danger of threatening biodiversity is the tropics, which are subject to factors of habitat loss and resource depletion, while Europe has been classified as an important center for pollution, (Harfoot *et al.,* 2021). It is difficult to rank global threats because they depend on the context, as the conditions and nature of threats differ between different regions and types, (Figs. 1 & 2).

3.2. Standard Molecular in biodiversity analysis

Molecular genetic parameters are a good way to know genetic diversity because they deal with a specific DNA sequence for a specific location on the chromosome that can be monitored and also heritable, (El-Mouhamady *et al.,* 2013C; 2014 A & B; 2021 A & B & C & D). This provides a database from which to know the relationships between organisms, (Hoshino *et al.,* 2012). For example, there are many molecular genetic markers available today to the scholarly community in plants. Although some of them can be similar, the methods of its use, applications and data differ in the way to determine the genetic differences between plants. These markers can also be used very effectively in genetic

mapping, molecular selection and phylogenetic tree which shows genetic diversity in plants, (Semagn *et al.,* 2006; El-Mouhamady 2012 B & C; Mishra *et al.,* 2014). One of the characteristics of a successful molecular marker is that must be characterized by genetic breadth, has the ability to identify a large number of genes quickly and more easily with a high percentage of polymorphism, (El-Mouhamady *et al.,* 2014 A & B & C and Mondini *et al.,* 2009). It is impossible to find a single molecular marker that satisfies all the previous requirements among different species, but that promising marker can be obtained after determining the type of study and the most successful technique, (El-Mouhamady *et al.,* 2022 A and Spooner *et al.,* 2005).

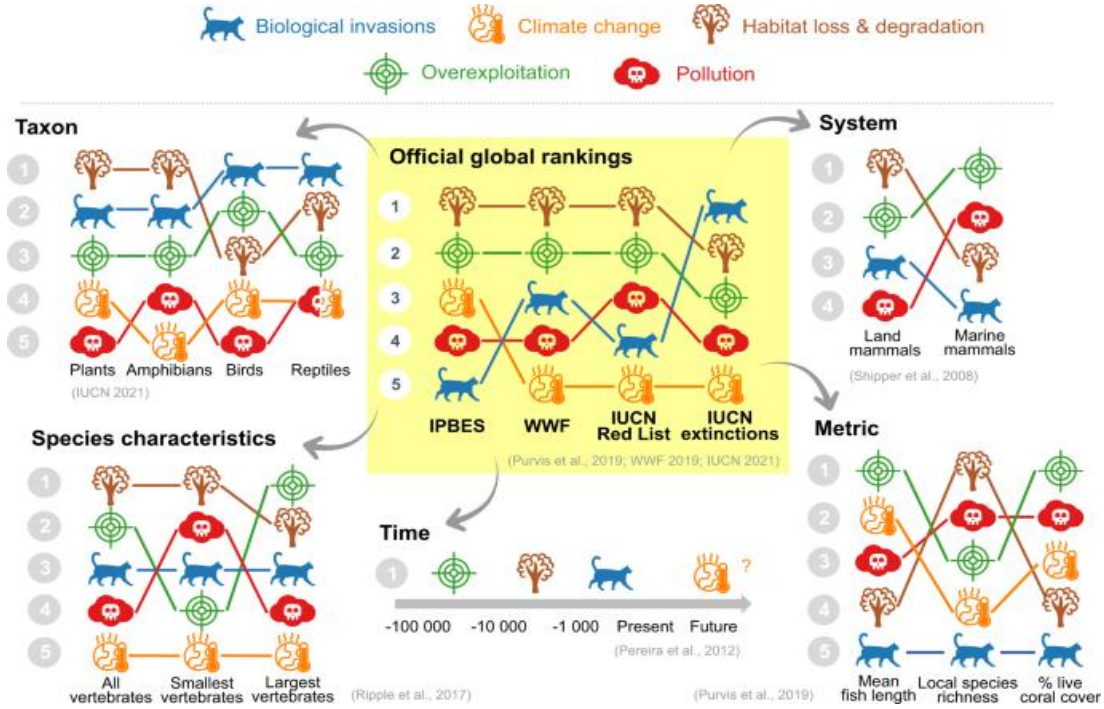


Fig. 1: Variation of global threats to biodiversity based on reports from international agencies according to Bellard *et al.* (2022).

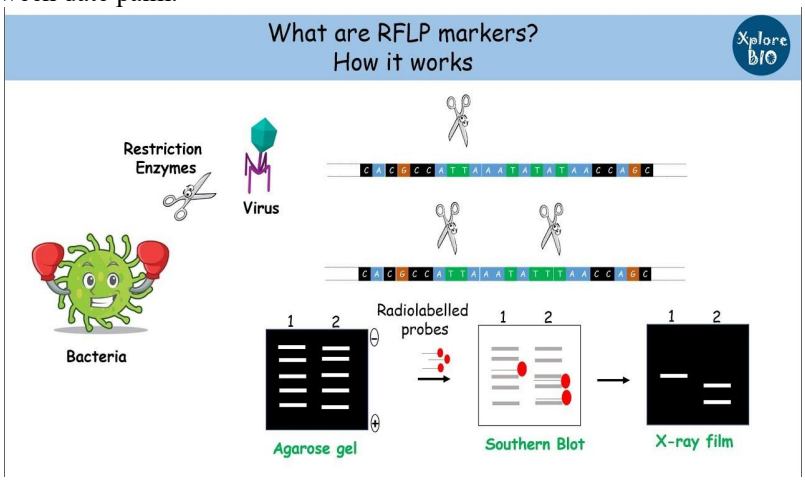
Fig. 2: Biodiversity in Plants and Animals according to Rosen (1997).

3.3. Promising Molecular and Genomic Techniques for Biodiversity Analysis

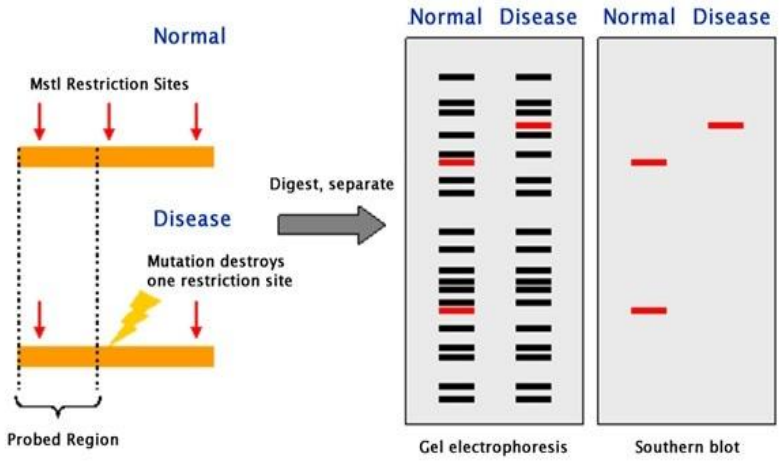
There are many techniques and genetic evidence at the molecular level that are used to know and analyze biological diversity in living organisms, whether plants, animals or microorganisms, and such mechanisms are considered one of the most important means concerned in this regard, as follows.

3.3.1. Restriction Fragment Length Polymorphism (RFLP)

This analysis is one of the genetic mechanisms used to analyze the difference between different species and distinguish between them using pieces of DNA or DNA fragments, (Fig. 3a & b). It is the inheritance of traits by easy Mendelian methods by the dominant alleles common (Agarwal *et al.,* 2008; El-Mouhamady *et al.,* 2013 D & 2014 D) to all the genotypes used, as it searches for the difference in one sample of different heterozygotes, and this confirms beyond any doubt that when rearranging their DNA sequences, evolutionary processes or certain mutations occur when Enzyme identification site or incompatible transit, (Kumar *et al.,* 2009; Mishra *et al.,* 2014; El-Mouhamady *et al.,* 2022 C & B). However, the number of studies that have been conducted to know the biodiversity in the Arabian Peninsula using RFLP markers is still few. Therefore, this method is considered a traditional method in surveying and determining the biodiversity in the Saudi domain, (Haliem and Al-Huqail, 2013 & El-Seidy *et al.,* 2013). (Al-Mahmoud *et al.,* 2012) used RFLP markers to distinguish and determine the genetic differences between some genotypes of the date palm (Phoenix dactylifera) during its initial stages of growth. The primary results showed that RFLP markers gave accuracy of more than 90% in distinguishing between date palm.



(A)



(B)

Fig. 3 A & B: Mode of action of RFLP Markers according to Cheriyedath (2019).

3.3.2. Amplified Fragment Length Polymorphism (AFLP)

This technology is considered one of the sensitive techniques that combines between RFLP and PCR, (Fig. 4.). Where, these markers are able to analyze the DNA of any living organism or any origin or any part of the DNA in the case if it is partially analyzed and it simply depends on the amplification of any part of the DNA by PCR, (Vos *et al.,* 1995 & Mueller and Wolfenbarger 1999). Further, these markers can successfully scan all regions of DNA randomly distributed around the genome at the same time, (Meudt and Clarke 2007 & Idrees and Irshad 2014). Accordingly, the molecular genetic differences or polymorphism % were determined by measuring the length of the amplified fragments, which were considered dominant markers, (Sunnucks 2000; Belaj *et al.,* 2003; Campbell *et al.,* 2003; Schlötterer 2004; Kumar *et al.,* 2009). Further, these markers can act as common, Co-dominant and controlling markers in certain sites, (Mishra *et al.,* 2014).

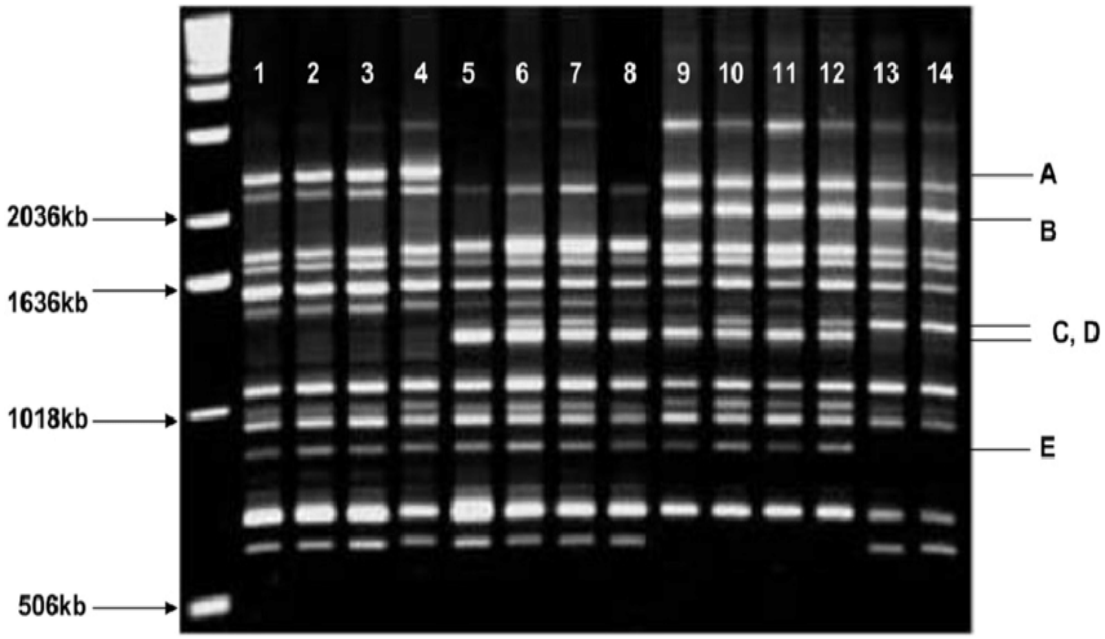


Fig. 4: AFLP Markers according to Mueller *et al.* (1999).

3.3.3. Random Amplified Polymorphic DNA (RAPD)

This genetic technique is considered one of the most effective ways to differentiate between varieties and lines of all living organisms, whether different plants or different animals under the same genus besides, finding genetic differences between them at the molecular level, (Fig. 5). Also, this markers is mainly based on PCR to identify genetic variation and is effective in screening for polymorphisms at many separate DNA loci, (Kumari and Thakur, 2014; El-Mouhamady *et al.,* 2015; Heiba *et al.,* 2016 A & B & Esmail *et al.,* 2017). In addition, it represents a genetic marker because it is prevalent and distributed randomly and can be used in more than one direction, for example at the level of one individual to the level of different species. It also determines the genetic diversity of multiple groups of plants, (Ndoye-Ndir *et al.,* 2008 and khatab *et al.,* 2017). Further, it is also used to determine genetic variance and linkage, (Akbulut *et al.,* 2009). One of the most famous scientific uses of this type of molecular genetic markers is to characterize and differentiate between 11 types of plants grown in Saudi Arabia, which are originally from desert origins and some of them are characterized by medicinal benefits, (Arif *et al.,* 2010 a). These plants have a great value, it was necessary to preserve and protect them and to protect their biological diversity until determining the aspects of benefit from it. Based on this, RAPD markers have already succeeded in producing certain genetic packages to distinguish between species, (Arif *et al.,* 2010 b).

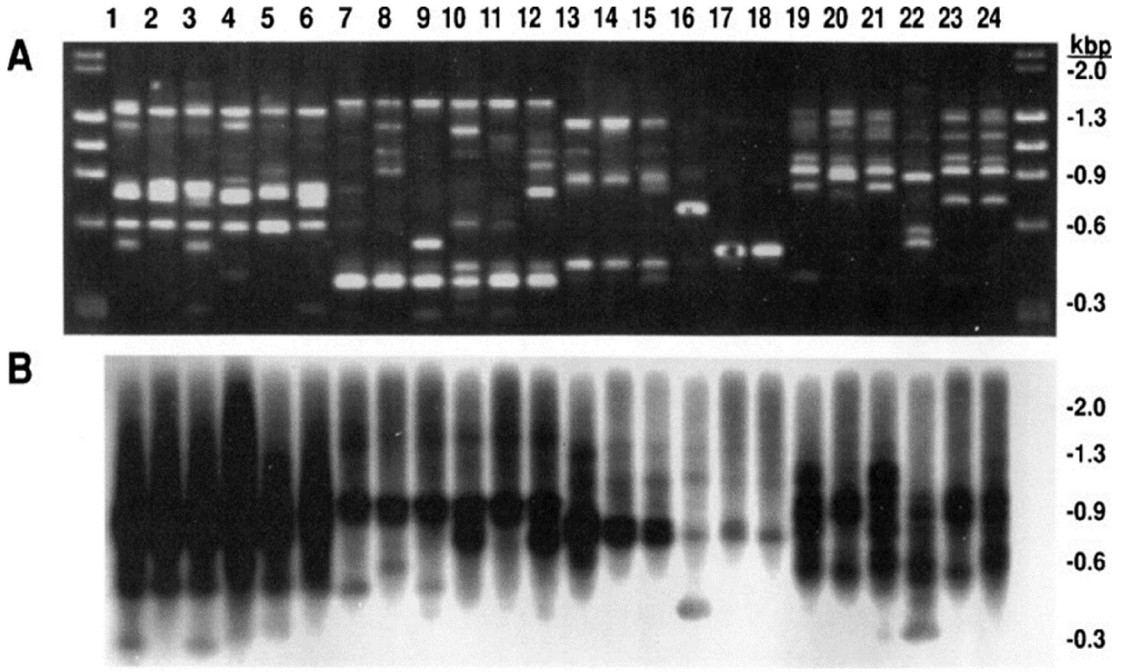


Fig. 5: RAPD markers according to Williams *et al.* (1995)

3.3.4. Sequence Characterized Amplified Region (SCAR)

This technique is defined as a portion of DNA or an amplified region with a specific sequence and polymorphism from a previously known sequence, which are parameters that can be transcribed and repeated, (Fig. 6). This is what makes it always available for the largest number of genetic applications and uses at the molecular level to distinguish between varieties or species, (Yuskianti & Shiraishi 2010; El-Mouhamady and Habouh 2019; Khatab *et al.,* 2021 A & B; Khatab *et al.,* 2022 & Khatab and El-Mouhamady 2022). As usual, these markers depend mainly on the PCR after identifying and amplifying a small part of the DNA using small sequences from (15 to 30 bp), which were originally designed from RAPD markers cloned sequences or any other markers with the same positive value, (Zian *et al.,* 2013; Ramadan *et al.,* 2016; Bhagyawant 2016 and Kishk *et al.,* 2017). These markers succeeded in identifying and distinguishing the most genetically related species. Also, this technique is considering a great benefit as a significant use in making DNA barcode in differentiating between different species, (Sheeja *et al.,* 2013).

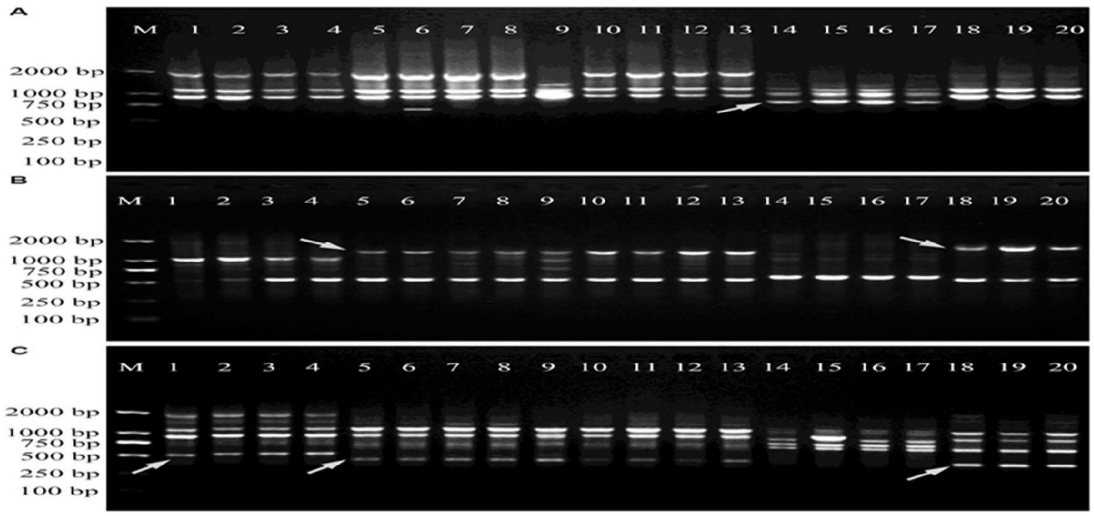


Fig. 6: Shows SCAR markers based on SCOT analysis according to Feng *et al.* (2018)

3.3.5. Microsatellites

Microsatellites are defined as fragments of DNA that are polymorphic, but with short sequences, ranging from (1 to 6 bp), (Fig. 7). While SSR markers is repeating small sequences and they are all synonyms with the smallest DNA sequences. These sequences can be obtained in the coding or non-coding parts of the whole genome and therefore are of great use for describing differences and variances within members of a single population or even at the level of the population, (Westman and Kresovich, 1997; Varshney *et al.,* 2005; Tawfik and El-Mouhamady 2019 and Khatab *et al.,* 2019).

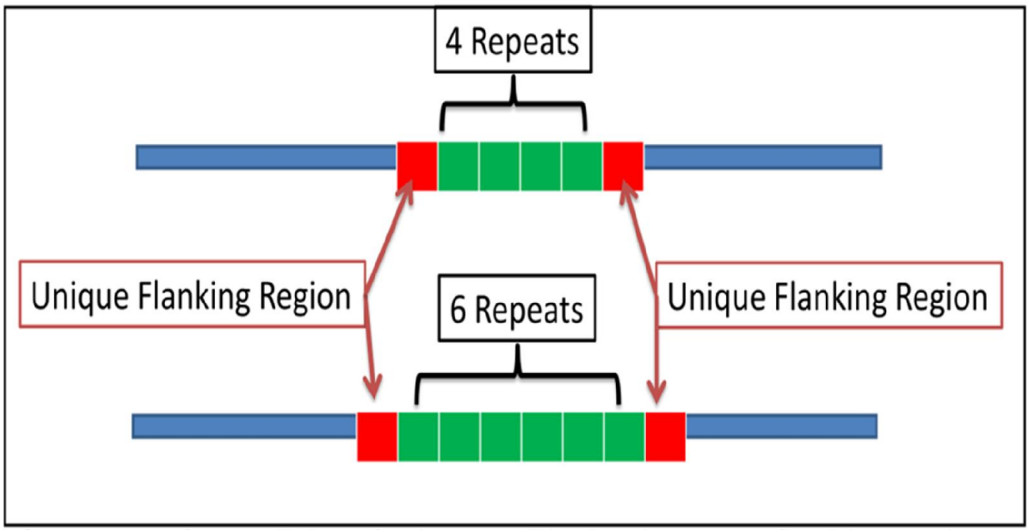


Fig. 7: Example of microsatellite according to Joshi *et al.* (2017).

3.3.6. Minisatellites

It is a fragment of DNA consisting of 10 to 60 small base pairs and is repeated throughout the genome of an organism and is distributed throughout. These moleculars are repeated differently in number between members of the plant or animal clan, and therefore they are used as a tool in detecting genetic variation between individuals and species, (Westman and Kresovich 1997). Fig.8 shows the comparison among microsatellites, minisatellites and microsatellites.

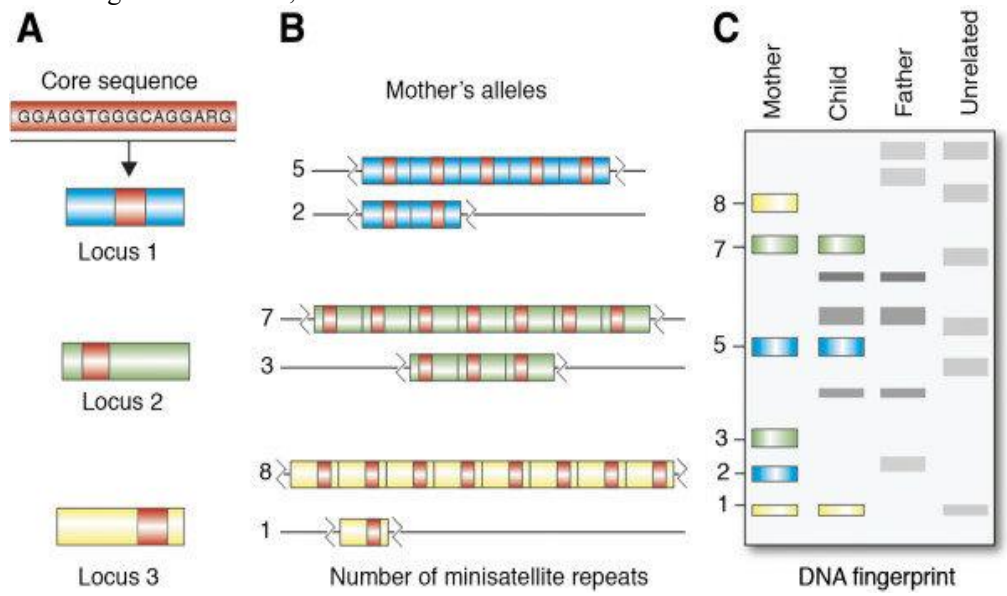


Fig. 8: Minisatellite repeat units are characterized by an approximate 16 bp core sequence in humans and other animals according to Chambers *et al.* (2014).

3.3.7. Expressed Sequence Tag (EST)

These markers are considered a discriminatory tool between cultivars and strains because they consist of 40 to 500 bp of a piece of cloned DNA (cDNA) so that corresponds to MRNA as a molecular marker, (Semagn *et al.,* 2006 & Idrees and Irshad 2014). Further, these markers have been used as an excellent taxonomic tool for a large number of crops and have been scientifically available, (Fig. 9). It is also considered an important reference for the development of molecular genetic parameters in studies, taxonomic and evolutionary tests, gene cloning and the discovery of new genes by previously cloned genes. Hence, it provides a great benefit in providing valuable information about the identification and identification of the different sequences of genes, the gene expression of a large number of them, and in regulating their work as well, (Semagn *et al.,* 2006; Sedlá˘cek *et al.,* 2010; Idrees and Irshad, 2014). Unfortunately, EST markers did not have a significant role in studying the biodiversity of plants, for example, in the Arabian Peninsula. Where (Miryeganeh *et al.,* 2014) studied the rates of migration among the population in a number of countries such as Oman, Jordan, Yemen, Kuwait and Saudi Arabia, and succeeded in proving that they had a close relationship with the rate of migration of seeds over long distances, especially by marine erosion, and this was what was credited with linking these human groups to each other.

3.3.8 Inter Simple Sequence Repeat (ISSR)

ISSR markers are considered simple, uncomplicated and spread randomly throughout the genome. As well, it succeed in showing a comparative advantage for SSR markers. Further, it does not require knowledge of the entire sequence of DNA in its work, and this is what makes it able to give multiple forms in different proportions (polymorphism), (Fig. 10). It can be defined as a segment or piece of DNA with a sequence length of 100 to 300 bp located between two identical sections of length from 16 to 25 bp for each section and directed in reverse, (Zietkiewicz *et al*., 1994, Culley and Wolfe 2001 & Reddy *et al*., 2002). ISSR markers are very important in giving reproducible results in the process of genetic differentiation, especially sex determination. In an attempt to genetically differentiate 14 genotypes for five different crops, namely rice, wheat, barley, sorghum and maize, (El Rabey *et al*., 2015) used 10 ISSR and 15 RAPD markers in order to determine the relationships and genetic differences between them at the molecular level as well, determining levels of Different evolution among them. The results were impressive in discovering 109 amplified fragments using ISSR, between 400 and 3000 bp and 130 markers using RAPD primers for the same accessions mentioned above.

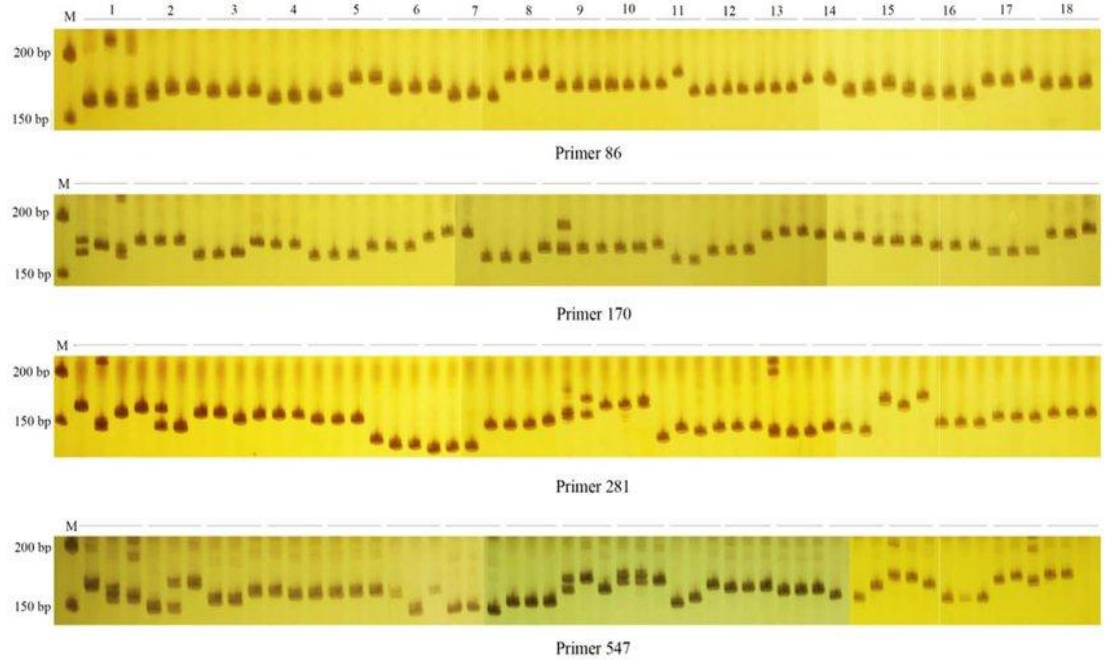


Fig. 9: EST-SSR marker variations of 18 Melilotus species according to Yan *et al.* (2017).

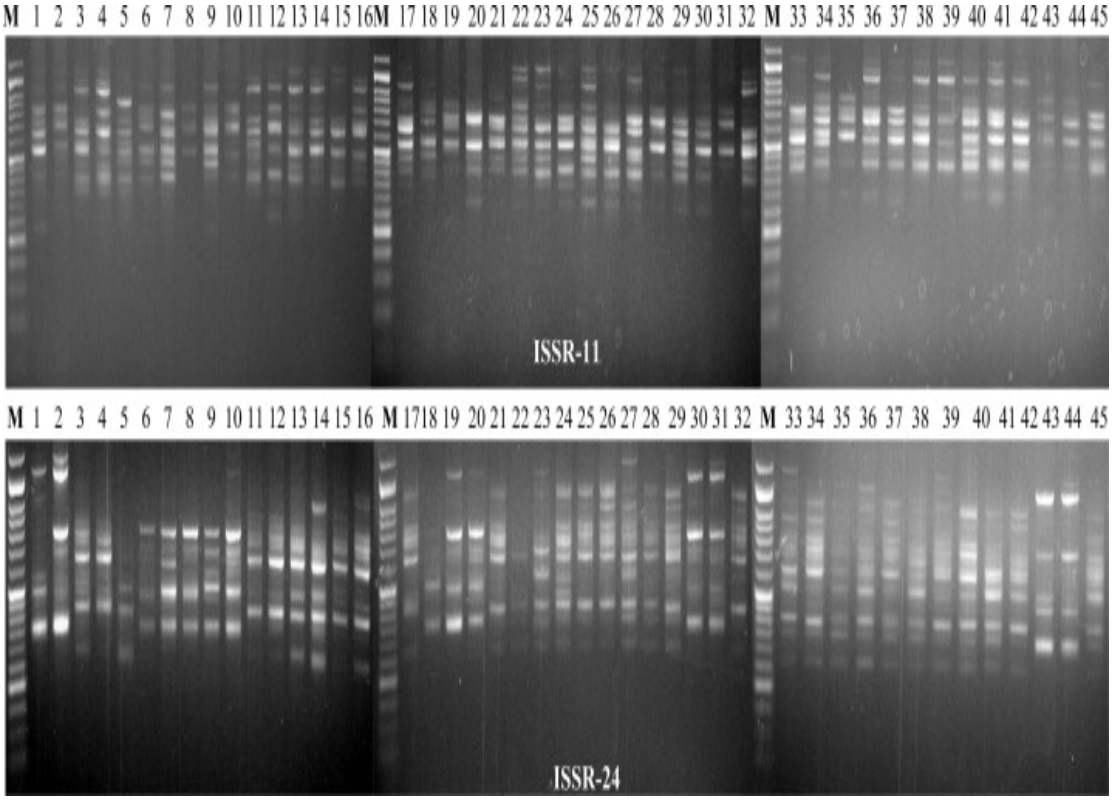


Fig. 10: ISSR primers for comparison among 45 purslane genotypes according to Alam *et al.* (2015)

3.3.9. Single Nucleotide Polymorphisms (SNPs)

This technique is effective in detecting a large number of variations associated with multiple forms within the same genome very quickly and its cost is simple, safe and reliable as well. Moreover, this technique has spread widely and has been marked by great interest as common markers, (Fig. 11). It is simply the appearance of variations in one site of a DNA nucleotide and appearing throughout the body of the genome, especially in protein coding sites and non-coding regions. On this basis, it is considered a great benefit in conducting a large number of genetic applications for the variations of clan members and the uses of the genome. In addition, it can analyze genetic diversity among individuals of the same species, (Mammadov *et al.,* 2012). These markers are mostly located in the sequence of between 100 and 300 bp of DNA sequences, especially in plants, (Lateef 2015). Also, these markers are considered robust because they depend on a large number of sites that can be evaluated. Where it is noted that species are of little variance, these markers have a great ability to discover rare differences, as the strength of differentiation between populations genetically is directly parallel to the number of sites in genetically different species, (Foster *et al.,* 2010). Many studies have been conducted using (SNPs) markers and their fruitful role in examining the genetic diversity between plants, for example, in barley by (Russell *et al.,* 2011; Hubner *et al.,* 2012) in Jordon and (Xia *et al.,* 2013) in 30 countries.

4. Promising and Advanced genomic Techniques for biodiversity

4.1. Transcriptomics:

It is noted that this technique depends on the complete set of copies of RNA produced from the genome under certain conditions or in certain tissues. These copies can be identified by high-precision and productivity mechanisms such as DNA microarray and RNA-Sequencing. Or in other words, genes can be identified and their function revealed in distinct cells differentially, after a comparison between transcriptomes or as a result of direct response to different treatments, (Mosa *et al.,* 2017). Therefore, it is very easy to analyze and detect genetic variation from a functional point of view of plants very effectively during exposure to stresses. Also, the scientists were able to discover a number of genetic expressions for a group of genes discovered during exposure to salt stress, such as pentatricopeptide repeat (PPR) proteins, (Hajrah *et al.,* 2017). One of the most famous examples of medicinal plants that

determined the importance of the transcriptome is a plant (*R.* Stricta) in Saudi Arabia. The transcriptome was able to detect genetic variations in the genome of this plant, determine which genes are better, and genetically distinguish between plants as well, (Park *et al.,* 2014).

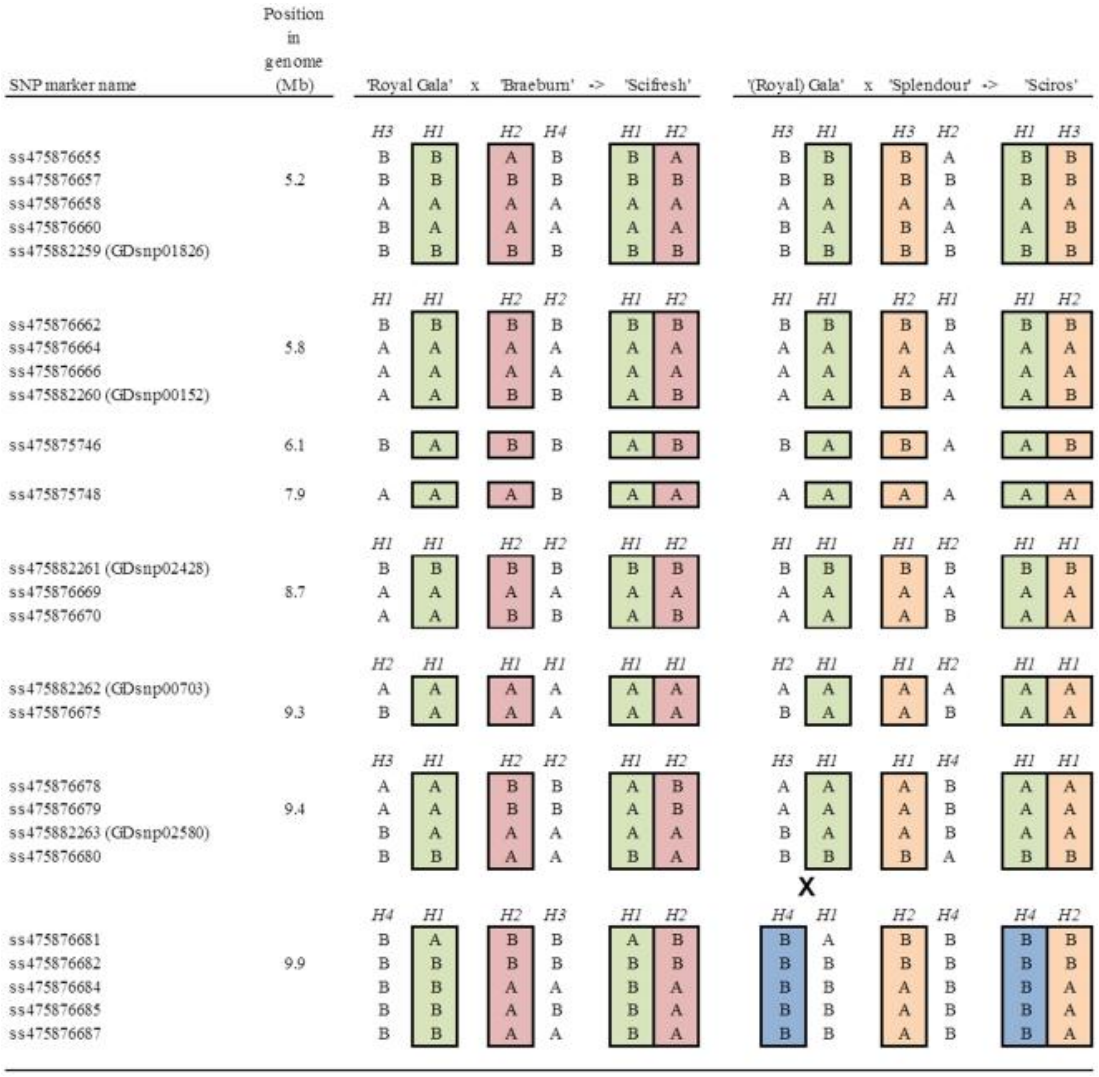


Fig. 11: Shows Single Nucleotide Polymorphisms (SNPs) according to Change *et al.* (2012).

4.2. Proteomics

Proteomics is an analysis of all the protein present in an organism under certain conditions or a specific biological system. There was a great leap in previous years in order to identify individual proteins through technological development, as this technology was the ideal and most widely used method for proteins to this day, (Yu *et al.,* 2010). One of the most important uses of this technique is mass spectrometry, bioinformatics techniques for collecting and analyzing a mass spectrometry database. Also, when the proteins were collected from different genotypes of plants, it was noticed that they were different in their genetic variance, (Alwhibi 2017). The proteins present in the stored seeds were distinguished from different samples from a tree in Saudi Arabia.

4.3. Metabolomics

This technique is very fruitful for studying the developments of metabolic processes and their receptors in all cells and tissues as a biological receptor and can be applied to spectroscopic analysis after the metabolic profiling of different plant species and various varieties such as mass spectrometry and nuclear magnetic resonance, (Mosa *et al.,* 2017). This technique has succeeded in developing an

unfamiliar group of plant varieties that are genetically and metabolic diverse. (Schauer *et al.,* 2005) evaluated the metabolic diversity of some metabolic genotype traits of Solanum lycopersicum which was classified as unfamiliar wild-bred species. Where this technique relied on identifying the biochemical signs and indicators associated with the desired trait and its effective contribution to the process of selecting the best plant lines, and even the selection of offspring as well. This is the real profitable leap in this direction. The scientists were also able to describe profiles in the genomic database of a number of secondary receptors, where they demonstrated the level of success achieved in increasing the levels of metabolism. In the same context, the problem of water stress led to an increase in activity in the levels of glycine betaine in maize, especially in the leaves, (Saneoka *et al.,* 1995 and Yang *et al.,* 1995). This proves its resistance to thirst and its transformation from a sensitive plant to one that is tolerant of this dangerous environmental factor. This technique also contributed to determining the genetic diversity of a number of wild breeds that are related in terms of kinship. Also, depending on the phenotype and this is useful in deriving new genetic traits that may be useful to the consumer.

5. DNA barcoding

5.1. What is DNA barcoding?

DNA barcoding is a rapid and effective genetic technique for the detection and identification of different species or one species by using a short sequence or a small section of DNA isolated from specific one gene or genes, (Fig. 12). This is exactly what happens about the use of the scanner in the supermarket to determine the identity of a specific commodity or stored item, compared to a database stored in a particular system, quickly and accurately, **(IBOL 2019).** From another angle, this technique is used to try to identify unknown species or any part of any living organism to make an archived database for the largest possible number of species or varieties, as well as comparing those species with the traditional type or variety to determine the natural limits of species.

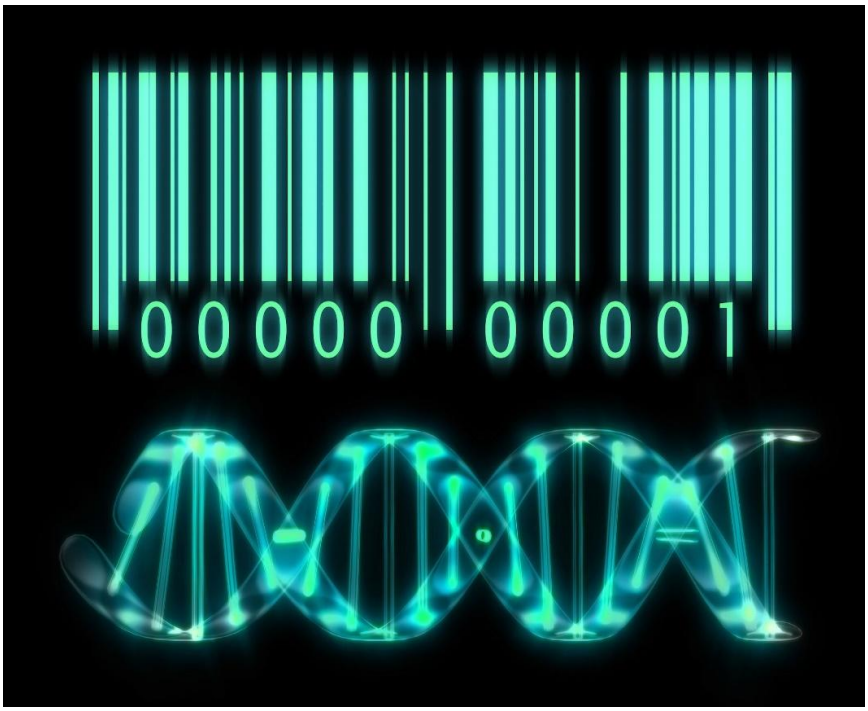


Fig. 12: DNA barcoding stages according to Yang *et al.* (2018).

5.2. Applications of DNA Barcoding

DNA barcoding technology is one of the latest and most successful genetic techniques to create a huge genomic library that includes a giant database for all living organisms. The issue is simply to determine the different genetic sequences of all living organisms, especially the unknown ones, and then upload those genetic codes or sequences in a reference database. This gigantic library is used later and when urgently needed to distinguish between different species and taxonomic for all organisms such as plants, animals and microorganisms. Also, this technique is selecting an unknown sample from a database for a previously stored classification. One of the most famous genes that achieved this goal in the biodiversity community, especially in animals and a region of the nuclear region, is the cytochrome c oxidase 1 gene, (Bandyopadhyaya *et al.,* 2013). Further, DNA barcoding protecting biological diversity against changes that threaten the survival of species, such as man-made threats, especially on the environment, the spread of illegal trade in animals and their various products. Also, overgrazing and illegal hunting. One of the most famous applications of DNA barcode is the cytochrome c oxidase 1 gene in mitochondria to distinguish between animals, fish and insects, especially when this gene is characterized by high mutation, which qualifies it to differentiate between different species, (Hebert *et al.,* 2003 a; Hebert *et al.,* 2003 b; Hebert *et al.,* 2004, Chase *et al.,* 2005 & Hogg and Hebert 2004). The most important uses of DNA coding can be summarized in the following points and this shows the size of the strategic role of this technology, (Fig. 13). 1):- Using a small piece of DNA to distinguish between different species, 2):- Taxonomic applications, 3):-DNA Barcode library, 4):-Phylogenetic analysis, 5):- Preserving the biodiversity of species, especially endangered species, 6):- The use of morphological traits as markers of different plant species and breeds in a taxonomic manner, 7):- Genetic Diversity Analysis, 8):-Ecological Applications, 9):- Identification of unknown species or hidden identification of species, 10):-Food Authentication, 11):- Detection and identification of commercial fraud in food, 12):- Illegal Trade Monitoring.

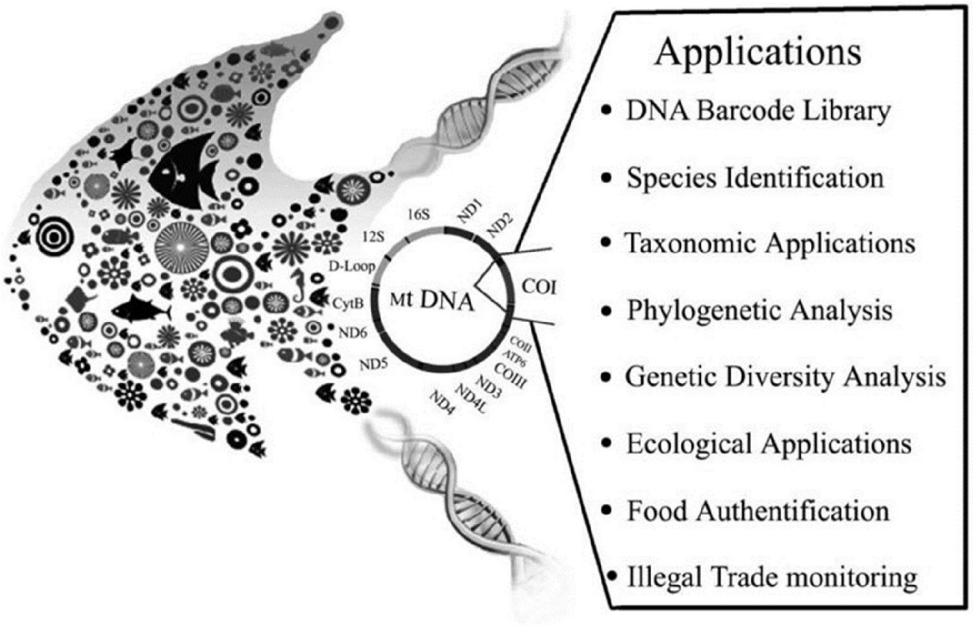


Fig 13: Most Common Applications of DNA Barcoding Using CO1 gene according to Padmavathi and Srinu, (2017).

5.3. DNA barcoding: Procedures and steps taken

Initially, the DNA barcoding process goes through a number of steps, starting with the process of collecting samples from different environmental areas. It is preferable to take fresh, mixed and dry samples or products manufactured according to the pre-planned direction of work, (Fig. 14). Then, isolate and extract the DNA using the available protocols and from them; CTAB (Doyle & Doyle 1987), SDS method, PVP method, Phenol- Chloroform method, etc. The extraction process is followed by amplification of DNA by appropriate molecular genetic markers through the PCR process, which is

known to go through three stages namely; denaturation, annealing and extension. One of the most important steps after that is the purification of the PCR product and the initiation of sequencing tape using the bands obtained. There are several types of work for making a sequencing, including the following:

1) Sanger sequencing, 2): - Next generation sequencing, 3): - Third-generation sequencing.

After obtaining the information and data for this new sequence of samples, the search and comparison process begins with a reference database of previously added sequences (Library References) using bioinformatics technology. The most famous database files obtained are NCBI, BLASR, etc.Further, The process of correct analysis of this new sequence is very important and there is no room for error because this sequence is the basis for creating a new DNA barcoding and for revealing the identity of the organism, whether it is a plant, animal or any other organism, and from this point the process of adding it to the database as a new bar code, (Yu *et al.,* 2020). Then, after making sure that the new sequence is statistically reliable, it will be added to the gene bank or reference library, (Clark *et al.,* 2016) to get the new membership number in this genetic library where the species are identified after extracting the data later from the reference database for the barcode of life, (Ratnasingham & Hebert 2007).

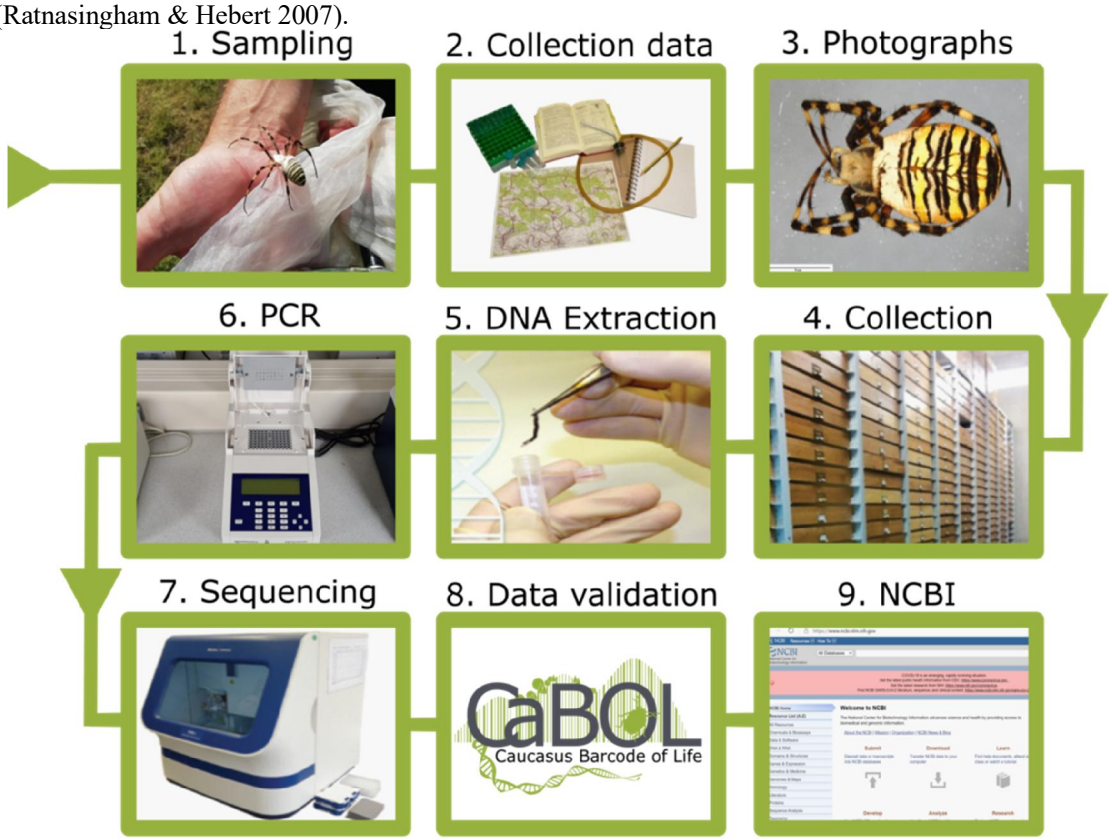


Fig. 14: Steps of DNA barcoding in Organisms according to Erhardt (2023)

It is worth mentioning that there are multiple programs that are used to make multiple sequencing and analyze data according to molecular genetics methodology such as MEGA 10 (Tamura *et al.,* 2021), ABGD (Puillandre *et al.,* 2012), Taxon DNA (Meier *et al.,* 2006), Geneious vR6.1.6, MAFFT v7.017 (Katoh *et al.,* 2002). The efficiency of genes is estimated by measuring the genetic distances between them within the same species, taking into account the presence or absence of a gap in the gsenetic barcode. Also, the difference between different species is the basis for determining this technique, as it is found that the average genetic distance is ten times smaller than the smallest genetic distance between different species, (Meyer & Paulay 2005).

5.4. Select of DNA Barcoding

It is noted that in order for the DNA barcode to be successful, it must be common and universal in terms of application, easy to sequence, capable of amplification and sufficiently diverse to be able to distinguish between the largest possible numbers of different types. Also, this variation must be low within individuals of one species. In addition, this sequence is preserved among specific species, easy to analyze and easy to retrieve, especially in the case of herbs and other damaged samples, (Chase *et al.,* 2007). Further, on this basis, there must be a global system, cheap and always capable of development by adding in the appropriate place to DNA barcode in the plant, for example, (Cowan and Fay 2012), (Fig. 15).

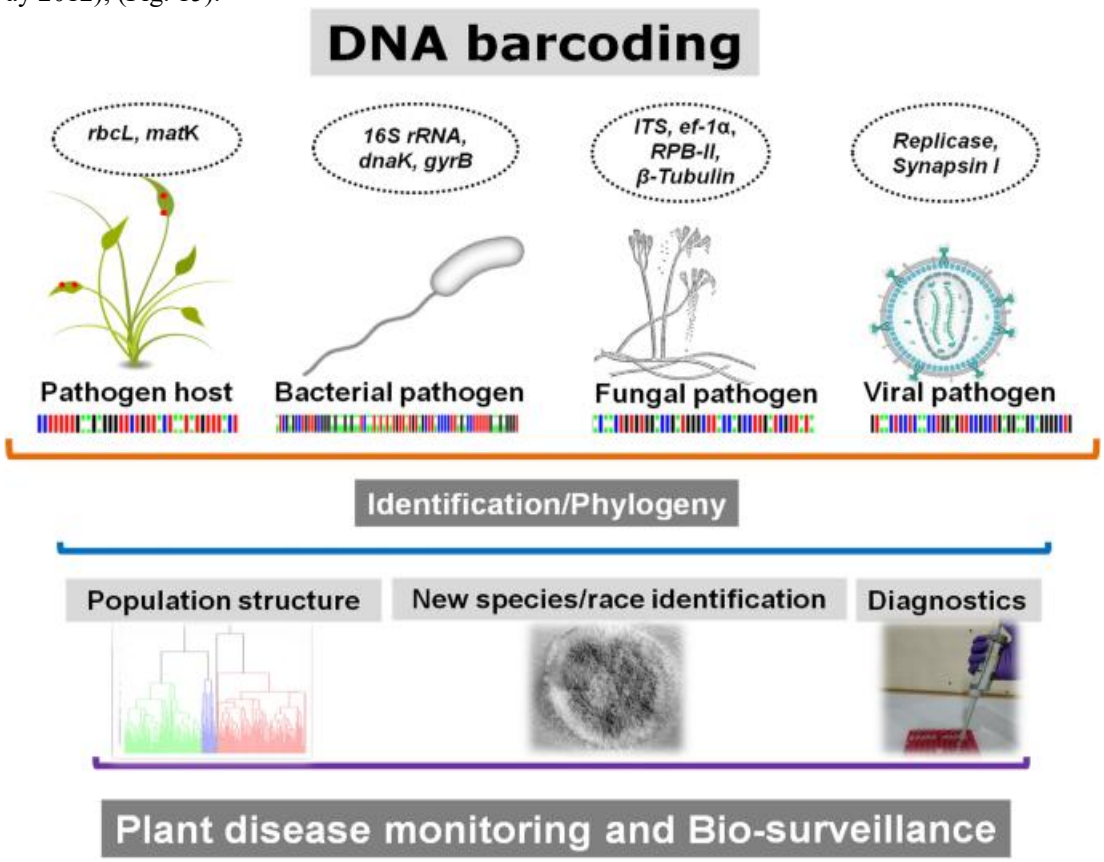


Fig. 15: Choosing of DNA Barcoding according to Choudhary *et al.* (2021).

5.5. DNA Barcoding in Animals

The DNA barcoding technique depends primarily on simplicity in identifying the sequence, ease of reading it, comparing it with the reference database as explained previously, scalability and standardization of the measurement method. On this basis, the main point for choosing the DNA barcoding gene is that it be a standard site of common function and reliable activity in a large number of samples under test, which are originally differentiated, so that the comparison with the reference database is more credible and acceptable to distinguish between different types or at least to distinguish a type One over the other. For example, in the vast animal community and the plurality of species and races, it is noted that the cytochrome C oxidase I gene near the end 5' or the end of the subunit, which is from 600 to 1000 bp, has a very high variance due to the level of mutation that occurs in it, which makes it suitable for differentiating and distinguishing between different animal species, (Kress & Erickson 2012). This gene is characterized by being a single locus inherited from the mother and a protein coding region with multiple copies of each cell so that it can restore the sequence in the case of poorly preserved samples, (Hebert *et al.,* 2003; Fazekas *et al.,* 2009; Hollingsworth *et al.,* 2011). COI genes are generally present in the mitochondria because they are very specific, powerful and can retrieve the 5 - end in the DNA of the desired animal whose identity is known besides, the region from which

this gene is derived is called the Folmer region in Fig. 16, (Folmer *et al.,* 1994). Since, there is an inverse relationship between the size of the genome and the rate of mutation in it. For example, whenever the size of the genome is small, such as what is an accident in mitochondria, we find that the rate of mutation in it is very high and vice versa in plants, it is observed that the size of the genome is large, this makes the proportion of variations and mutations in the COI is very slim. Further, this qualifies it to be a global model for differentiating between types of animals, unlike rbcl and matK genes, which are more successful in the plant community, (Drake *et al.,* 1998 &, 2007). The percentage of variances that can result from a COI gene sequence is often less than 10% of the variance between species because additions or exclusions are often rare, (Blaxter 2004), (Fig. 16).

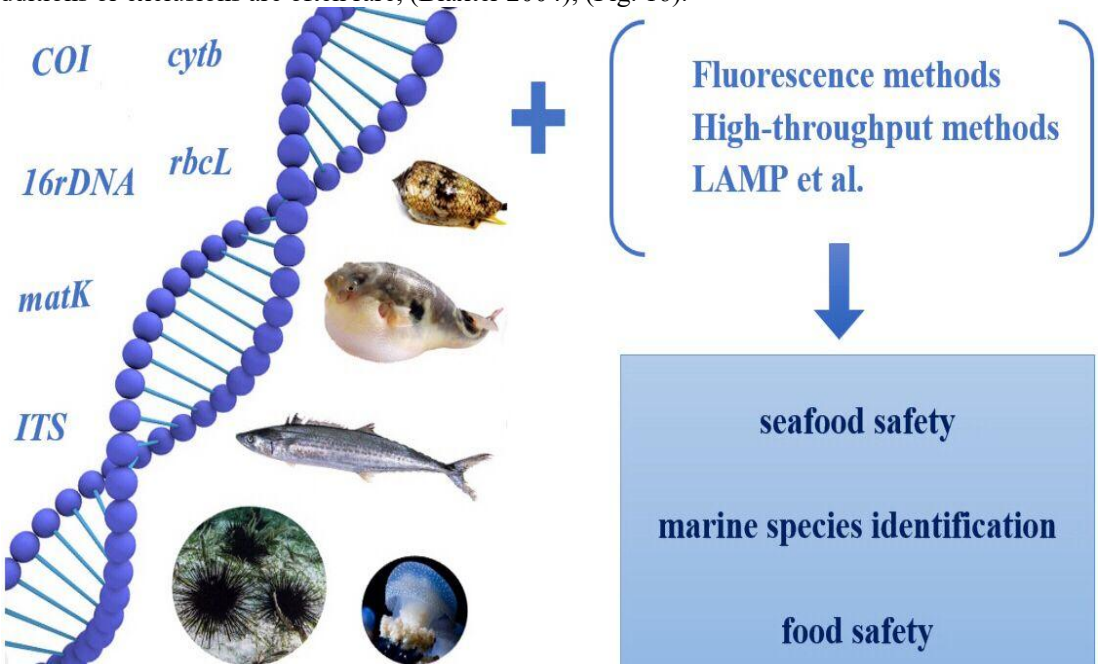


Fig. 16: DNA Barcoding Region of COI in Animals according to Gong *et al.* (2018)

5.6. DNA Barcoding in Plants

There is no doubt that the different types of plants are under imminent danger due to a number of challenges, including the factors of climate change. The factors of climate change and a number of other risks, such as overgrazing, began to decrease the plant density to unprecedented limits, and the human element played a major role in this dangerous pattern. Therefore, preserving the biodiversity of plants has become one of the priorities of international conferences because of their great benefits to humans, animals and birds as basic food. This prompted the urgent call for advanced molecular genetics methods to record, identify and identify plant species, especially wild ones. As a serious attempt to preserve it and identify ways to benefit from it in the future in many areas, including the medical and food fields, the DNA Barcoding had a primary role in this task.As mentioned earlier, the DNA barcoding in the animal community is easy due to the small size of the genome. In plants, the process of DNA barcoding is very difficult because of the large size of the genome and this calls for multiple sites instead of being a single site in animals because the rate of nucleotide replacement is little in the plant genome, (Cho *et al.,* 1998; Mower *et al.,* 2007; Kress & Erickson 2007; Fazekas *et al.,* 2008). Figure 17 shows the DNA Barcoding in plants Vs. animals. Many studies and tests have been conducted on a number of genetic sites of the plant genome to distinguish between plant species, (Fazekas *et al.,* 2009). But the Thumb Rule feature did not work in plants, unlike animals, because in the animal it works on the basis of the position of the individual gene, and this is impossible in plants to make DNA barcoding. In order to get the best possible results, a number of genes were tested for making DNA barcodes, such as trnHpsbA, rpoB, rpoCI, ITS, 23S rDNA to find some markers that could be a successful measure of differentiation between different plant species or genera. While, some of them were unclear and more ambiguous, (Janarthanan *et al.,* 2020). Thus, the encoded genes, the most

successful in this regard in encoding DNA such as rbcL, rpoB and matK have been put together with other non-coding genes like trnH-psbA and atpF-atpH as an important checklist for separating and differentiating different plant species, (Fazekas *et al.,* 2008). The ITS nuclear DNA barcoding genes are considered to be one of the most important markers used successfully to differentiate between different plant species, as they showed a high rate of variation in the plant world, (Chen *et al.,* 2010 & Schoch *et al.,* 2012).

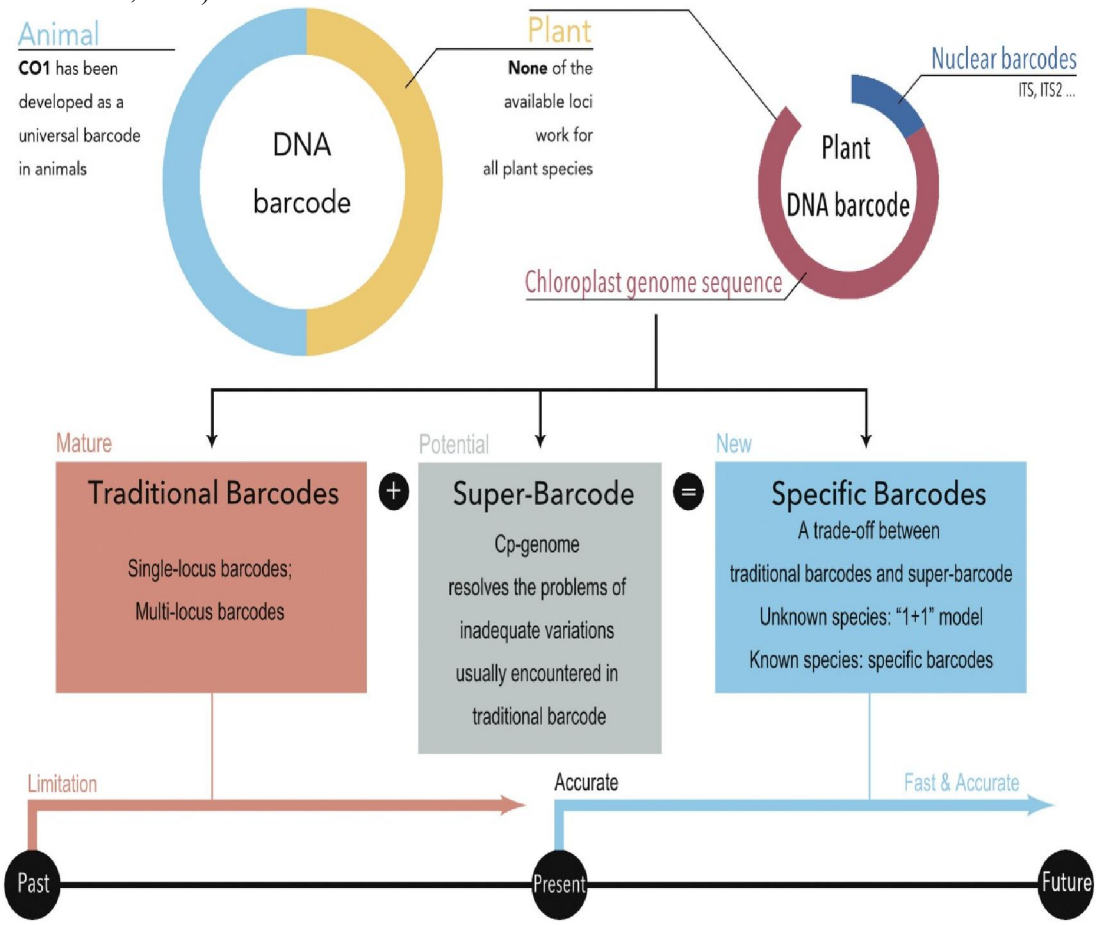


Fig. 17: Viewed the DNA Barcoding in Plants Vs. Animals according to Xiwen *et al.* (2014).

5.7. DNA Barcoding in Bacteria

As previously explained, the goal of DNA barcoding using a small part of DNA or gene but specific part is to index life to distinguish one type of living organism from another, (Guerra-Garcia *et al.,* 2008). While, the situation is completely different in the case of microbes, as it is noted that most of their types are unknown and remain in a hidden state. Therefore, the DNA barcoding strategy represents the ideal way to provide a database for each microbe after its identification through its DNA sequence, and this gives a good idea of the environmental situation and conservation priorities in this case, (Begerow *et al.,* 2010). It is worth mentioning that when we mention bacteria, we must talk about phytoplasma, which are the pathogens that affect the agricultural production sector and cause great losses. For this reason, Makarova *et al.,* (2012) designed a DNA barcoding using Tu (tuf gene) which succeeded in identifying the phytoplasma and designed a group of primers at molecular weights ranged from 420-444 bp in all 91 strains of phytoplasma are (16S rRNA groups -I through -VII, -IX through -XII, -XV, and -XX). One of the most successful DNA Barcodes for distinguishing bacterial strains is (16S rRNA, rpoB, dnaK, gyrB and recA) and these DNA Barcodes can be used very effectively both taxonomically and evolutionarily.

5.8. DNA Mini-Barcoding

The short DNA barcoding in terms of length is very easy in the process of amplification and PCR analysis due to its small size as it is less than 200 bp. These barcodes are used to identify the types of herbal plants to control the commercial fraud of products manufactured from them. But on condition that the number of herbal species does not exceed 10 in the herbal mixture, (Meusnier *et al.,* 2008, Särkinen *et al.,* 2012, Srirama *et al.,* 2014 & Gao *et al.,* 2019).

5.9. DNA Metabarcoding

With the tremendous progress made by DNA barcoding technology, the trend is now available for a DNA barcode to represent metabarcoding by making a certain initiator in the PCR interaction to make sure of the biodiversity of different species, its affiliation, and its composition to a sample represented for one environment only, (Taberlet *et al.,* 2012). Therefore, it can be said that the DNA Metabarcoding is a sequence of a uniform sample of a number of species in the same environment. One of the most famous examples in this context is the determination of plants type in the fruit trees used in the manufacture of 55 commercial products using ITS1 and ITS2 IN DNA metabarcoding, (De Boer *et al.,* 2017). As for forensic medicine, this technology had a large share of success, as (De Boer *et al.,* 2017) were able to determine many different types of mammals with a success rate of 99.9 % using 16SrRNA DNA Metabarcoding as optimum standard in this regard.

6. Conclusion

DNA barcoding was able to detect species with cryptic classification, collect biological samples from crime scenes in forensic surveys, as well, identify new unclassified microbes in a sample and other useful uses. Therefore, DNA barcoding technique will help in the coming years in drawing better pictures of life and determining the processes of development in light of the threats threatening biodiversity, especially in light of the climate change crisis that is hitting the planet with the utmost severity. Therefore, DNA barcoding is considered one of the most important modern scientific techniques to know and study the biodiversity of living organisms in any environmental area.

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