Innovations

Genetic Integrity of Invitro Conserved Medicinal Plants

[1]Himani Choudhary; [2]Shailika Sharma; [3]Papiya Mukherjee
[1]Department of Biosciences (UIBT), Chandigarh University, Punjab, India
[2,3]Department of Botany, Panjab University, Chandigarh, India

Corresponding Author: Shailika Sharma

Abstract: *The conservation of medicinal plants is essential for ensuring the continues availability of bioactive compounds used in pharmaceuticals and traditional medicine. Ex situ conservation techniques like tissue culture and cryopreservation have become crucial tools for maintaining these valuable plants as over-harvesting, habitat loss, and climate change threaten many therapeutic plant species. Ensuring the genetic integrity of plants is must in in vitro conservation because extended culture times can result in somaclonal variations or unintentional genetic variations that could compromise the therapeutic qualities of plants. This paper reviewed the molecular and phenotypic stability of medicinal plants that have been conserved in vitro, determining whether the method results in genetic drift, somaclonal variation, or other changes to the genetic composition of the plant. Molecular tools like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Inter-Simple Sequence Repeats (ISSR) markers have been used in numerous studies to show that while in-vitro methods can maintain most of the genetic integrity of plant, some variations may still happen, especially in long-term cultures or under stressful surroundings. The main aim of this review is to emphasize how crucial it is to preserve genetic integrity in medicinal plants that have been conserved in order to assure their continuous availability for use in pharmacological and therapeutic applications.Bridging existing knowledge gaps and refining these molecular methods are essential for conserving the valuable genetic resources, contributing to sustainable agriculture and biodiversity conservation.*

Key Words: *Genetic Stability, Molecular markers, Somaclonal variations and Medicinal plants*

Introduction

Medicinal plants contain bioactive substances that can be used therapeutically to treat, prevent, or manage a wide range of illnesses and ailments. Bioactive chemicals found in medicinal plants are abundant and can be utilized to treat a wide range of illnesses. Traditional medicine and medicinal plants have a major

role in the health care systems of the majority of underdeveloped countries. Many significant secondary metabolites employed in cosmetically, nutraceuticals, and medicines are mostly sourced from plants (Bishwas et al. 2022). Natural plant-based products are widely used in traditional and folklore medicinal systems as powerful therapeutic agents in herbal mixtures (Dey et al. 2017). The World Health Organization estimates that 80% of people worldwide still receive their primary and basic medical care from traditional medicine on a daily basis (Bodeker and Ong 2005). The primary causes of the indiscriminate exploitation of these species and the swift decline in their wild populations are plant-based pharmaceuticals, nutraceuticals, and industrial trials (De Silva and Senarath 2009; Lal and Singh 2010; Nazir et al. 2020). Factors contributing to the depletion of some species include limited seed viability, a lack of vegetative replication techniques, low germination rates and seasonal variations (Long et al. 2015).

The two basic ways to preserve genetic diversity are ex situ and in situ conservation. In situ conservation can lead to conflicts between humans and wildlife, especially when wildlife encroaches on agricultural land or human settlements. Ex situ strategy used to preserve and safeguard biodiversity, especially when conservation inin situ, or inside the species natural environments, is impractical or insufficient. Short-term advancements in genetics in ex situ conserved can be maximized, but genetic diversity will be diminished over generations of selective breeding of such genetically limited populations. This limits the ability of populations to genetically adapt to future demands (Yang et al. 1992).A different approach for the conservation of those priceless and endangered medicinal plants is the tissue culture technique due to a lack of vegetative propagation techniques, low seed viability, and a low germination rate (Rout et al.2008; Bantawa et al.2011; Swarna and Ravindhran 2012). Somaclonal variations greatly hinder the utilization of tissue culture, despite the fact that it is recognized as one of the most crucial areas of biotechnology for enormous scale multiplication and conservation (Larkins and Scowcroft 1981). Therefore, the validity of this in vitro conservation technique can only be established if the genetic stability of plant material is unaltered. For that cryopreservation i.e. long-term conservation of plant cell, tissue and organ at -196°C following exposure to liquid nitrogen. In this extremely low temperature, all the metabolic and biochemical activities of cells and tissues are stopped, they may be stored for extended periods of time without any serious deterioration (Parihar et al. 2023).

In addition to conservation, the secondary goal of in vitro conservation and cryopreservation techniques is to protect medicinal plant material's genetic integrity (Pandian et al. 2024).In vitro culture produces clonedcells and tissues by rapid mitotic division with the exact same genetic makeup as the parent plant (Cardoso et al. 2019). Maintaining plant genetic homogeneity is crucial because variation in genetics can lead to biochemical heterogeneity, which reduces the efficacy of medications, and cross-pollination stops seed-derived plant progenies from creating a homogenous population. (Vieira et al. 2001).Reliable in vitro

cultivation techniques can meet the growing demand for innovative products generated from plants have therapeutic and commercial value in the marketplace. Despite the many benefits of in vitro propagation, there are instances where the micro-propagated plants develop genetic instability due to the plant tissues constant exposure to various stressors during in vitro growth (Krishna et al. 2016). Somaclonal variation may arise from a alter in the genetic makeup of the in vitro grown clones including excessive growth hormone concentration, prolonged culture period, unequal transplant regeneration and unfavourable culture circumstances. For medicinal plants, this is especially crucial because even minor genetic alterations might modify the biochemical makeup and therapeutic effectiveness of the plant (Canter et al. 2005). Thus, it is necessary to evaluate the genetic fidelity of the regenerated plants that were grown via plant tissue culture after some period. By utilizing various DNA markers to evaluate the regenerants' clonal fidelity, genetically stable plants can be generated. Molecular markers are tools used in genetics and molecular biology to identify specific sequences in DNA, RNA, or proteins. Several micro-propagated plants have been successfully genetically homogenized using molecular markers such as Restricted Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) (Gupta et al. 2013; Thakur et al. 2016; Tikendra et al. 2019; Dey et al. 2020a; b).

Molecular markers have established themselves as a standard tool in a wide range of plant genetic applications, such as genetic diversity assessment estimation, genetic identity determination, linkage map development, trait variation marker detection, and desirable individual selection in breeding programs. Most of the same applications have been documented in tissue culture, and the use of markers to evaluate the degree of somaclonal variation seen in the in vitro grown culture. In the regenerated plants, the somaclonal alteration could lead to molecular, biochemical, cytological, morphological, and cytological modifications (Rani et al. 2000). Since there are chances that the regenerated plants will lose their integrity, clonal fidelity analysis of in vitrogrown plants is required to produce genetically stable, superior plants (Biswas and Kumar 2023). A new chapter of in vitro research has been made possible by the development of DNA recombinant technology, which uses several DNA-based markers to detect clonal fidelity.

This paper focuses on the application of various markers to determine the genetic fidelity of medicinally significant plants that are produced in vitro which show sometimes somaclonal variations. To make sure that the conserved plants stay genetically identical to the original wild-type plants, these markers aid in the detection of any genetic changes, mutations, or genetic drift that may occur throughout the tissue culture procedure.

Somaclonal Variation

The main benefit of clonal replication by tissue culture is the production of genetically homogeneous individual plants. However, spontaneous variability may result from temporary or permanent changes in the genetic components of plant cells and tissues during in vitro plant multiplication. Temporary, reversible, and non-heritable alterations are caused by epigenetic and physiological influences, whereas permanent changes reflect heritable variation that already existed in the source plants (Larkins and Scowcroft 1981).The genetic variation seen in plants that results from tissue culture methods is known as somaclonal variation. A noteworthy example would be the banana, where the percentage of Cavendish cultivars that had off-types from tissue-cultured plantlets varied from 6% to 38% (Sahijram et al. 2003), though it could be as high as 90 % (Smith 1988). Somaclonal variation pertains to the variations that emerge in plant tissues that have undergone regeneration and their next progeny (Brar et al. 1998). Somaclonal variability is a major issue when it comes to the establishment of commercial plantations and the standardization of the micro propagation procedure for economically important species, as it is essential to rigorously maintain the characteristics of the individuals chosen for the in vitro culture start in these circumstances. Simultaneously, somaclonal variability provides breeders with a source of genetic variation to help create new plants with improved economic qualities (Mehta et al. 2000). Plants with these variants may have beneficial characteristics, such as increased yield, stress tolerance, or disease resistance, that were absent from the original genotype. It is possible to introduce novel features into crops through somaclonal variants, which could result in new varieties with improved resistance or performance.

Somaclonal variation can have either an epigenetic or genetic origin and can manifest at the phenotypic level (Duta et al. 2023). The genetic changes could be at the DNA sequence level, where they generally include point mutations, or at the chromosomal level, where they primarily involve changes in chromosomal number (polyploidy or aneuploidy) or organization (insertions, duplications, deletions, or translocations).The most frequent epigenetic modifications are gene amplifications and modifications to the normal methylation patterns of DNA and histones (Bhatia et al. 2015). Consequently, it is an essential to use methods for evaluating somaclonal variation to determine which true-to-type replicants were produced after in vitro propagated material (Modi et al. 2020). The scientific community concurs and suggests evaluating the phenotypic, cytological, phytochemical, and genetic characterstics of the in vitrogrown regenerated plants using a variety of methodologies (Chatterjee et al. 2020).

Assessment of Genetic Fidelity

High levels of genetic homogeneity among the in vitro grown plants are necessary for clonal propagation for the survival of elite genotypes identified for their superior features. In vitro propagation is linked to both genetic and

epigenetic changes, which might result in phenotypic outcomes. These changes are referred to as somaclonal variation. As a result, ensuring the genetic homogeneity of plants grown in vitro from an early age is crucial (Guo et al. 2007).The identification and characterisation of somaclonal variants, which are mostly based on physical differences, cytogenetical analysis can be used to determine the amount and kind of chromosomal variation can be done using a wide range of assays and instruments such as biochemical and molecular DNA markers.

Morphological markers

Visual observations and comparisons of in vitro grown plants can be made of morphological traits like plant height, leaf shape, and leaf colour patterns. Plants that clearly differed morphologically from their parents can be marked, compared and closely observed. Petiole diameter at the midsection of the petiole, and leaf thickness, the leaf lamina between the main veins using an electronic digital calliper have been used as measurement method for morphological change (Yu et al. 2022). In some reports, the foliar micro-morphological features under in vitro and in vivo conditions were compared with the mother plant and showed morphologically stable plantlets development through gradual acclimatization (Sandhya et al. 2021).

Cytological Markers

The structural properties of the chromosomes can be recognised in the karyotypes and chromosomal bands which are defined as cytological markers. The chromosomal bands created by using different strains, such as the G banding, are known as cytological markers and are associated with changes in the number, size, and shape of chromosomes (Mohiiuddin et al. 2023) Using the root tip squash method for counting chromosomes at mitotic metaphase, the relative nuclear DNA content from the flow cytometry study can be calibrated. Cyto-molecular techniques like flow cytometry were employed to study the genetic homogeneity of regenerated medicinal plant Carthamus tinctorius (Mallon et al. 2010).

Plants have been classified over time using morphological traits, chemical content, and cytological data. These methods have considerable limitations since environmental and developmental factors may have an impact. Over the past few decades, new biochemical and DNA-based markers have been made possible by developments in molecular biology and biochemistry, which have assisted in overcoming these limitations. These markers, which are more common than phenotypic characteristics and typically unaffected by environmental factors, provide a comprehensive image of the underlying variation in the organism's DNA (Alizadeh et al. 2015).

Biochemical markers

The first biochemical marker technique was isozyme analysis, which was rapid, somewhat inexpensive, and simpler because it did not require DNA extraction. Isozyme electrophoresis is one of the protein-based markers (direct products of genes) that has been identified as a potentially useful method for identifying any genetic variation among plants grown in vitro (Adhikari er at. 2017). Isomers, also known as isoenzymes, are protein indicators that catalyse the same chemical process while having different amino acid sequences.

Significant changes in biochemical parameters were noted during somatic embryogenesis utilizing biochemical markers, in medicinally important plant Silybum marianum L. which could serve as indicators for tracking the various processes occurring during somatic embryogenesis and influenced by the explants type, basal media and PGRs (Abbasi et al. 2016). Retention of biochemical stability was demonstrated by Ahuja et al. (2002) using HPLC to analyze the diosgenin content of Dioscorea floribunda cryopreserved plants. Additionally, the assessment of bacoside a in cryopreserved plants revealed a retention of biochemical stability in Bacopa monnieri plants (Sharma et al. 2017). Similarly, the biosynthetic stability of the compound valepotriates in regenerated plants of Valeriana jatamansiwas estimated by Sharma et al. (2021).

But the main drawback of the isozyme marker is that it produces less genetic variation and is rather rare. These restrictions were removed by DNA markers, which could easily track inheritance and identify differences in nucleotide sequences between individuals (Malgaonkar et al. 2020).

Molecular Markers

Molecular markers have several applications in genetic resource management and agricultural development. Their applications include: i) germplasm characterization; ii) genetic diversity assessment; iii) genetic relationship validation; iv) marker-assisted selection (MAS); v) clonal fidelity testing and varietal identification (Anand 2000).

Since DNA recombinant technology has advanced, several DNA-based markers have been created which are more effective and dependable than isozymes in identifying the genetic fidelity of numerous significant, uncommon, and remarkable plants (Alizadeh et al. 2015). Table1 showing different molecular markers (RAPD & ISSR) used to check the mono morphism of plants such as Acorus calamus (Meetei et al. 2024) and Centella asiatica (Mohapatra et al. 2021).

There are basically two types of DNA based marker, hybridization-based DNA marker and PCR based DNA marker. The molecular marker known as restriction fragment length polymorphism (RFLP) is based on hybridization. Furthermore, PCR-based molecular markers include Inter-Simple Sequence Repeat (ISSR), Micro-satellites or Short Tandem Repeats (STRs), Single Nucleotide Polymorphism (SNPs), Simple Sequence Repeats (SSRs), Amplification Length Polymorphism (ALP), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment

Length Polymorphism (AFLP), Sequence Tagged Sites (STS), Number of Tendom Repeats (VNTRs), Single Polymorphic Amplification Test (SPLAT), Single Strand Conformation Polymorphism (SSCP), and Random Amplified Polymorphic DNAs (RAPD), Start Codon Targeted (SCoT), Intron-exon splice junction (ISJ) (Shahid et al. 2012).

Restriction Fragment Length Polymorphism (RFLP)

It is a hybridization-based method that was one of the first methods for DNA analysis in several fields, which include forensic science. This method is predicated on the idea that restriction enzyme digestion produces DNA fragments of varying sizes. Due to point mutation, insertion/deletion, translocation, inversion, and duplication, the genetic makeup of individuals within a single species will produce distinct segments of DNA after restriction digestion (Yang et al. 2013). In RFLP process, using a restriction enzyme, genomic DNA is sliced into pieces of varying sizes. Six base pair cutter restricting enzymes are commonly used for RFLP research because they are more readily available, less expensive, and yield product segments (200–20,000 bp) that are simple to separate on agarose gels. Separated DNA fragments are transferred to the nitrocellulose membrane using the Southern blot technique. By hybridizing with a complementary radioactively tagged probe, fragments of interest are found, and following autoradiography, the particular banding pattern is visible (Blears et al. 1998). In addition to having excellent reproducibility, codominant inheritance, and simple data transferability between labs, RFLP is easy to evaluate due to its large size differences. Additionally,it also gives locus-specific markers (Grover and Sharma 2016).

The following are the technique's drawbacks: it is time-consuming, includes the laborious Southern blotting process, calls for expensive radioactive instruments, high-quality DNA, and requires previous sequence knowledge in order to create a radiolabelled probe (Varshney et al. 2000).

Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique combines the flexibility of PCR-based technology with the effectiveness of RFLP by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh 1998). The amplification of particular fragments and subsequent restriction digestion of an organism's entire genomic DNA form the basis of AFLP. AFLP detects polymorphism because of insertions, duplications, or deletions in the amplification fragments, alterations at the restriction region itself, and changes in the flanking sequences of the restriction sites themselves (Paun and Schonswetter 2012). Using a mix of rare cutter (EcoRI or PstI) and frequent cutter (MseI or TaqI) restriction enzymes, genomic DNA is restriction digested for AFLP analysis. Following agarose gel electrophoresis, the separated amplified AFLP fragments are seen by autoradiography or AgNO3 staining, correspondingly (Mishra et al. 2014). With AFLP, marker segments for any

organism can be quickly generated without the need for previous DNA sequencing (Kumar et al. 2009).

Random Amplified Polymorphic DNA (RAPD)

RAPD was developed by Welsh and McClelland (1990) along with Williams et al. (1990). It does not require any previous DNA sequencing because it is based on the PCR assay. With this method, many complementary genomic DNA sections are amplified at random using a single arbitrary primer, resulting in a large number of distinct products made from DNA that can be separated on an agarose gel. A single species of primer, ten nucleotide bases long, is annealed to the genomic DNA at two different sites on the opposing strands of the DNA template. Both forward and reverse primer functions are provided by this primer. When the distances between the oppositional primer sites are within the amplifiable ranges, these primers bind to complementary sequences present across the genome (Idrees et al. 2014). The genome has several complementary binding sites for a single primer, which results in an amplification product consisting of multiple distinct DNA segments of varying lengths. The polymorphism generated by RAPD markers is brought by changes in the chromosome, such as insertions or deletions, or changes in the nucleotide sequence located at the primer binding location (Nadeem et al. 2018). The PCR buffer's concentration of magnesium chloride, the primer-to-template ratio, the temperature at which it is annealed, the brand and source of the Taq DNA polymerase, the quantity and quality of the DNA template, and the thermal cycle brand are only a few of the variables that affect the reproducibility of the RAPD markers (Wolff and Peters-van Rijn 1993). RAPD is more common and faster than AP-PCR and DAF, although having a lower resolving power. Standardizing these circumstances or figuring out a set of conditions that produces consistent findings is crucial to getting trustworthy results. This is due to the ease of use and inexpensive cost of agarose gel electrophoresis (Idrees et al. 2014).

Genetic stability of an endangered medicinal orchid Dendrobium nobile Lindl., was investigated using Randomly Amplified Polymorphic DNA (RAPD) and start codon targeted (SCoT) markers which detected 97% of genetic fidelity among the in vitroregenerants (Bhattacharyya et al. 2014). Some in vitro grown plants which showed 100% monomorphism was assessed by RAPD molecular markers such as Acorus calamus, Celastrus paniculatus, Decalepis hamiltonii, Gentiana kurroo, Nepenthes khasiana, Podophyllum hexandrum, Uraria picta (Meetei et al. 2024; Senapati et al. 2013; Saminathan et al. 2023; Kaur et al. 2009; Devi et al. 2013; Tariq et al. 2015; Ahire et al. 2011)(Table1).

Inter-Simple Sequence Repeat (ISSR)

This method involves amplifying DNA segments that are situated at an amplifiable distance between two similar microsatellite repeat sections which are oriented in opposite orientations. ISSR order typically, 16–25 bp primers are used in a single

primer PCR process to target several genomic loci and amplify various inter-SSR sequence length. Either di-or tri-nucleotide microsatellite repeats can be utilized as primers. High polymorphism ISSR markers are employed in research of genome mapping, phylogeny, genetic diversity, and gene tagging (Reddy et al. 2002). Because ISSR primers are lengthier (15–35 bp) than RAPD primers, their high annealing temperature causes them to be more stringent (Amom et al. 2018). Since anchored primers can anneal at particular locations on the template DNA to produce distinct bands, they are used more frequently than unanchored primers (Nilkanta et al. 2017; Nadeem et al. 2018). The 200–2000 bp long amplified fragments are divided using polyacrylamide or agarose gel electrophoresis. Autoradiography or AgNO3 staining are used to visualize the resulting ISSR banding pattern. Like RAPD, ISSR primers have no need for the template DNA's prior sequence knowledge. The dominant inheritance pattern is displayed by ISSR markers, which are easy to use and need less DNA to analyze (Longya et al. 2020). ISSR is rapid, easy, and very reproducible. It doesn't require radioactivity, and it doesn't require previous knowledge of the genome sequence (Bornet and Branchard 2001).

The genetic fidelity of the in vitro regenerated plants of Artemisia absinthium were estimated using the intersimple sequence repeat (ISSR) and sequence-specific amplification polymorphism (SSAP) molecular markers. In both the markers, no variation was detected in the plants regenerated from the nodal explants (100% monomorphism) while a little polymorphism (2%) was detected in the callus regenerated plants (Kour et al. 2014). Inter Simple Sequence Repeats analysis revealed a homogeneous profile of amplification for micropropagated plants use to analyse the genetic stability of the in vitro regenerated plants such as Aconitum heterophyllum, Bergenia ligulate, Centella asiatica, Lilium davidii, Pterocarpus marsupium (Belwal et al. 2016; Deeba et al. 2024; Mohapatra et al. 2021; Yang et al. 2021; Tippani et al. 2019) (Table.1).

Single nucleotide polymorphism (SNP)

A single nucleotide polymorphism (SNP) is a variation in a single nucleotide (A, T, G, or C) that occurs among members of a species SNP has emerged as the new norm for molecular markers for a variety of applications since it is the most common marker system found in plant and animal genomes. They are easily able to differentiate among homozygous and heterozygous alleles due to their binary form, or co-dominant, condition. Additionally, its efficacy is derived from the vast number of loci that may be evaluated rather than the quantity of alleles, in contrast to microsatellites (Foster et al. 2010). Most notably, SNPs may be genotyped quickly and effectively on a large number of samples because of high throughput technology (Tsuchihashi and Dracopoli 2002). SNPs in plants can be created using single-stranded pyrosequencing (Miller et al. 2003) and ESTs (Coles et al. 2005). Plant genetic diversity can be ascertained using SNP, especially in species with

low genetic diversity but not commonly used in genetic stability studies (Idrees et al. 2014).

Simple Sequence Repeats (SSRs)

Single Sequence Repeats (SSRs), often known as microsatellites, are frequently employed in plant genetics studies that employ both high-throughput and low-throughput genotyping methods. Since SSRs are co dominant, multi-allele genetic markers with characteristics that are highly informative, reproducible in experiments, and transferable among closely related species, they have been the most popular markers for plant genotyping over the past 20 years (Mason 2015). Specifically, SSRs are helpful for wild species in (i) diversity research based on genetic distance, (ii) crossing over rates and estimating gene flow, (iii) most importantly, inferring infraspecific genetic links in evolutionary investigations. However, SSRs are frequently utilized for cultivated plants in order to: (i) create linkage maps, (ii) map loci implicated in quantitative characteristics (QTL), (iii) calculate the level of genetic kinship, (iv) define cultivar DNA fingerprints, and (v) use marker-assisted selection (Jonah et al. 2011; Kalia et al. 2011). These markers are extremely useful when researching genetic mapping, population organization, and processes associated with evolution.SSR marker along with ISSR marker confirmed the genetic uniformity of in vitro raised regenerants of plant Trillium govanianum (Kumar et al. 2024) (Table.1).

Start Codon Targeted (SCoT)

Start Codon Targeted (SCoT), a novel and potent marker, was used in accordance with the brief conserved region that surrounds the ATG start codon in plant genes. As efficient polymorphic markers, SCoT markers generate more consistent and dependable bands that remarkably illustrate individual differences (Amirmoradi et al. 2012). Additionally, studies of the genetic diversity and population dynamics of many plant species have found success with this marker (Tiwari et al. 2016). To detect the genetic variation is the main goal of the SCoT marker, which can be useful for diversity research, genetic mapping, and marker-assisted selection (MAS) in crops. It is usually preferable to use several molecular markers while assessing the genetic uniformity of micro propagated plants (Muthukumar et al. 2016; Jogam et al. 2020; Rohela et al., 2019). Due to the outstanding reliability and specificity in genetic fidelity investigations, SCoT markers have been getting a lot of attention nowadays. In vitro grown plants such as Decalepis salicifolia, Malaxis acuminata, Nardostachys jatamansi, (Wasi et al. 2024; Bhattacharyya et al. 2023; Bose et al. 2016) shows 100% monomorphism when assessed by SNP marker. Therefore, finding genetic variation is the main goal of the SCoT marker, which can be useful for diversity research, genetic mapping, and marker-assisted selection (MAS) in crops.

Intron-exon splice junction (ISJ)

Semi-specific intron-exon splice junction (ISJ) markers are essential for post-transcriptional DNA processing and are predicted on sequences that are frequently present in plants (Weining and Langridge 1991). In microsatellite-based analyses, ISJ markers can offer alternative perspectives. Because these markers are dominating and the primers are partially equivalent to the exon-intron border sequences, amplification outputs may contain parts that include both coding and non-coding elements (Rafalski 1997). Because functional gene fragments may be present in the amplified sequences, ISJ markers may be considered selectively non-neutral. One of the advantages of ISJ markers is that they don't need previous knowledge about the DNA sequence of species (Sawicki and Szczecinska 2007). Genetic stability among the in vitro derived regenerants was indicated by ISJ markers, which showed 4.76% clonal variability in plant which are grown by explants which is taken from 8-9 months old green purplish capsules of Malaxis wallichii (Bose et al. 2017) shows in Table1. However,one of the advantages of ISJ markers is that they don't need previous knowledge about the DNA sequence of species (Sawicki and Szczecinska 2007).

Discussion and Conclusion

In vitro conservation is a great way to meet commercial demand while minimizing overexploitation of plant species from their native environments. In order to boost secondary metabolite production, a variety of elicitation methods, plant growth regulators, and biological and physiological traits can be combined (Narayani and Srivastava 2017). Although genetic diversity can be preserved through in vitro conservation over several generations, there is a chance that genetic drift, somaclonal variation, mutations can lead to the potentially change in the plant's genetic makeup. Their therapeutic qualities, which are especially valuable for pharmaceutical applications, may be impacted by these modifications(Bhattacharyya et al. 2018).The in vitro propagation technique, which employs a range of explants, comprising seeds, shoot tips, roots, leaves, as well as nodal and rhizome sections, is an essential tool for rapidly growing vast numbers of medicinal plants. Compared to cultivated plants from adventitious or fully established meristematic tissues, the produced plantlets derived through callus culture show poorer genetic stability. Meristem (axillary) proliferation is thought to be the most dependable technique, while the callus phase is thought to be the least dependable (Bhatia et al. 2015). For plant clonal multiplication, adventitious shoot production straight from the removed organs is superior to callus creation but less dependable than axillary bud proliferation. Explants and their origin, as well as various regeneration techniques, are responsible for the genetic diversity in tissue culture grown plants.

Conservation initiatives should include continuous genetic evaluations utilizing methods like DNA markers, RAPD (Random Amplified Polymorphic DNA), and SSR (Simple Sequence Repeats) to guarantee the genetic integrity of in vitro

conserved medicinal plants (Fig.2). Furthermore, incorporating molecular technologies like genetic transformation and cryopreservation might reduce genetic deterioration during extended storage. Researchers employed a variety of molecular marker methods for different plant species, , to evaluate clonal fidelity such as RAPD (Bhardwaj et al. 2018; Prakash et al. 2019; Sarkar and Banerjee 2020), SSR (Mehraj et al. 2019), ISSR (Kurup et al. 2018;Dey et al. 2020b, Chandrika and Rai 2009), AFLP (Singh et al. 2002), SCoT (Bhattacharyya et al. 2017; Rath et al. 2020), SRAP (Rajput and Agrawal 2020), IRAP (Shingote et al. 2019), DAMD (Konar et al. 2019), CBDP (Sharma et al. 2019a,b), IJS (Bose et al. 2016), etc. Although the most commonly utilized markers are RAPD, ISSR, and SCoT markers due to their cost-effectiveness, substantial amount of knowledge, and dependability (Fig.2). Since the findings of one marker type's variability analysis can be supported by those of the other, utilizing two marker arrays is more dependable for precise genetic evaluation than using just one. The majority of findings using various molecular markers showeda significant amount of monomorphism, suggesting that the genetic components of plants grown in vitro differed little, demonstrating that the plants were clonally identical (Devarumath et al. 2002).

In conclusion, medicinal plants can still be effectively preserved by invitro conservation; nevertheless, it is critical to constantly assess and manage any genetic differences that might affect the plants' prospective medical utility. Assessment of genetic integrity of in vitro conserved medicinal plant species could be analysed by regulatory bodies that monitor medicine integrity as well as the pharmaceutical sector to control the quality of the drugs derived from these species. Molecular markers which fill the maximum gap in solving the problem of authentication, off-course is a boom to the molecular biology for obtaining clonally stable true-to-type plantlets for sustainable commercial use. Now, it is possible to preserve the long-term genetic integrity and therapeutic potential of conserved plants with appropriate management and genetic monitoring. Assessing the genetic integrity or variability of in vitro medicinal plants is crucial to establishing effective conservation strategies in future.

Conflicts of Interest

The authors declare no conflict of interest.

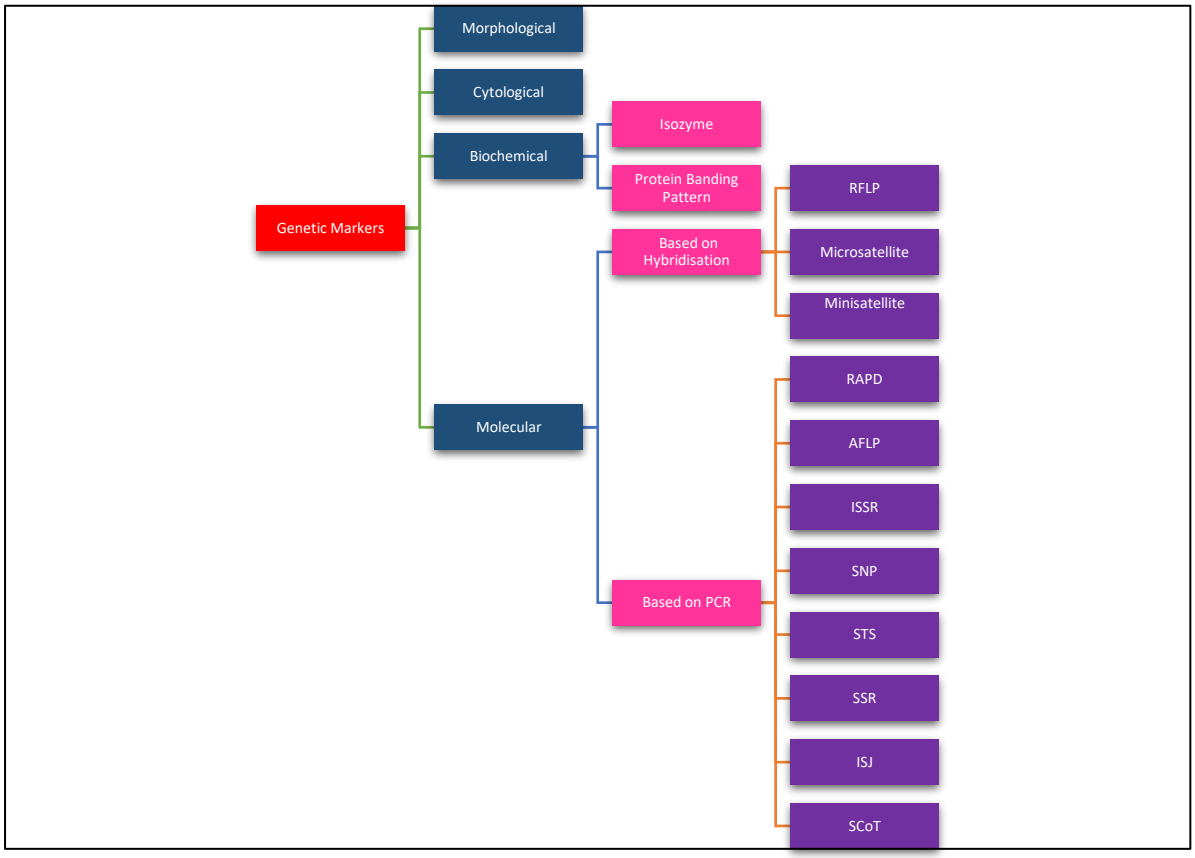
Data Availability Statement

Data supporting this study are included within the manuscript and no new data was created or analysed. Any supporting file will be made available upon request from the corresponding author.

Acknowledgement

This work was supported by Biosciences department (UIBT) of Chandigarh University and Department of Botany, Panjab University, Chandigarh.

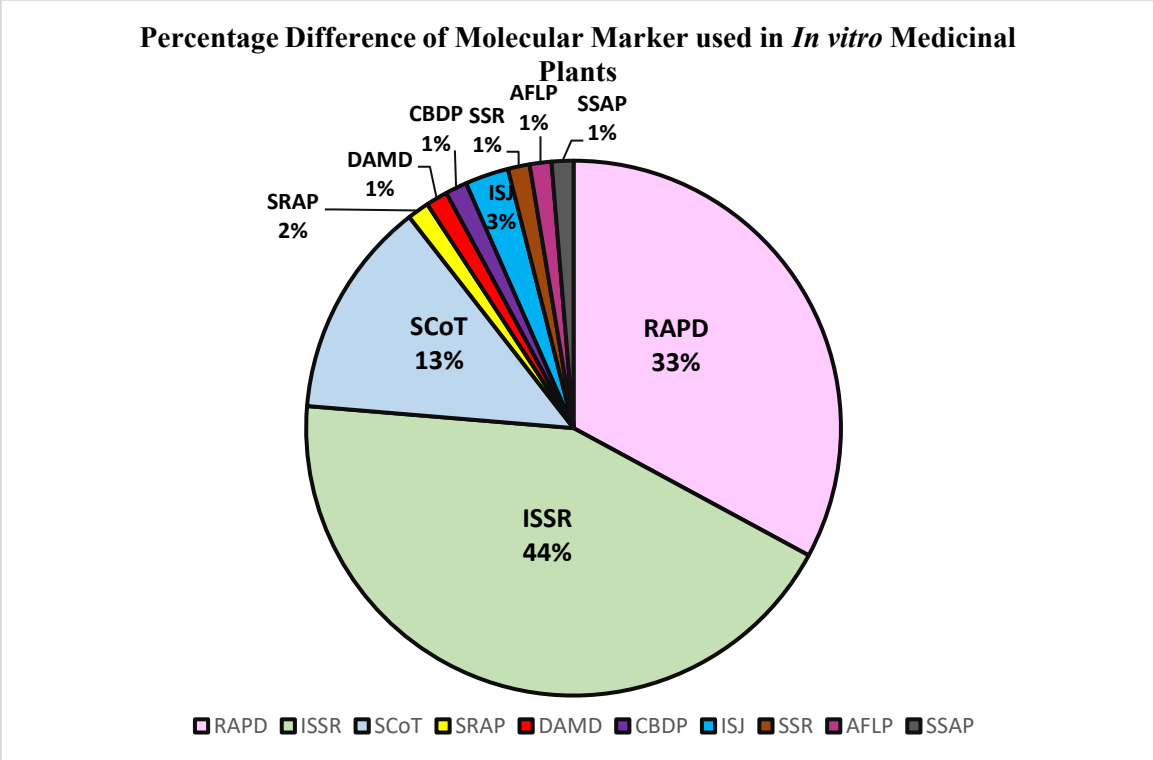
Fig. 1 Flowchart showing Different types of Genetic Markers assessed in the Genetic stability of In vitro grown medicinal plants



Genetic Markers Abbreviations:

RFLP - Restriction Fragment Length Polymorphism
RAPD - Random Amplified Polymorphic DNA
AFLP - Amplified Fragment Length Polymorphism
ISSR - Inter-Simple Sequence Repeat
SNP - Single Nucleotide Polymorphism
STS - Sequence-Tagged Site
SSR - Simple Sequence Repeat
ISJ - Intersimple Sequence Repeat (ISJ can sometimes be used interchangeably with ISSR)
SCoT - Start Codon Targeted Polymorphism
ISJ - Intron Splice Junction
SCoT - Start Codon Targeted polymorphism
DAMD - Directed Amplification of Mini satellite region DNA
SRAP - Sequence-Related Amplified Polymorphism
CBDP - CAAT Box- Derived Polymorphism

Fig 2. Pie chart showing comparative analysis of different types of molecular markers in Genetic stability of in vitro grown medicinal plants



References

- *Abbasi BH, Ali H, Yucesan B, Saeed S, Rehman K, Khan MA (2016) Evaluation of biochemical markers during somatic embryogenesis in Silybum marianum L. 3 Biotech 6:1-8.*
- *Adhikari S, Saha S, Biswas A, Rana TS, Bandyopadhyay TK, Ghosh P (2017) Application of molecular markers in plant genome analysis: a review. The Nucleus 60:283-297.*
- *Ahire ML, Ghane SG, Lokhande VH, Suprasanna P, Nikam TD (2011) Micropropagation of Uraria picta through adventitious bud regeneration and antimicrobial activity of callus. In Vitro Cell Dev Biol Plant 47:488-495.*
- *Ahmad N, Wali SA, Anis M (2008) In vitro production of true-to-type plants of Vitex negundo L. from nodal explants. J Hortic Sci Biotechnol 83(3):313-317.*
- *Ahuja S, Mandal BB, Dixit S, Srivastava PS (2002) Molecular, phenotypic and biosynthetic stability in Dioscorea floribunda plants derived from cryopreserved shoot tips. Plant Sci 163(5):971-977.*
- *Alizadeh M, Krishna H, Eftekhari M, Modareskia M, Modareskia M (2015) Assessment of clonal fidelity in micro propagated horticultural plants. J Chem Pharm Res 7(12):977-990.*
- *Amin S, Wani TA, Kaloo ZA, Singh S, John R, Majeed U, Shapoo GA (2018) Genetic stability using RAPD and ISSR markers in efficiently in vitro regenerated plants of Inula royleana DC. Meta Gene 18:100-106.*

- *Amirmoradi B, Talebi R, Karami E (2012) Comparison of genetic variation and differentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. Plant SystEvol 298(9):1679-1689.*
- *Amom T, Tikendra L, Rahaman H, Potshangbam A, Nongdam P (2018) Evaluation of genetic relationship between 15 bamboo species of north-East India based on ISSR marker analysis. Mol Biol Res Commun 7:7-15.*
- *Anand LALITHA (2000) Molecular markers and their application in horticultural crops. Biotechnology in Horticultural and Plantation Crops In: Chadha KL, Ravindran PN, Sahijram L, (eds) Malhotra Publishing House, New Delhi, India, pp-120-137.*
- *Bantawa P, Saha-Roy O, Kumar S, Ghosh R, Mondal KT (2011). In vitro regeneration of an endangered medicinal plant Picrorhiza scrophulariiflora. Biol Plant 55:169-172.*
- *Belwal NS, Kamal B, Sharma V, Gupta S, Kumar Dobriyal A, Jadon VS (2016) Production of genetically uniform plants from shoot tips of Aconitum heterophyllum Wall. a critically endangered medicinal herb. J hortic sci biotechnol 91(5):529-535.*
- *Bhardwaj AK, Singh B, Kaur K, Roshan P, Sharma A, Dolker D, Naryal A, Saxena S, Pati PK, Chaurasia OP (2018) In vitro propagation, clonal fidelity and phytochemical analysis of Rhodiola imbricate Edgew: a rare trans-Himalayan medicinal plant. Plant Cell Tissue Organ Cult 135:499-513.*
- *Bhatia S, Sharma K, Dahiya R, Bera T (2015) Modern applications of plant biotechnology in pharmaceutical sciences. Academic press.*
- *Bhattacharyya P, Kumar S, Lalthafamkimi L, Sharma R, Kumar D, Singh D, Kumar S (2023) Molecular and phytomedicinal stability of long term micropropagated Malaxis acuminata: an endangered terrestrial orchid of biopharmaceutical importance. S Afr J Bot 155:372-382.*
- *Bhattacharyya P, Kumar V, Van Staden J (2017) Assessment of genetic stability amongst micropropagated Ansellia africana, a vulnerable medicinal orchid species of Africa using SCoT markers. S Afr J Bot 108:294-302.*
- *Bhattacharyya P, Kumar V, Van Staden J (2018) In vitro encapsulation based short term storage and assessment of genetic homogeneity in regenerated Ansellia africana (leopard orchid) using gene targeted molecular markers. Plant Cell Tissue Organ Cult 133:299-310.*
- *Bhattacharyya P, Kumaria S, Diengdoh R, Tandon P (2014) Genetic stability and phytochemical analysis of the in vitro regenerated plants of Dendrobium nobileLindl., an endangered medicinal orchid. Meta gene 2:489-504.*
- *Bishwas P, Nandy S, Dey A, Tikendra L, Nongdam P (2022) Molecular markers in assessing genetic clonal fidelity for In Vitro propagated endangered medicinal plants. In: Molecular genetics and genomics tools in biodiversity conservation, Singapore: Springer Nature Singapore, pp 97-149.*

- *Biswas P, Kumar N (2023) Application of molecular markers for the assessment of genetic fidelity of in vitro raised plants: current status and future prospects. Molecular Marker Techniques 233-256.*
- *Blears MJ, De Grandis SA, Lee H, Trevors J T (1998) Amplified fragment length polymorphism (AFLP): a review of the procedure and its applications. Ind Microbiol Biotechnol 21(3):99-114.*
- *Bodeker G, Ong CK (2005) WHO global atlas of traditional, complementary and alternative medicine. WHO Vol-1.*
- *Bornet B and Branchard M (2001) Nonanchored inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. Plant Mol Biol Report 19(3):209-215.*
- *Bose B, Kumaria S, Choudhury H, Tandon P (2016) Assessment of genetic homogeneity and analysis of phytomedicinal potential in micropropagated plants of Nardostachys jatamansi, a critically endangered, medicinal plant of alpine Himalayas. Plant Cell Tissue Organ Cult 124:331-349.*
- *Bose B, Kumaria S, Choudhury H, Tandon P (2017) Insights into nuclear DNA content, hydrogen peroxide and antioxidative enzyme activities during transverse thin cell layer organogenesis and ex vitro acclimatization of Malaxis*
- *wallichii, a threatened medicinal orchid. Physiol Mol Biol Plants 23:955-968.*
- *Brar DS, Jain SM (1998) Somaclonal variation: mechanism and applications in crop improvement. In: Somaclonal variation and induced mutations in crop improvement, Dordrecht: Springer Netherlands, pp 15-37.*
- *Canter PH, Thomas H, Ernst E (2005) Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol 23(4):180-185.*
- *Cardoso JC, Oliveira MEBD, Cardoso FDC (2019) Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. Hortic Bras 37(2):124-132.*
- *Cepkova PH, Vitamvas J, Viehmannova I, Kisilova J, Cusimamani EF, Milella L (2015) Simplified in vitro propagation protocol for Tacca leontopetaloides (L.) Kuntze and assessment of genetic uniformity of regenerated plantlets. Emir J Food Agric 27(10):736-743.*
- *Chandrika M, Rai VR (2009) Genetic fidelity in micropropagated plantlets of Ochreinauclea missionis an endemic, threatened and medicinal tree using ISSR markers. Afr J Biotechnol 8:2933-2938.*
- *Chatterjee T, Ghosh B (2020) Micropropagation of medicinal plants: A review Int j economic plants 7(2):066-072.*
- *Chuengpanya R, Muangkroot A, Jenjittikul T, Thammasiri K, Umpunjun P, Viboonjun U, Chuenboonngarm N (2022) In vitro propagation and genetic fidelity assessment of Hedychium longicornutum Griff. Ex Baker, a vulnerable zingiberaceous plant of Thailand. Curr Appl Sci Technol 22(6):1-21.*

- *Coles ND, Coleman CE, Christensen SA, Jellen EN, Stevens MR, Bonifacio RA, Rojas-Beltran JA, Fairbanks DJ, Maughan PJ (2005) Development and use of an expressed sequenced tag library in quinoa (Chenopodium quinoa Willd.) for the discovery of single nucleotide polymorphisms. Plant Soc 168:439-447.*
- *De Silva MAN, Senarath WTPSK (2009) Development of a successful protocol for invitro mass propagation of Celastrus paniculatus willd. A valuable medicinal plant. Trop Agric Res 21:21-29.*
- *Deeba F, Singh P, Warsi ZI, Husain Z, Kumari M, Rahman LU (2024) Bergenia ligulata (Wall.): micropropagation, genetic fidelity, and SEM studies. In Vitro Cell Dev Biol Plant 60(1):75-84.*
- *Devarumath R, Nandy S, Rani V, Marimuthu S, Muraleedharan NRAPD, Raina S (2002) RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing Camellia sinensis (China type) and C. assamica ssp. Assamica (Assam-India type). Plant Cell Rep 21:166-173.*
- *Devi SP, Kumaria S, Rao SR, Tandon P (2013) In vitro propagation and assessment of clonal fidelity of Nepenthes khasiana Hook. f.: a medicinal insectivorous plant of India. Acta Physiol Plant 35:2813-2820.*
- *Dey A, Bhattacharya R, Mukherjee A, Pandey DK (2017) Natural products against Alzheimer's disease: Pharmaco-therapeutics and biotechnological interventions. Biotech Adv 35:178-216.*
- *Dey A, Nandy S, Nongdam P, Tikendra L, Mukherjee A, Mukherjee S, Pandey DK (2020a) Methyl jasmonate and salicylic acid elicit indole alkaloid production and modulate antioxidant defence and biocidal properties in Rauvolfia serpentina Benth ex Kurz. in vitro cultures. S Afr J Bot 135:1-17.*
- *Dey A, Nongdam P, Nandy S, Mukherjee S, Mukherjee A, Tikendra L, Hazra AK, Pandey DK (2020b) Polyamine elicited aristolochic acid production in in vitro clonally fidel Aristolochia indica L: an ISSR and RAPD markers and HPTLC based study. S Afr J Bot 140:326-335.*
- *Duta-Cornescu G, Constantin N, Pojoga DM, Nicuta D, Simon-Gruita A (2023) Somaclonal variation—Advantage or disadvantage in micro propagation of the medicinal plants. Int J Mol Sci 24(1):838.*
- *Fatima N, Ahmad N, Anis M, Ahmad I (2013) An improved in vitro encapsulation protocol, biochemical analysis and genetic integrity using DNA based molecular markers in regenerated plants of Withania somnifera L. Ind Crops Prod 50:468-477.*
- *Foster JT, Allan GJ, Chan AP, Rabinowicz PD, Ravel J, Jackson PJ, Keim P (2010) Single nucleotide polymorphisms for assessing genetic diversity in castor ean (Ricinus communis). BMC Plant Biol 10:13-23.*
- *Gantait S, Das S, Mahanta M, Banerjee M (2023) Influence of in vitro culture age on morphology, antioxidant activities, reserpine production, and genetic fidelity in Indian snakeroot (Rauvolfia serpentina (L.) Benth. ex Kurz.). S Afr J Bot 162:864-872.*

- *Goto S, Thakur RC, Ishii K (1998) Determination of genetic stability in long-term micropropagated shoots of Pinus thunbergii Parl. using RAPD markers. Plant Cell Rep 18:193-197.*
- *Grover A, Sharma PC (2016) Development and use of molecular markers: past and present. Crit Rev Biotechnol 36:290–302.*
- *Guo WL, Wu R, Zhang YF, Liu XM, Wang HY, Gong L, Liu B (2007) Tissue culture-induced locus-specific alteration in DNA methylation and its correlation with genetic variation in Codonopsis lanceolata Benth. et Hook. f. Plant Cell Rep 26:1297-1307.*
- *Gupta K, Dey A, Gupta B (2013) Plant polyamines in abiotic stress responses. Acta Physiol Plant 35:2015–2036.*
- *Gurme ST, Jadhav PP, Pawar KD, Bapat VA, Jadhav JP (2018) Somatic embryogenesis and evaluation of genetic fidelity in Amorphophallus paeoniifolius (Dennst.) Nicolson. J Crop Improv 32(6):801-811.*
- *Idrees MUHAMMAD, Irshad MUHAMMAD (2014) Molecular markers in plants for analysis of genetic diversity: a review. Eur J Acad Res 2(1):1513-1540.*
- *Jiang GL (2013) Molecular markers and marker-assisted breeding in plants. In: Andersen SB (ed) Plant breeding from laboratories to fields 3:45-83.*
- *Jogam P, Sandhya D, Shekhawat MS, Alok A, Manokari M., AbbaganiS, Allini VR (2020) Genetic stability analysis using DNA barcoding and molecular markers and foliar micro-morphological analysis of in vitro regenerated and in vivo grown plants of Artemisia vulgaris L. Ind Crop and Prod 151(11)24:76.*
- *Jonah P, Bello L, Lucky O, Midau A, Moruppa S (2011) Review: the importance of molecular markers in plant breeding programmes. GJSFR 11(5):5-12.*
- *Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK (2011) Microsatellite markers: an overview of the recent progress in plants. Euphytica 177:309-334.*
- *Kapoor B, Kanwar K, Sharma P, Sharma R, Thakur K (2019) In-vitro propagation and genetic stability analysis of Swertia chirayita: an endangered high-value medicinal plant of Himalayas. Med Plants Int J Phytomed Relat Ind 11(3):328-337.*
- *Karwasra RK, Nazir R, Pandey DK (2024) Meta-topolin mediated improved micropropagation, phytochemical profiling, and assessment of genetic fidelity in Chlorophytum borivilianum Sant. et Fernand., an endangered medicinal herb. Ind Crops Prod 214:118463.*
- *Kaur R, Panwar N, Saxena B, Raina R, Bharadwaj S V (2009) Genetic stability in long-term micropropagated plants of Gentiana kurroo an endangered medicinal plant. J New Seeds 10(4):236-244.*
- *Konar S, Adhikari S, Karmakar J, Ray A, Bandyopadhyay TK (2019) Evaluation of subculture ages on organogenic response from root callus and SPAR based genetic fidelity assessment in the regenerants of Hibiscus sabdariffa L. Ind Crop Prod 135:321-329.*
- *Koppula T, Sandhya D, Rohela GK, Kommidi S, Mohammed M (2023) Micropropagation and assessment of genetic homogeneity of regenerants by*

ISSR and SCoT markers in Solena amplexicaulis (Lam.) Gandhi—a threatened medicinal cucurbit. In Vitro Cell Dev Biol Plant 59(6):724-733.

- *Kour B, Kour G, Kaul S, Dhar MK (2014) In vitro mass multiplication and assessment of genetic stability of in vitro raised Artemisia absinthium L. plants using ISSR and SSAP molecular markers. Adv Bot 1-7.*
- *Krishna H, Alizadeh M, Singh D, Singh U, Chauhan N, Eftekhari M, Sadh RK (2016) Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech 6:1-18.*
- *Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK (2009) Potential of molecular markers in plant biotechnology. Plant omics 2(4):141-162.*
- *Kumar V, Garima Sharma R, Thakur A, Sharma P, Sharma YP, Sharma S (2024) Conserving industrially important endangered medicinal herb Trillium govanianum (Himalayan Trillium) through biotechnological based interventions. Plant Cell, Tissue Organ Cult 159(1):3.*
- *Kurup SS, Purayil FT, Alkhaili MMS, Tawfik NH, Cheruth AJ, Kabshawi M, Subramaniam S (2018) Thidiazuron (TDZ) induced organogenesis and clonal fidelity studies in Haloxylonpersicum (Bunge ex Boiss& Buhse): an endangered desert tree species. Physiol Mol Biol Plants 24:683–692.*
- *Lal D, Singh N (2010) Mass multiplication of Celastrus paniculatus Willd: an important medicinal plant under in vitro conditions via nodal segments. Int J Biodivers Conserv 2:140–145.*
- *Largia MJV, Shilpha J, Pothiraj G, Ramesh M (2015) Analysis of nuclear DNA content, genetic stability, Bacoside A quantity and antioxidant potential of long term in vitro grown germplasm lines of Bacopa monnieri (L.). Plant Cell, Tissue Organ Cult 120:399-406.*
- *Larkins P, Scowcroft WR (1981) Somaclonal variation, a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197-214.*
- *Long RL, Gorecki MJ, Renton M, Scott JK, Colville L, Goggin DE, Finch-Savage WE (2015) The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. Biological Reviews 90(1):31-59.*
- *Longya A, Talumphai S, Jantasuriyarat C (2020) Morphological characterization and genetic diversity of rice blast fungus, Pyricularia oryzae, from Thailand using ISSR and SRAP markers. J Fungi 6:38.*
- *Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associates 1:535-557.*
- *Malgaonkar M, Shirolkar A, Murthy SN, Mangal AK, Pawar SD (2020) DNA based molecular markers: a tool for differentiation of ayurvedic raw drugs and their adulterants. Pharmacogn Rev 14:72–81.*
- *Mallon R, Rodriguez-Oubina J, Gonzalez ML (2010) In vitro propagation of the endangered plant Centaurea ultreiae: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis. Plant Cell Tissue Organ Cult 101:31-39.*

- *Mason AS (2015) SSR Genotyping. In: Batley J (ed) Plant Genotyping. Springer, New York, pp 77-89.*
- *Meetei NT, Purushottam PS, Kishor B (2024) Evaluation of genetic fidelity of in vitro regenerated Sweet flag (Acorus calamus L.) using molecular markers. Indian J Exp Biol 62 (02):133-139.*
- *Mehraj U, Panwar S, Kanwar PS, Namita RP, Amolkumar US, Mallick N, Kumar S (2019) Assessment of clonal fidelity of doubled haploid line of marigold (Tagetes erecta) using microsatellite markers. Indian J Agric Sci 89:1162-1166.*
- *Mehta YR, Angra DC (2000) Somaclonal variation for disease resistance in wheat and production of dihaploids through wheat × maize hybrids. Genet Mol Biol 23:617-622.*
- *Miller PT, Henry R (2003) Single-nucleotide polymorphism detection in plants using a single stranded pyrosequencing protocol with a universal biotinylated primer. Anal Biochem 317:166-170.*
- *Mishra KK, Fougat RS, Ballani A, Vinita T, Yachana J, Madhumati B (2014) Potential and application of molecular markers techniques for plant genome analysis. Int J Pure Appl Biosci 2:169-188.*
- *Mitra M, Das A, Ghorbanpour M, Malik S, Mandal N (2024) High-frequency shoot regeneration, assessment of genetic fidelity, and histochemical analysis of forskolin production in Coleus forskohlii Briq. Protoplasma 1-20.*
- *Modi A, Kanakala S (2020). Molecular Markers for the Evaluation of Clonal Fidelity in Medicinal Plants. In: Protocols used in Molecular Biology Bentham Science Publishers,pp 153-161.*
- *Mohapatra P, Ray A, Sandeep IS, Nayak S, Mohanty S (2021) Tissue-Culture-Mediated Biotechnological Intervention in Centella asiatica: A Potential Antidiabetic Plant. Biotechnol Anti Diab Med Plant 89-116.*
- *Mohiuddin S, Chahota R, Bhatia A, Mohiuddin H, Mohiuddin I, Shabir B, Chintey K, Bhatia A (2023) Cytological Markers in Genome Mapping. Int j innov sci res Technol 8(1):282-289*
- *Muthukumar M, Kumar TS, Rao MV (2016) Organogenesis and evaluation of genetic homogeneity through SCoT and ISSR markers in Helicteresisora L., a medicinally important tree. S. Afr. J. Bot. 106:204–210.*
- *Nadeem MA, Nawaz MA, Shahid MQ, Dogan Y, Comertpay G, Yıldız M, Hatipo Glu R, Ahmad F, Alsaleh A, Labhane N, Ozkan H, Chung G, Baloch FS (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 32:261-285.*
- *Nandi SK, Rawat S (2019) In vitro propagation of selected Himalayan medicinal herbs: conservation and sustainable utilization. In: Medicinal plants of India: conservation and sustainable use. Today & Tomorrow's Printers and Publishers, New Delhi, pp 395-407.*

- *Narayani M, Srivastava S (2017) Elicitation: a stimulation of stress in in vitro plant cell/tissue cultures for enhancement of secondary metabolite production. Phytochem Rev 16:1227-1252.*
- *Nazir R, Gupta S, Dey A, Kumar V, Yousuf M, Hussain S, Dwivedi P, Pandey DK (2020) In vitro propagation and assessment of genetic fidelity in Dioscorea deltoidea, a potent diosgenin yielding endangered plant. S Afr J Bot 140:349-355.*
- *Nilkanta H, Amom T, Tikendra L, Rahaman H, Nongdam P (2017) ISSR marker based population genetic study of Melocanna baccifera (Roxb.) Kurz: a commercially important bamboo of Manipur, North-East India. Scientifica 2017(1):3757238.*
- *Pandian A, Sivalingam AM, Ramasubbu R, Ashok kumar K (2024) Progress and Prospects of Cryopreservation Techniques for Long-Term Conservation of Critically Endangered Medicinal Plant Species. In: Propagation to Pharmacopeia, 1st edn. pp 317-328.*
- *Parihar A, Kumar A, Panda U, Khan R, Parihar DS, Khan R (2023) Cryopreservation: a comprehensive overview, challenges, and future perspectives. Adv Biol 7(6):2200285.*
- *Paun O, Schons wetter P (2012) Amplified fragment length polymorphism: an invaluable finger printing technique for genomic, transcriptomic, and epigenetic studies. Methods Mol Biol 862:75–87.*
- *Pradhan S, Paudel YP, Qin W, Pant B (2023) Genetic fidelity assessment of wild and tissue cultured regenerants of a threatened orchid, Cymbidium aloifolium using molecular markers. Plant Gene 34:100418.*
- *Prakash S, Sharma S, Kajla S (2019) In vitro multiplication and genetic fidelity studies in Cleopatra mandarin (Citrus reshni Tanaka). Curr J Appl Sci Technol 33:1-6.*
- *Purohit S, Jugran AK, Bhatt ID, Palni LMS, Bhatt A, Nandi SK (2017) In vitro approaches for conservation and reducing juvenility of Zanthoxylum armatum DC: an endangered medicinal plant of Himalayan region. Trees 31:1101-1108.*
- *Purohit S, Rawat V, Jugran AK, Singh RV, Bhatt ID, Nandi SK (2015) Micropropagation and genetic fidelity analysis in Valeriana jatamansi Jones. J Appl Res Med Aromat Plants 2(1):15-20.*
- *Rafalski JA (1997) Randomly amplified polymorphic DNA (RAPD) analysis. In: DNA Markers Protocols, Application and Overviews. New York, Wiley, pp 75-83.*
- *Rajput S, Agrawal V (2020) Micropropagation of Atropa acuminata Royle ex Lindl. (a critically endangered medicinal herb) through root callus and evaluation of genetic fidelity, enzymatic and non-enzymatic antioxidant activity of regenerants. Acta Physiol Plant 42:1-17.*
- *Rani V, Raina SN (2000) Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. In Vitro Cell Dev Biol Plant 36:319-330.*

- *Rath SC, Seth S, Mishra SK, Yadav PK, Gupta AK, Panigrahi J (2020) Genetic homogeneity assessment of in vitro-regenerated plantlets of Nyctanthes arbor tristis L. and comparative evaluation of bioactive metabolites and antioxidant activity. In Vitro Cell Dev Biol Plant 56:72-87.*
- *Rautela I, Dhiman M, Sharma MD, Kumar N, Misra P (2017) Assessment of genetic fidelity of mother plant and in vitro raised medicinal plant Ephedra gerardiana through molecular markers. J plant dev sci 9(3):191-200.*
- *Rawat B, Rawat JM, Mishra S, Mishra SN (2013) Picrorhiza kurrooa: current status and tissue culture mediated biotechnological interventions. Acta Physiol Plant35:1-12.*
- *Rawat JM, Bhandari A, Mishra S, Rawat B, Dhakad AK, Thakur A, Chandra A (2018) Genetic stability and phytochemical profiling of the in vitro regenerated plants of Angelica glauca Edgew.: an endangered medicinal plant of Himalaya. Plant Cell, Tissue Organ Cult135:111-118.*
- *Rawat JM, Rawat B, Agnihotri RK, Chandra A, Nautiyal S (2013) In vitro propagation, genetic and secondary metabolite analysis of Aconitum violaceum Jacq.: a threatened medicinal herb. Acta Physiol Plant 35:2589-2599.*
- *Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128:917.*
- *Rohela GK, Jogam P, Bylla P, Reuben C (2019) Indirect regeneration and assessment of genetic fidelity of acclimated plantlets by SCoT, ISSR, and RAPD markers in Rauwolfia tetraphyllaL.: an endangered medicinal plant. Bio Med Res Int 2019(1):3698742.*
- *Rout GR, Mahato A, Senapati SK (2008) In vitro clonal propagation of Nyctanthesarbor-tristis. Biol Plant 52:521-524.*
- *Sahijram L, Soneji J, Bollamma K (2003) Analyzing somaclonal variation in micropropagated bananas (Musa spp.). In Vitro Cell Dev Biol Plant 39:551-556.*
- *Saminathan M, Muruganandam A, Arumugam M, Kolar AB (2023) Micropropagation and assessment of genetic fidelity of Decalepis hamiltonii wight & arn using RAPD markers. Biochem Cell Arch 23(2).*
- *Sandhya, D., Jogam, P., Manokari, M., Shekhawat, M. S., Jadaun, J. S., Allini, V. R., & Abbagani, S. (2021). High-frequency in vitro propagation and assessment of genetic uniformity and micro-morphological characterization of Origanum majorana L.–A highly traded aromatic herb. Biocatalysis and Agricultural Biotechnology, 34, 102024.*
- *Sarkar J, Banerjee N (2020) Influence of different cytokinins on micropropagation of an important medicinal plant, Solanum erianthum D. Don, and assessment of the genetic fidelity of the regenerants. In Vitro Cell Dev Biol Plant 56:480-490.*
- *Sawicki J, Szczecinska M (2007) Semi-specific intron-exon splice junction markers in bryophyte studies. Biodiv Res Conserv 5-8:25-30.*

- *Senapati S, Aparajita S, Rout G (2013) Micropropagation and assessment of genetic stability in Celastrus paniculatus: an endangered medicinal plant. Biologia 68(4):627-632.*
- *Shahid M, Srivastava M, Ali S, Pathak N, Rastogi S, Ali SS (2012) An overview of molecular marker techniques. Adv life sci 1(1):13-19.*
- *Sharma C, Mukherjee P (2023) Biotechnological approaches for conservation and secondary metabolites production in medicinally important species of genus Saussurea. S Afr J Bot 161:696-710.*
- *Sharma N, Singh R, Pandey R, Kaushik N (2017) Genetic and biochemical stability assessment of plants regenerated from cryopreserved shoot tips of a commercially valuable medicinal herb Bacopa monnieri (L.) Wettst. In Vitro Cell Dev Biol Plant 53:346-351.*
- *Sharma S, Kaur R, Kumar K (2019a) Studies on genetic fidelity of long term micropropagated culture derived plants of Ofra strawberry using molecular markers. Indian J Hortic 76:596-603.*
- *Sharma S, Parasher K, Mukherjee P, Sharma YP (2021) Cryopreservation of a threatened medicinal plant, Valeriana jatamansi Jones, using vitrification and assessment of biosynthetic stability of regenerants. Cryoletters 42(5):300-308.*
- *Sharma U, Rai MK, Shekhawat NS, Kataria V (2019b) Genetic homogeneity revealed in micropropagated Bauhinia racemosa Lam. using gene targeted markers CBDP and SCoT. Physiol Mol Biol Plants 25:581-588.*
- *Shingote PR, Mithra SA, Sharma P, Devanna NB, Arora K, Holkar SK, Khan S, Singh J, Kumar S, Sharma TR, Solanke AU (2019) LTR retrotransposons and highly informative ISSRs in combination are potential markers for genetic fidelity testing of tissue culture-raised plants in sugarcane. Mol Breed 39:1-13.*
- *Shukla SP, Sharma A (2017) In vitro seed germination, proliferation, and ISSR marker-based clonal fidelity analysis of Shorea tumbuggaia Roxb.: an endangered and high trade medicinal tree of Eastern Ghats. In Vitro Cell Dev Biol Plant 53:200-208.*
- *Singh A, Negi MS, Moses VK, Venkateswarlu B, Srivastava PS, Lakshmikumaran M (2002) Molecular analysis of micropropagated neem plants using AFLP markers for ascertaining clonal f idelity. In Vitro Cell Dev Biol Plant 38:519-524.*
- *Slazak B, Sliwinska E, Saluga M, Ronikier M, Bujak J, Slomka A, Kuta E (2015). Micropropagation of Viola uliginosa (Violaceae) for endangered species conservation and for somaclonal variation-enhanced cyclotide biosynthesis. Plant Cell, Tissue Organ Cult120:179-190.*
- *Smith MK (1988) A review of factors influencing the genetic stability of micropropagated bananas. Fruits 43:219-223.*
- *Swarna J, Ravindhran R (2012) In vitro propagation and assessment of genetic integrity of Talinum triangulare (Jacq.) Wild: a valuable medicinal herb. Acta Physiol Plant 10: 1007-1017.*

- *Tariq A, Naz S, Shahzadi K, Ilyas S, Javed S (2015) Study of genetic stability in in vitro conserved Podophyllum hexandrum using RAPD markers. J Anim Plant Sci 25 (4):1114-1120*
- *Tavakoli M, Mousavi A, Haghbeen K (2021) Evaluation of somaclonal variation of Arnebia pulchra (Boraginaceae) calli versus seeds using ISSR markers. IJGPB10(1).*
- *Thakur J, Dwivedi MD, Sourabh P, Uniyal PL, Pandey AK (2016) Genetic homogeneity revealed using SCoT, ISSR and RAPD markers in micropropagated Pittosporum eriocarpum Royle-an endemic and endangered medicinal plant. PLoS One 11(7):e0159050.*
- *Tikendra L, Koijam AS, Nongdam P (2019) Molecular markers based genetic fidelity assessment of micropropagated Dendrobium chrysotoxum Lindl. Meta Gene:20.*
- *Tikendra L, Potshangbam AM, Dey A, Devi TR, Sahoo MR, Nongdam P (2021) RAPD, ISSR, and SCoT markers based genetic stability assessment of micropropagated Dendrobium fimbriatum Lindl var oculatum Hk f-an important endangered orchid. Physiol Mol Biol Plants 27:341-357.*
- *Tippani R, Nanna RS, Mamidala P, Thammidala C (2019) Assessment of genetic stability in somatic embryo derived plantlets of Pterocarpus marsupium Roxb. using inter-simple sequence repeat analysis. Physiol Mol Biol Plants 25:569-579.*
- *Tiwari G, Singh R, Singh N, Choudhury DR, Paliwal R, Kumar A, Gupta V (2016) Study of arbitrarily amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers for genetic diversity and population structure in Kalmegh [Andrographis paniculata (Burm. f.) Nees]. Ind Crops Prod 86:1-11.*
- *Tsuchihashi Z, Dracopoli NC (2002) Progress in high throughput SNP genotyping Pharmacogenomics J 2:103-110.*
- *Upadhyay S (2024) In vitro rapid mass multiplication and molecular validation of Rheum emodi, Doctoral dissertation, SRHU.*
- *Varshney A, Dhawan V, Srivastava PS (2000) A protocol for in vitro mass propagation of Asiatic hybrids of lily through liquid stationary culture. In Vitro Cell Dev Biol Plant 36:383-391.*
- *Vieira RF, Grayer RJ, Paton A, Simon JE (2001) Genetic diversity of Ocimum gratissimum L. based on volatile oil constituents, flavonoids and RAPD markers. Biochem Syst Ecol 29:287-304.*
- *Wani T, Rana S, Bhat WW, Pandith S, Dhar N, Razdan S, Lattoo S (2016) Efficient in vitro regeneration, analysis of molecular fidelity and Agrobacterium tumifaciens-mediated genetic transformation of Grewia asiatica L J Plant Biochem Physiol 4(167):2.*
- *Wasi A, Shahzad A, Tahseen S (2024) Synthesis of nonembryonic synseed, short term conservation, phytochemical evaluation and assessment of genetic stability through SCoT markers in Decalepis salicifolia. Plant Cell, Tissue Organ Culture 156(3):101.*

- *Weining S, Langridge P (1991) Identification and map ping of polymorphisms in cereals based on polymerase chain reaction. Theor Appl Genet 82:209-216.*
- *Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213-7218.*
- *Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531-6535.*
- *Wolff K, Peters-van Rijn J (1993) Rapid detection of genetic variability in Chrysanthemum (Dendranthema grandiflora Tzvelev) using random primers. Heredity 71:335-341.*
- *Yang L, Chao L, Su X, Wang C, Dong C, Chen S (2021) High-frequency invitro plantlet regeneration in Lilium davidiivar. Unicolour Salisb, an important edible and medicinal plant, and confirmation of genetic fidelity of regeneration plantlets using ISSR markers. Plant Biotechnol Rep 15(4):435-446.*
- *Yang RC, Yeh FC (1992) Genetic consequences of in situ and ex situ conservation of forest trees. For Chron 68(6):720-729.*
- *Yang W, Kang X, Yang Q, Lin Y, Fang M (2013) Review on the development of genotyping methods for assessing farm animal diversity. Journal of animal science and biotechnology 4:1-6.*
- *Yu S, Zhao X, Wang Y, Jiang D, Zhang Y, Hu L, Cai X (2022) Morphological, cytological, and molecular-based genetic stability analysis of in vitro-propagated plants from newly induced aneuploids in caladium. Agriculture 12(10):1708.*