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# Novel Approach towards DNA Barcoding as a Tool in Molecular Biology and Biological Activities of Cyclotides with Particular Emphasizes at Molecular Level

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**ABSTRACT: DNA barcoding categorized the plant materials based on short, standardized gene sequence in precise manner. Four plant DNA barcode markers called rbcL, matK, trnHpsbA, and ITS2 have been developed for making revolutionary advancements in the field of ecology, evolutionary biology and conservation, including community assembly, species interaction networks, natural conservation, taxonomic discovery, and assessing priority areas for environmental protection and also potential applications in the field of forensics. DNA barcoding uses particular regions of DNA making helping in categorization and recognizing unidentified species. Researchers now interested to generate DNA barcodes designed for all living organisms and to build up data accessible to public to help in understanding of natural biodiversity of world. Cyclotides are peptides derived from plants with particular head to tail cyclic backbone that have three disulphide bonds by forming a cystine knot. Recent information about DNA barcoding can be used for detection of unidentified biological specimens to a taxonomic group, accurate detection of phytomedicinals, and in the biodiversity of living organisms. Study on DNA barcoding indicates that is no single universal barcode for recognition of all plant groups. Therefore, relative analysis of plant barcode loci is fundamental for to choose ideal candidate for specific medicinal plant genus and families. Current advances in genomics have promoted the development in plant DNA barcoding by introducing of high throughput techniques resembling next generation sequencing that provided a new way for complete plastome sequencing which is now called as super DNA barcodes. All features that exhibit by cyclotides used in molecular imaging and diagnostic tools. Most important future challenges focusing on construction of global plant DNA barcode library and making genomic sequencing technologies in most efficient by applying these genetic markers to the sub branches of biological sciences.**

**Keywords:** DNA barcoding, Genomic Sequencing, Cyclotides, Evolutionary biology, Genetic Markers.

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## INTRODUCTION

Molecular biologist, biochemist, plant biologist, plant breeder and genetics microbiologist, field ecologist, evolutionary biologist, conservationist, or applied forensic specialist is to find out the exact recognition of a plant in a rapid, repeatable and reliable approach (Kress *et al*., 2011). DNA barcodes are short DNA sequences with base pairs in range 400-800 with the purpose that they can be simply separated and undergo characterization for every one species of plant on planet (Costion *et al*., 2016).

Through combining the efforts of molecular genetics, sequencing technologies, and bioinformatics, DNA barcodes tender a rapid and precise way to distinguish earlier known, classify biological species and to retrieve sequence about them. This implementation furthermore has the prospective to rapidity the finding of the thousands of biological species up till now to be named (Cowan *et al*., 2006). DNA barcoding is a practice with the purpose to discover the varieties based on species specific differences in short sequences of their DNA (Hebert *et al*., 2003). DNA barcoding utilizes principles of biochemistry, microbiology and biotechnology to recognize plant species in most efficiently detection method that is faster and accurate as compared to other traditional methods (Lahaye *et al*., 2008). This skill is now adopted in morphological characteristics, physiological conditions and allows species discovery without individual taxonomic information. This has enabled research scientists especially in field of molecular biology to put efforts on DNA barcoding technique to estimate the herbal plant and related biological products accuracy (Webb *et al*., 2002).

While techniques used in DNA barcoding are most efficient for characterization and identification of medicinal plants but research data clarify that more superior and recently developed techniques of high throughput sequencing (Van *et al*., 2006). The next generation sequencing technology provides tools to make advancements in DNA barcoding process (Armstrong *et al*., 2005). DNA barcoding generally target short regions of DNA strand surrounded by genome and not need of complete data of genome (Hamilton *et al*., 2012). New DNA barcode principally rely on next generation sequencing to identify several plant species in herbal products (Techen *et al*., 2014). Hence, the next generation sequencing a useful technique toward introducing multiple advancements in DNA barcoding research (Kress *et al*., 2007).

Cyclotides are absorbing proteins some scientists called it cyclopeptides because it is composed of 30 amino acid residues with long specific sequence present in a number of families of land plants. Cyclotides that are naturally active exhibit several biological activities such as protease inhibition action, activity against microbes, act as insecticidal, acting as cytotoxic and worked against toxic compounds by showing expression, activity against HIV and related viral infections and also show similar activities like hormone (Gould *et al*., 2011). These naturally active cyclotoides contribute to specific head and tail spherical knotted from head to tail structure with three disulfide linkages also with one disulfide bond linked from side to side a macro cycle shaped by the two other disulfides bond and resulted in inter linking peptide backbone form a structure called cystine knot topology. Cyclotides considered as natural combinatorial peptide with structurally contributed by cystine-knot scaffold and from head to tail cyclization (Austin *et al*., 2010).

In recent times Gruber and their friends reported new type of cyclotides called a point-mutated cyclotide (T20K) kalata B1, with the purpose of activity in oral cavity of experimental mouse model with multiple sclerosis (Thell *et al.*, 2016). That research work followed on previous research studies that had been as a conventional role for the specific cyclotides in natural immune system with more particularly in immune suppression action.

## PRINCIPLES OF DNA BARCODING

The method of generating and to apply DNA barcodes for the principle of recognition of biological specifies involve two steps one is the creating of the DNA barcode library of identified species and second one is the matching of DNA barcode sequence of an unidentified sample against the DNA barcode library. The primary step is to select single to a number of individuals per species to provide as reference sample in library of DNA barcode that is called DNA barcode library (Kress *et al*., 2009). Tissue samples obtained starting specimens by now housed in herbaria or be able to be in use directly from live specimens in field with properly pressed, tagging, and put into voucher specimens. These specimens can give out as a vital permanent documentation that can matched with the DNA barcode to a selective species of plant (Kress *et al*., 2005).

### *Species Discovery*

Plant DNA barcodes are especially important applications in biological discovery of supplementary in order to detect new taxa and biodiversity new groups related to plants. In several research studies, surprising sequence differences led to reconsideration of morphological and environmental dissimilarity that has then results in prescribed recognition of innovative species (Kress *et al*., 2010). In additional cases, morphological and natural variants that activated to generate sequence records to set up whether nearby is at the substructure of genetic substantiation for recognizing of different taxa and subfamilies of ecologically important species.

Discoveries of biological species is an important and revolutionary step towards all related information getting of the filled spectrum of class from comparatively small in addition to natural poor groups such as bryophytes all the way through to striking ecologically as well as ethnically important vegetation (Liu *et al*., 2013). Some fungi also act as pathogens and cause specific diseases. Fungi exist in nature with several varieties with genome sequences that can detected by DNA barcoding technique (Sharma *et al.,* 2019).

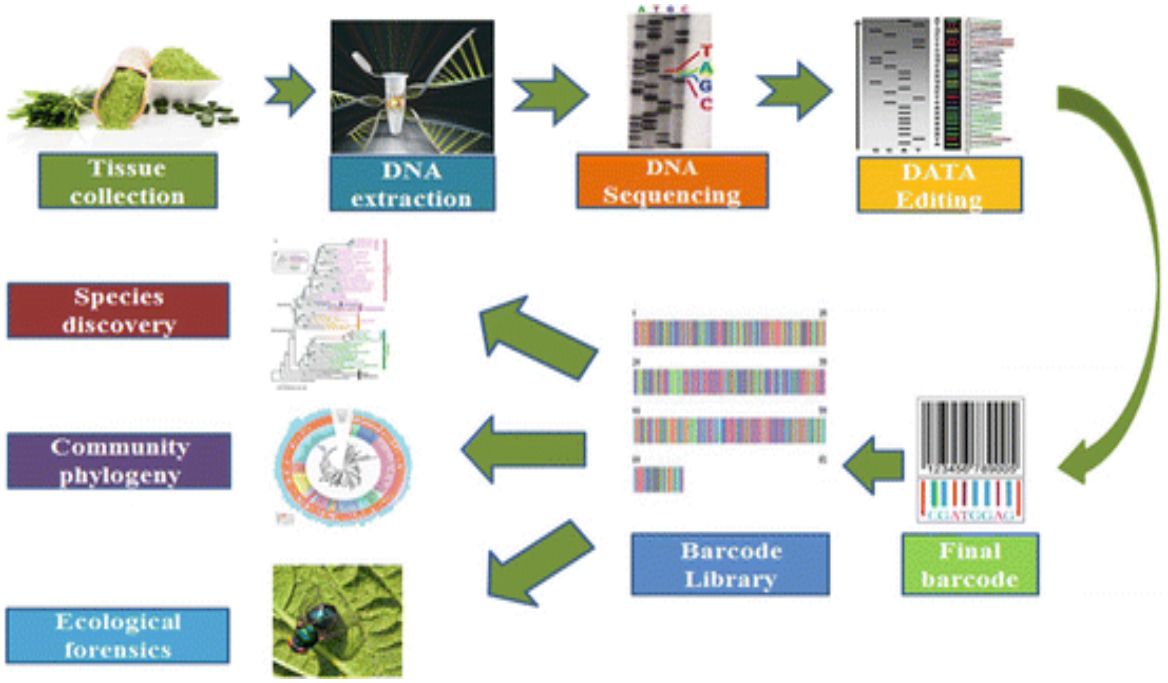


**Fig.1.** Steps involved in DNA Barcoding.

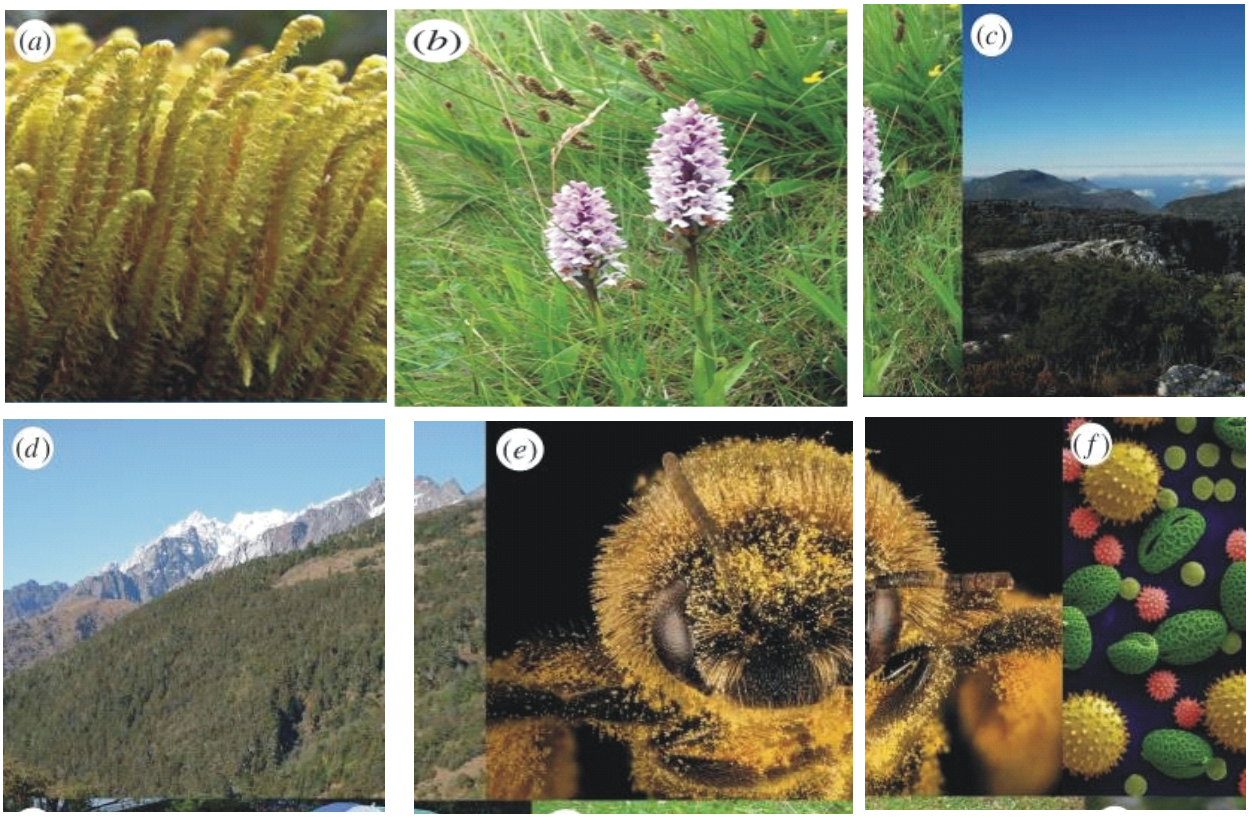


**Fig. 2**(a) Species Dicovery of Bryophyte (Herbertus); (b) DNA barcode of flora of Wales; c) Floristic bareding of Cape Flora; (d) DNA barcode of flora of China; (e) Study of pollen Movement; (f) Species identification of historical pollen.

***Functional Traits and Species Assembly***

Quantitative data on practical and genetic traits collectively by means of determined evolutionary history give ecologist a prevailing a tool intended for considerate the process of population assembly (Swenson *et al*., 2013). Biological applications of the DNA barcodes for relationship between beetles and hosts include in quantitative determination of ecological communication. Advantages of by means of a multi locus genetic material that the beetles be able to notorious to species at several stages of life and (Chen *et al.,* 2015). When the fundamental network of food web chain recognized using the DNA barcodes, compare across where living organism inhabitants. This has been revealed in several cases with the intention of DNA barcodes be able to detect the occurrence of biological food seb species such as cryptic species principally that relationship seen in insects. (Hebert *et al*., 2003).

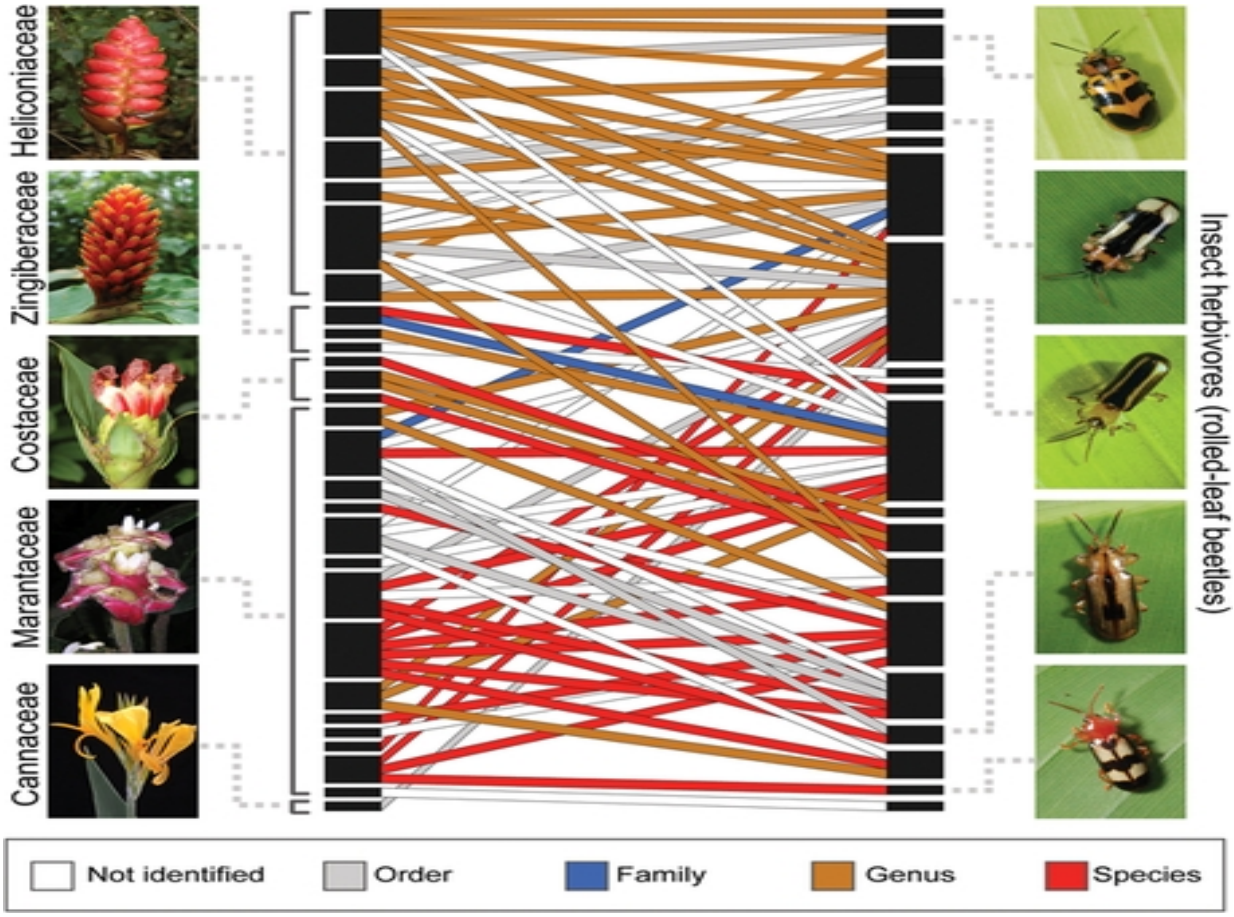


**Fig. 2.** Plant Herbivore Network based on DNA Barcodes.

***Role of DNA Barcoding in Forensics***

Bacterial infections becomes severe when their spread into the body and body immune system defend against a particular microbes and tried to kill them. As a result of this antigen enter in body and antibody reaction occur and defense system produce useful antibodies in the body (Dao *et al*., 2019).The correct identification of plants and animals is equally important in the non scientific, commercial world as it is to ecologists and taxonomists. Broadly termed "DNA barcode forensics," genetic markers are being employed to insure commercial product identity and purity, to protect endangered species in illegal trading, and to document the use of forest plants by local people (Nicole *et al.,* 2012).

Hepatitis B patients diagnosed by Polymerase Chain Reaction (PCR) that is most valuable analysis for the prevention of viral borne disease in blood especially Hepatitis B (Dawood *et al.,* 2019). Traditional medicines, teas, and herbal supplements together are an important and large component of the commercial market in biodiversity, locally, nationally, and internationally. It is estimated that medicinal plants account for over US$60 billion in annual revenues in the United States (Newmaster *et al.,* 2013), for a review of statistics on markets and use).


**Fig. 3.** Forensic Tools in DNA Barcoding Technique.

From the early development of plant DNA barcodes, applications to monitor this market have been in development. Many of these investigations in which DNA barcodes have been applied to commercial medicinals and herbal supplements have concluded that in some cases the genetic markers used, which varied quite widely among studies, were not able to discriminate among species States (Newmaster *et al.*, 2013).

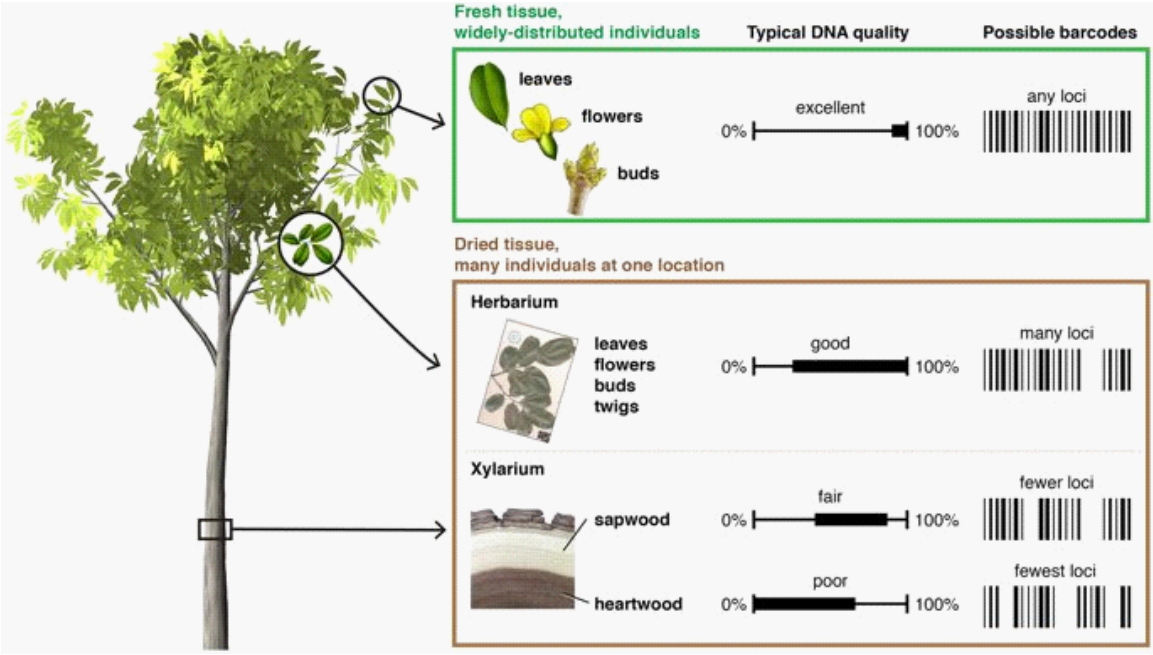


**Fig. 4.** Plant Forensic Tools in DNA barcoding technique.

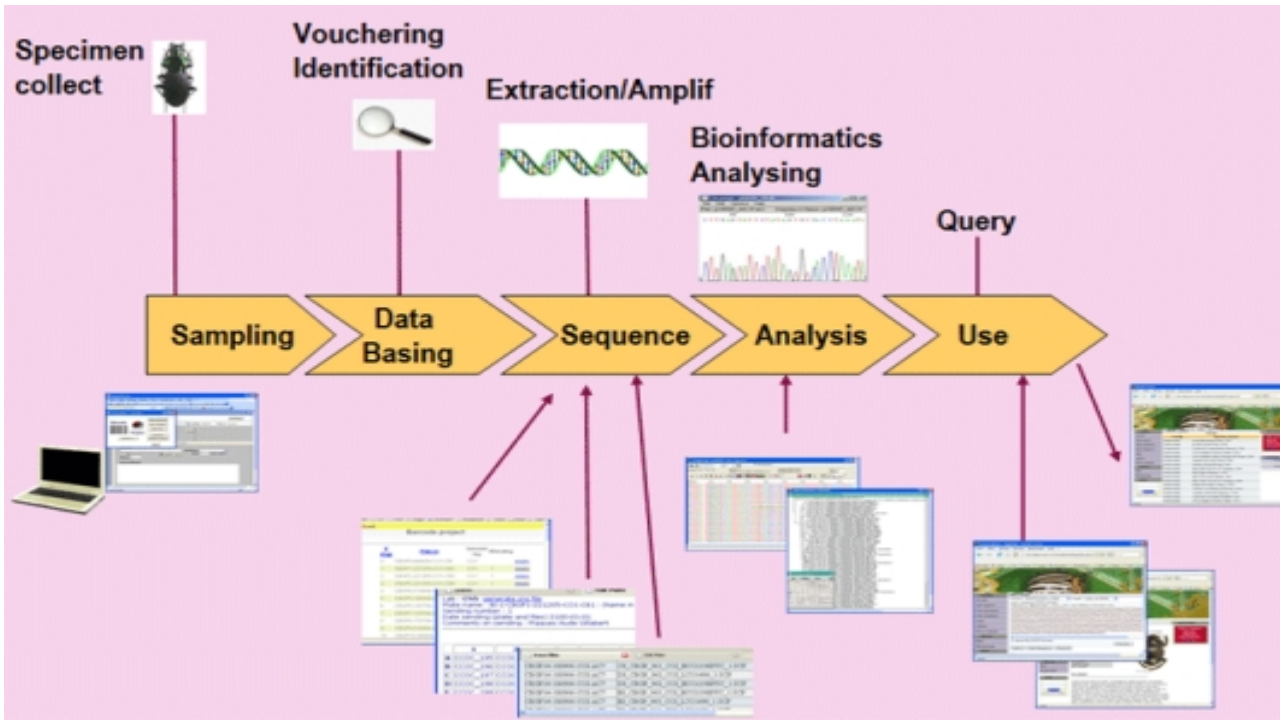


**Fig. 5.** Tools of Constrcution of DNA barcode library.

***Herbal Drug Authentication by DNA Barcoding***

Herbal medicines have a widespread and well-documented history of use in the prevention and treatment of various diseases, which continue to gain global influence in modern medical and health services. The international trade in herbal products is a major force in the global economy and the demand is increasing in both developing and developed nations. Recent reports indicate that herbal products available to consumers in the market place may be contaminated or substituted with alternative plant species and ingredient substitutions that are not listed on the labels (Newmaster *et al*., 2013).

***Building the Global Plant DNA Barcode Library***

Eventually DNA barcode sequence data (rbcL, matK, and trn H-psbA) were generated and compared across 15 forest plots in the CTFS/Forest GEO network representing 1347 species of trees in both temperate and tropical habitats in seven different countries (Craik *et al*., 2009). Two additional avenues for developing the library for plants include lineage based efforts and floristic efforts (Kress *et al*., 2014). Individual taxonomists are also generating DNA barcodes for specific groups of plants as either trial for sequencing success using the standard markers or as part of their basic molecular phylogenetic investigations in which the DNA barcode markers are used for understanding evolutionary relationships. Although many of these "DNA barcodes" may not receive the official Gen Bank DNA barcode designation, they are all adding to the library of sequences that complement the standard DNA barcode markers (Wang *et al*., 2017).

***Advancements in DNA Markers and New Sequencing Technologies***

Speculation and predictions on the future direction of plant DNA barcoding began almost simultaneously with the initiation of studies applying these markers to questions in taxonomy, evolution, and ecology, including the relationship between loci based DNA barcodes and genomic approaches to species identification (Kress *et al.,* 2008a). The need for both advanced sequencing technologies as well as efficient database design and search strategies for species identification were recognized.

***Implication of Genomic Tools in DNA Barcoding Technique***

In recent years, genomic approaches have been introduced in DNA barcoding technology. Many recent papers on DNA barcoding in plants have been published.

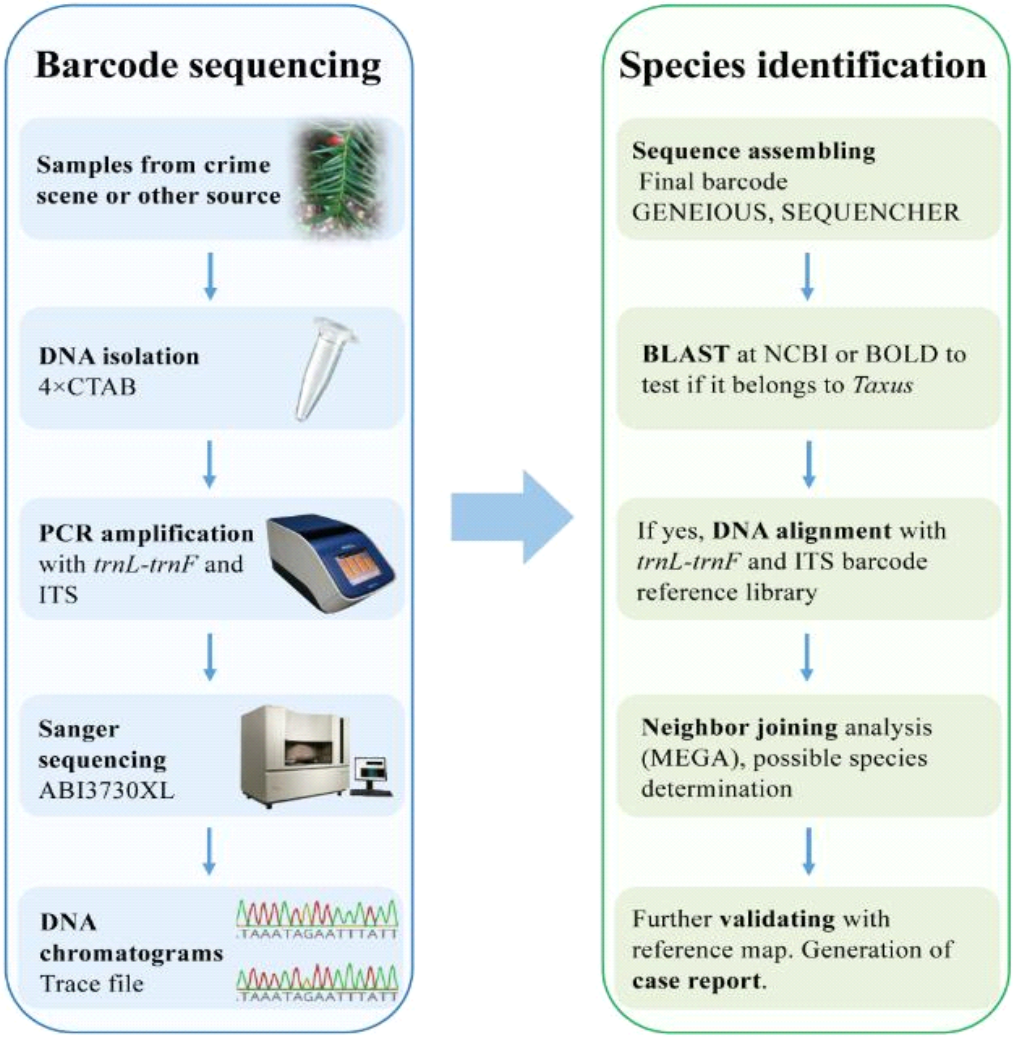


**Fig. 6.** Genetic Tools in DNA barcoding technique.

Due to these advances in DNA barcoding technique, multiple applications like ecological surveys, cryptic taxon identification, authentication of phytomedicinals, and herbal drugs have been employed. These applications can be extended further if new genomic technologies are introduced into this technique (Newmaster *et al*., 2013).

***Metabarcoding***

One exciting modification of DNA barcoding is appropriately called metabarcoding also called "eDNA" which employs genetic markers for the identification of organisms in environmental samples, such as soil, sea water, or coral reefs (Leray *et al.*, 2015).

***Next Generation Sequencing Principles in DNA Barcoding Technique***

The combination, complementation, and extension of employing the standard single or multilocus DNA barcodes with next generation sequencing (NGS) technologies has been inevitable. The divide between specimen based DNA barcoding and environment based metabarcoding as described above has been in part responsible for this turn to NGS. It has been suggested that genome skimming of both plastid and nuclear regions as an "extended DNA barcode" may serve as the bridge between standard DNA barcoding and whole genome sequences as the ultimate in species identification Such "megabarcoding" will not only circumvent the need for PCR, but will also provide an increased level of genetic data that can serve other purposes besides species identification (Coissac *et al*., 2016).

***Super DNA Barcoding: Genomic Tool in Plant Discrimination***

As sequencing technology and bioinformatics continue to advance, complete plastome sequencing has revolutionized the technique of barcoding, which is termed as "Super-barcodes". These plastid-genome-based species classification and identification have been progressively accepted by taxonomists. The analysis of this super-barcode also resolved the problems of sequence retrieval usually encountered in traditional barcoding studies. Compared with the nuclear genome, the cp-genome is small in size and has a higher interspecific and lower intraspecific divergence, which makes it more suitable as a genome-based barcode( Li *et al.*, 2015).

***Genetic Origin of Cyclotides in Plants***

Plants from Rubiacea and Violaceae have committed genes for production of cyclotides. The genes encoded protein precursorcontain an ER signals peptide, an N-terminal pro-region, the N-terminal repeat, the mature cyclotide domain and a C-terminal flanking region (Craik *et al.*, 2013). The protein precursors for cyclotides from the Solanaceae family are encoded in genes similar to those found in the Rubiacea and Violaceae plants with dedicated precursor proteins that have an ER signal, a pro-region, the linear peptide precursor, and end with a hydrophobic tail.(Craik *et al*., 2015). General structural features of the cyclic cystine knot (CCK) topology found in all cyclotides. Detailed three-dimensional structure of the cyclic cystine knot (CCK) and the connecting loops found in cyclotides. The six Cys residues labeled I through VI whereas loops connecting the different Cys residues are designated as loop 1 through 6, in numerical order from the N- to the C-terminus. B. Möbiuscyclotides contain a cis-Pro residue in loop 5 that induces a local 180° backbone twist, whereas bracelet cyclotides do not.

***Biosynthesis of Cyclotides***

Design showing major events consideration to take place during biosynthesis of the cyclotides. The case for the processing of kalata B1 is shown. It has been proposed that the cyclization steps is internality mediated by anasparaginylendopeptidase, the common Cystine protease found in plants. The cyclic steps takes place at the same time while the cleavage of the Carboxy-terminal pro-peptide or inactivated form of protein called pro form from the cyclotide precursor protein through a transpeptidation reaction.

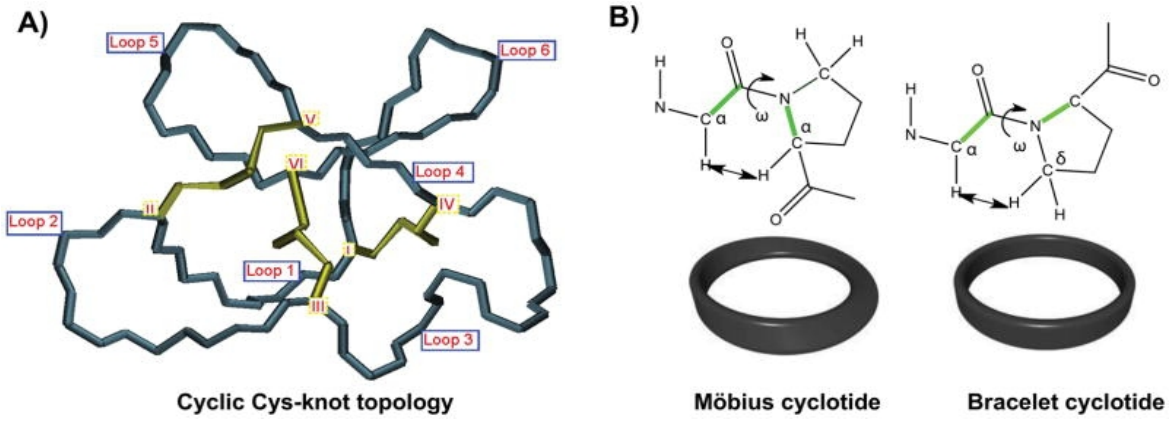


**Fig. 7.** Structural Features of cyclic cystine knot.

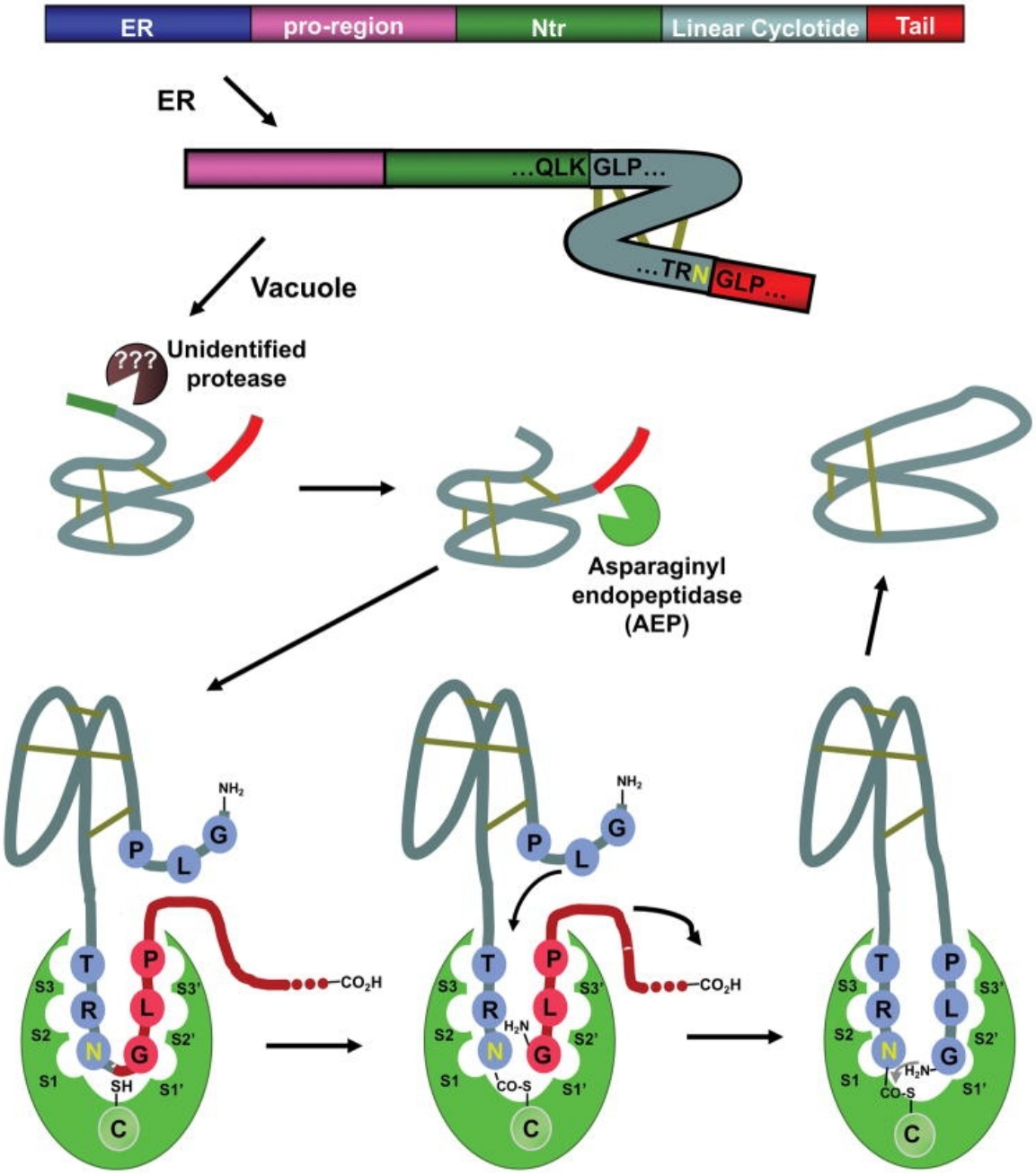


**Fig. 8.** Biosynthesis of cyclotides.

The transpeptidation reaction involves an acyl-transfer step from the acyl-AEP intermediate to the N-terminal residue of the cyclotidedo main (Craik *et al*., 2013). The active uterotonic ingredient was found to be a peptide called kalata B1 that was reported to comprise ~30 amino acids, but its sequence, structure, or cyclic nature could not be defined using the protein chemistry techniques available in the early 1970s. It was not until 1995 that the macrocyclic and knotted nature of kalata B1 was delineated ( Saether *et al*., 1995).

The upper panel shows the amino acid sequences using one-letter codes, with the conserved cysteine residues numbered using Roman numerals I–VI. The cyclic backbone is schematically illustrated by brackets around the sequences. Beta-strands are illustrated as arrows in the structure, and the six loops between conserved cysteine residues are labelled with Arabic numbers 1–6. These loops are typically hyper variable in different cyclotide structures and expose amino acid side chains that are responsible for the various activities of cyclotides (Eliasen *et al*., 2012).

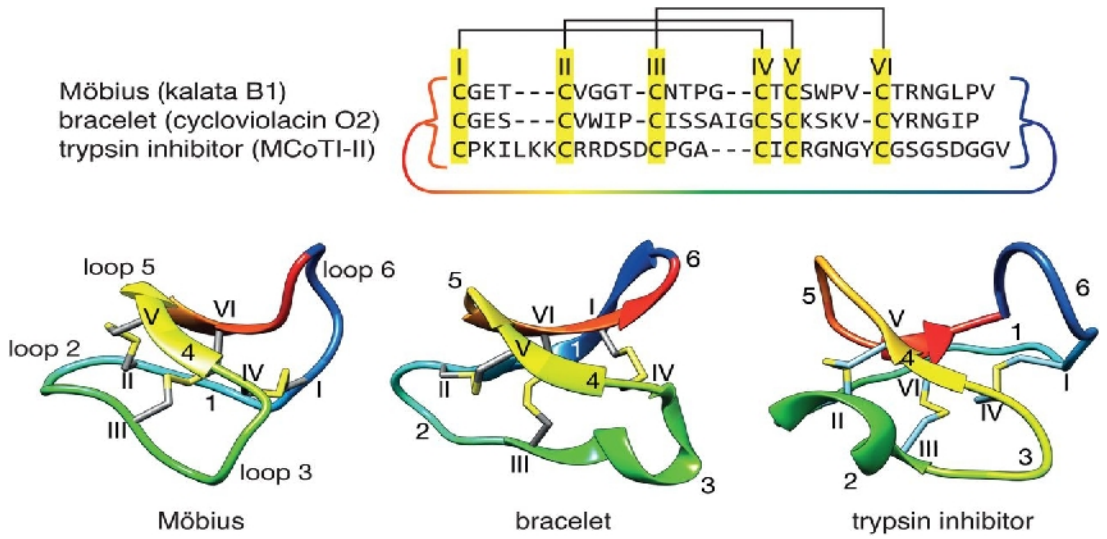


**Fig. 9**. Cyclotides from the Möbius (kalata B1), bracelet (cycloviolacin $O_2$), and trypsin inhibitor (MCoTI-II) subfamilies of cyclotides.

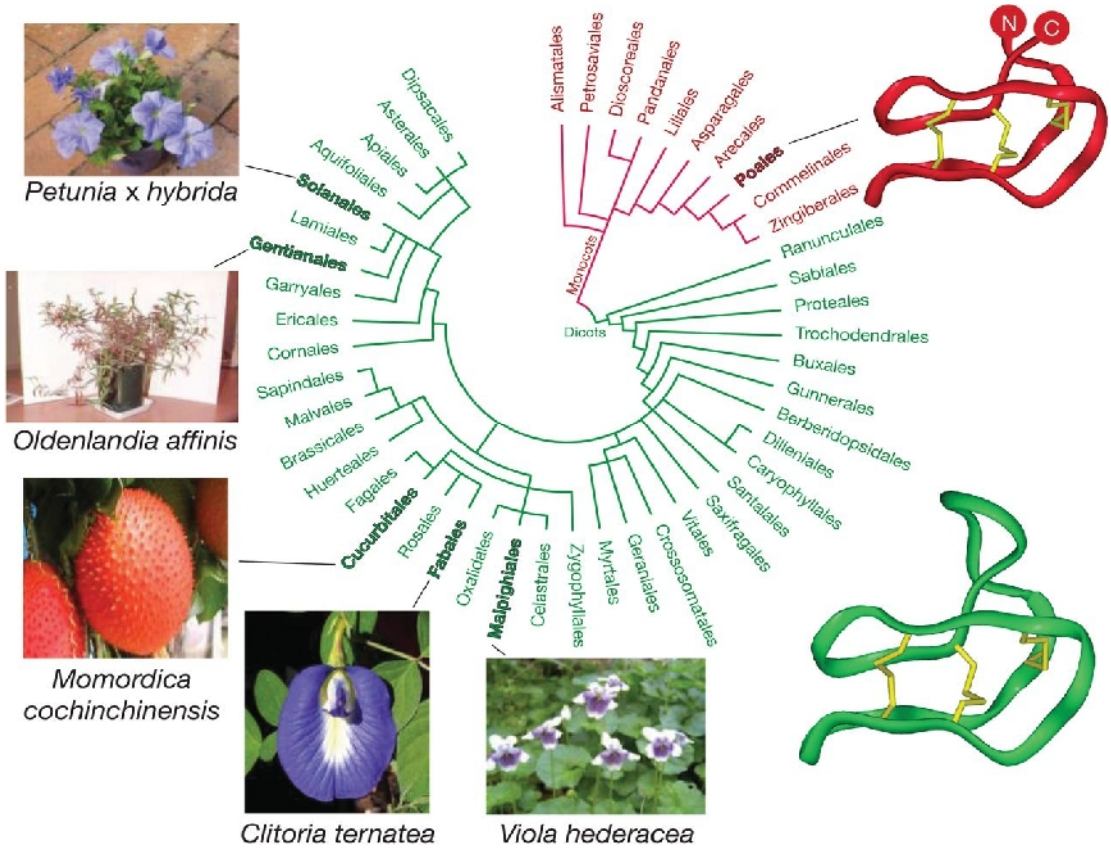


**Fig. 10.** Shows the currently known distribution of cyclotides in five major families of angiosperms plants.

Currently known distribution of cyclotides in five major families of angiosperms plants, illustrating that they are found in five major families of angiosperms, namely the Rubiaceae, Violaceae, Cucurbitaceae, Solanaceae, and Fabaceae. Cyclotides are ubiquitous in the Violaceae, having been found so far in the >35 species in this family that have been screened where relatively small numbers of plants have been screened so far. Several articles have discussed the distribution and evolution of cyclotides in plants (Craik *et al*., 2009) and this is a continually expanding topic.

Phylogenetic tree showing the distribution of known cyclotides in the orders Solanales (Solanaceae family), Gentianales (Rubiaceae family), Cucurbitales (Cucurbitaceae family), Fabales (Fabaceae family), and Malpighiales (Violaceae family). Thus far, cyclotides have been only show in dicotyledons (green), as illustrated by the green cyclotide structure. In monocots (red), linear cyclotide analogues (red structure) with the same fold (cystine knot) but lacking the head-to-tail cyclic backbone have been described. Typical cyclotide-containing representatives of each family are shown for each order (Clark *et al*., 2006).

## MECHANISM OF ACTION OF CYCLOTIDEKALATA B1 ON BIOLOGICAL MEMBRANES

The cyclotideskalata B1 (top left) and MCoTI-II (top right) are shown using space-filling surface models derived from their NMR solution structures. Kalata B1 initially binds to the membrane by interaction between the Glu7 side chain (red) and phosphatidylethanolamine phospholipid before it is internalized into the membrane using a hydrophobic surface patch (green). Although the exact mechanism is yet to be discovered, bulges form in the membrane and intracellular components leak from pores. In contrast, the arginine-rich (blue) basic surface of MCoTI-II does not bind membranes.

### *Pharmaceutical Applications*

A wide range of pharmaceutically relevant activities have been found in screening studies of natural cyclotides and numerous synthetic 'designer' cyclotides have also been made for applications in drug design. In terms of pharmaceutical applications of cyclotides, there are three broad classes of molecules that have been considered: (i) natural cyclotides; (ii) point-mutated cyclotides; and (iii) 'grafted' cyclotides. Because of the great interest from the pharmaceutical industry in oral activity, we focus mainly on applications where cyclotides have shown oral activity, as foreshadowed in the original African medicinal tea applications for uterotonic activity (Craik *et al*., 2015).

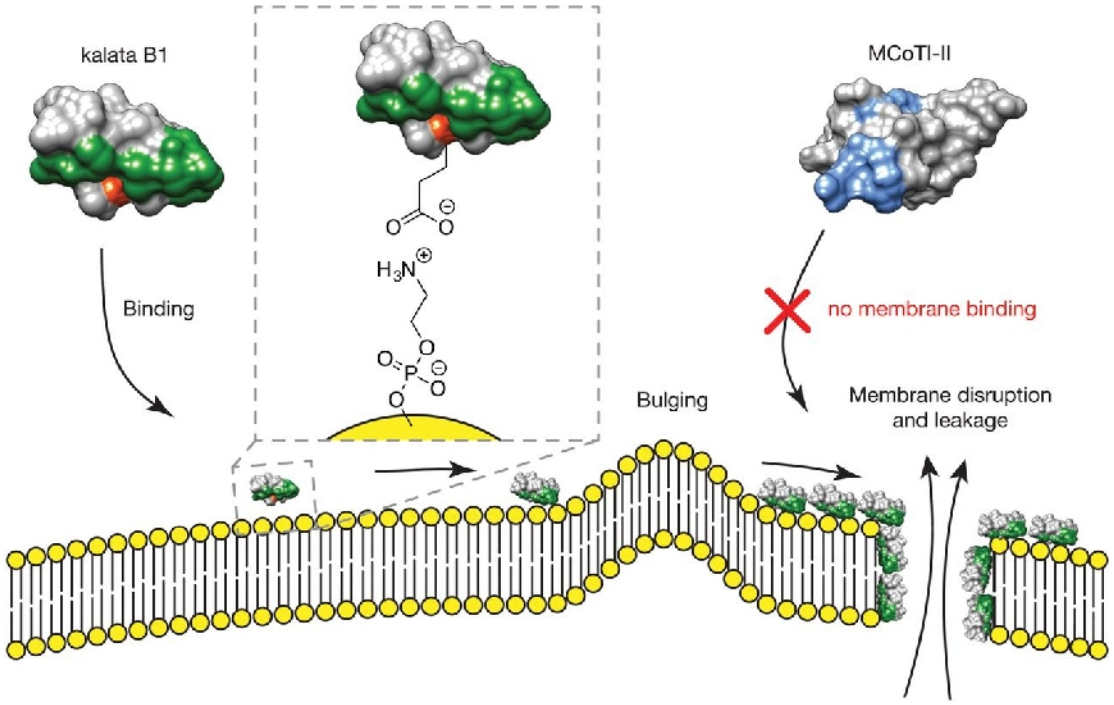


**Fig. 11.** The proposed mode of action of cyclotidekalata B1 on biological membranes.

### *Natural Cyclotides*

Early reports of the pharmaceutical applications of natural cyclotides mainly concerned their anti-HIV activity, reported in a series of papers in the 1990s and 2000s. Later structure–activity studies examined the effect of opening the circular backbone of kalata B1 on the anti-HIV activity (Daly *et al.* , 2004 ). Interestingly, despite the acyclic analogues having basically similar structures to the natural cyclic derivatives, they were devoid of anti-HIV activity, suggesting an important role for the cyclic backbone. In a more recent study, Gruber and colleagues identified the molecular targets of labour-accelerating cyclotidekalata B7 and engineered derivatives: the G protein-coupled oxytocin and vasopressin V 1a receptors ( Daly *et al.* , 2004).

*Point Mutated Cyclotides*

Recently Gruber and co-workers reported a point-mutated cyclotide, (T20K) kalata B1, that has oral activity in a mouse model of multiple sclerosis ( Thell *et al*., 2016). This work followed on from earlier studies that had established a role for cyclotides in the immune system, more specifically in immunosuppressant action. Initially, it was shown that the prototypic cyclotidekalata B1 has inhibitory properties on primary activated human lymphocytes which was not caused by cytotoxicity. A later study identified an interleukin-2-dependent mechanism as a cause of immunomodulation and, subsequently, similar effects were attributed to cyclotides isolated from Viola tricolor .The potential of grafted cyclotides in the context of multiple sclerosis has also been demonstrated using intravenous delivery. The immunosuppressive properties of cyclotides and other small peptides have been recently reviewed (Thell *et al*., 2014).

***Grafted Cyclotides***

In the context of cyclotides, the exceptional chemical and thermodynamic stability of the scaffold (analogous to the root stock) can be combined with beneficial properties of small peptides (analogous to the scion). To achieve grafting, peptide sequences of varying lengths are typically inserted into loops 2, 3, 5, or 6 of the cyclotide scaffold. Loops 1 and 4 are important for the cystine knot fold and hence typically are not well suited for peptide engineering.

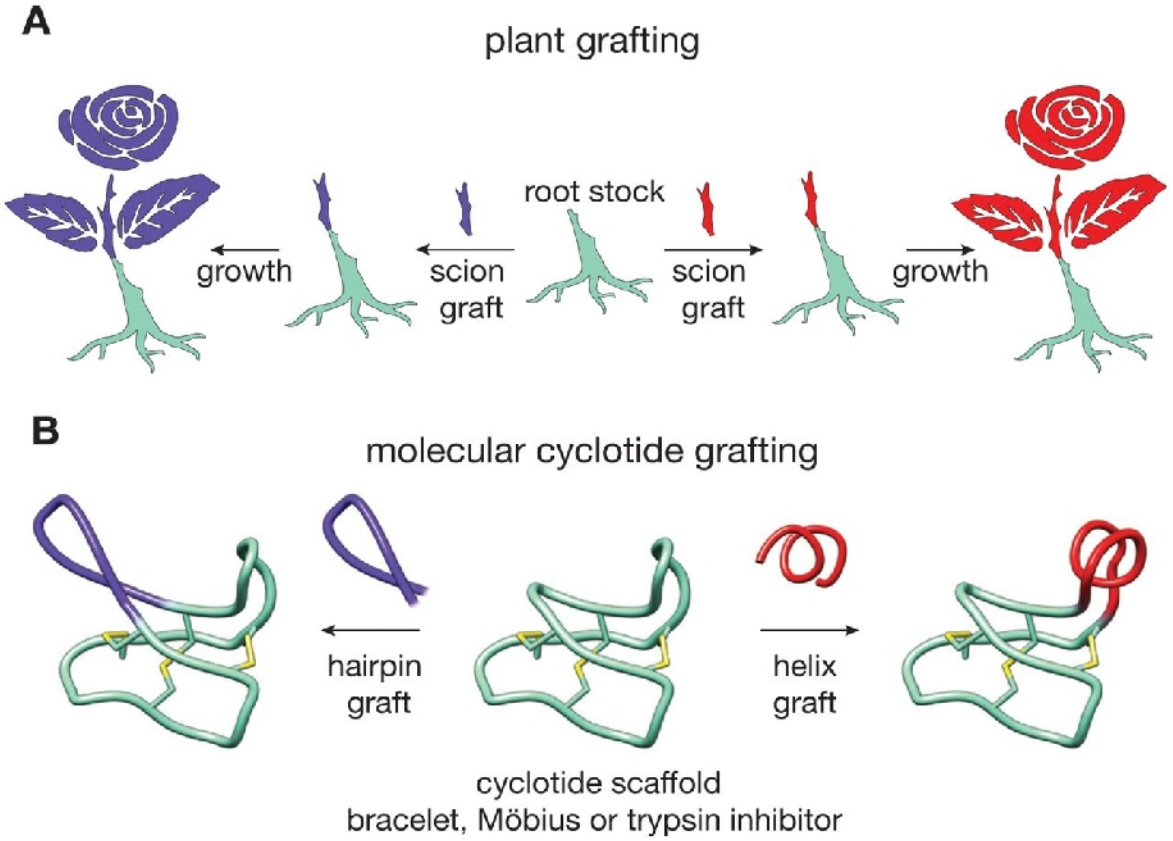


**Fig. 12.** Role of cyclotides in Grafting.

The Fig. 12 showed that upper panel (A) shows grafting between two plants of the same species. A scion with given traits and properties is permanently attached to a rootstock with different traits and properties. The resulting plant combines the traits of the individuals that gave rise to it. The lower panel (B) shows the concept of grafting applied to the cyclotide scaffold. Insertion of peptide epitopes into cyclotide loops gives rise to novel molecules, which combine the stability of the cyclotide scaffold with the desired activity of the grafted epitope (Kimura *et al*., 2012).

## CONCLUSION

The achievement goals of sequencing technology such as operation of micro fluidic PCR basis with improvement in traditional techniques with the intention to offer a quicker and less exclusive option at huge scale multi locus on plant DNA barcoding are diagnostic tool of current state of discoveries in genomics. Studies on clinical activities of cyclotides needed more experimentation and research on clinical projects. Various cyclotides showed that they are orally active except small literature about their oral activity.

It is estimated that more research needed on biopharmaceutical activities of these cyclotides determination be accessible in the laboratory examination in coming years.

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