

MICROPROPAGATION AND GENETIC FIDELITY ASSESSMENT OF HORTICULTURAL CROPS USING MOLECULAR MARKERS: A REVIEW

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ABSTRACT

Micropropagation is one of the important biotechnological methods used for the rapid and large scale propagation of elite genotypes of horticulture throughout the year. The method has some advantages over traditional methods of propagation such as rapid multiplication, production of disease-free plant materials, conservation of germplasms and propagation of plants which cannot be multiplied by seed or vegetatively. Nevertheless, the in vitro conditions may cause genetic and epigenetic changes that are referred to somaclonal variations. In commercial micropropagation, somaclonal variation is of particular importance because the main goal of clonal propagation is to produce genetically uniform plants. Morphology, biochemistry and cytology can provide useful information about uniformity; however, the use of DNA based molecular markers provides the most accurate way to determine genetic variation. A variety of marker techniques like Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP), and Start Codon Targeted (SCoT), markers have been used to detect genetic fidelity of horticulture crops in micropropagation. Marker choice will depend on the particular crop, genetics, genomic information, facilities, cost and resolution requirements. Developments in next-generation sequencing, genome-wide SNP screening, DNA methylation profiling and transcriptomics will make the application field of fidelity evaluation much wider than classical DNA fingerprinting. This review gives an overview of the principles of micropropagation, sources and origins of somaclonal variation, approaches to genetic fidelity evaluation, properties and use of molecular marker systems, applications of the technique in horticultural crops, factors affecting genetic stability, and technologies of high throughput quality control. The combination of optimized micropropagation techniques and molecular quality control systems may contribute to genetic stability and commercialization of micropropagated horticultural planting material.

KEYWORDS: Micropropagation, genetic fidelity, somaclonal variation, molecular markers, tissue culture, horticultural crops

1. INTRODUCTION

Horticulture crops are very significant to agriculture around the world owing to their role in food production, nutrition, income generation, creation of employment opportunities, industry and exportation. Horticulture crops, especially the fruits, vegetables, plantation, medicinal and ornamental crops, have often been grown using the vegetative propagation technique due to the inability of seed propagation to maintain desirable genotypes or because of such problems as long juvenile period, dormancy, low viability of seeds, heterozygosity, apomixis or other biological reasons. Micropropagation presents a different means of achieving rapid multiplication of the selected genotypes under controlled environment, (Alizadeh et al., 2015).

Micropropagation refers to the growing of plant tissues/organs in a sterile nutrient medium and subsequent multiplication, rooting and acclimatization of plantlets produced. The process can potentially produce a very large number of plants from a small amount of initial material and is especially useful in the case of horticultural crops that require rapid multiplication. The importance of micropropagation to horticulture crops and other crops has been acknowledged by FAO.

The main merit of micropropagation is that it allows obtaining clones that are genetically identical to the mother plant. Nevertheless, genetic homogeneity of the regenerated plant is not always guaranteed. In fact, plants obtained from micropropagation often show genetic or epigenetic modifications during the in vitro cultivation period (Abdalla, et al., 2022). These are known as somaclonal variations. The appearance of somaclonal variation is

beneficial when it produces valuable characters. Otherwise, it is problematic, especially when micropropagation is used for commercial cloning or germplasm conservation purposes, (Majumder et al., 2025).

The importance of off-type occurrence becomes evident when we consider crop species such as banana, strawberry, grape, citrus, papaya, pineapple and ornamental plants whose productivity strongly depends on genetically homogenous planting material. Therefore, genetic homogeneity control is one of the crucial issues in modern micropropagation, (Debnath, 2010).

While conventional methods based on morphological characters require a certain degree of plant development and may be influenced by environmental conditions, molecular markers allow detecting DNA level differences and usually remain unaffected by the environment, (Bhardwaj, et al., 2025). Therefore, RAPD, ISSR, SSR, AFLP, SCoT and many other marker systems become very useful for studying the genetic stability of micropropagation-derived plants.

2. Micropropagation of Horticultural Crops

Micropropagation Concept

Micropropagation is defined as the in vitro multiplication of plants using very tiny portions of the plant as explants like shoot tips, nodal explants, meristematic tissue, leaves, embryos or other tissues. The method is usually carried out under aseptic conditions with the help of a suitable basal medium containing plant growth regulators (Bhardwaj, et al., 2025). The traditional procedure of micropropagation may include the following steps:

Selection of superior mother plant → Collection of explants → Surface sterilization → Initiation of culture → Shoot multiplication → Root formation → Acclimatization → Nursery establishment → Field establishment

3. Main stages of micropropagation

3.1. Choice and maintenance of the donor plant

The choice of the donor plant is the basis of any efficient programme of clonal propagation. It should have all the necessary horticultural traits, i.e., high productivity, quality, proper morphology, freedom from diseases and adaptability. Wherever possible, the donor should be kept under controlled conditions so that the source material would be elite in its genotype, (Bhojwani, and Dantu, 2013).

The physiological state of the donor plant may affect the process of culture initiation and regeneration, since young and actively developing tissues are sometimes different from those which are mature, and seasonal changes may affect the endogenous hormone level, phenolic oxidation and contamination.

3.2. Choice of the explant and surface sterilization

The choice of the right explant is important, since various tissues have different capacities for regeneration and different genetic stability. The shoots tip and nodal segments with the existing meristems are usually chosen for clonal propagation since they allow to obtain the shoots without going through the callus stage, (Kumar, et al., 2023).

The chosen explant has to be surface sterilized before inoculation, since it allows to kill all microorganisms without damaging the tissue itself. The sterilization procedure must be carefully optimized because inadequate sterilization results in contamination, whereas excessive sterilization can damage the explant.

3.3 Culture establishment

In the culture establishment stage, explants are placed on a suitable nutrient medium. The Murashige and Skoog (1962) medium continues to be one of the commonly used basal media for plant tissue culture despite modifications being needed for some crops.

The nutritional needs of horticultural crops show wide differences. As such, micropropagation processes may vary in terms of minerals, vitamins, carbon sources, plant growth regulators, pH, and culture conditions, (Sing, A. 2015).

3.4 Shoot multiplication

Shoot multiplication usually involves repeated induction of axillary buds or adventitious shoot development. Cytokinins like 6-benzylaminopurine, kinetin, thidiazuron, and meta-topolin can be used, depending on the crop and genotype. While a higher multiplication rate may be desirable commercially, too much multiplication pressure can increase the likelihood of abnormal growth or genetic instability in certain cases. Hence, maximum multiplication is not always the only goal of protocol optimization, (Butt, et al., 2015).

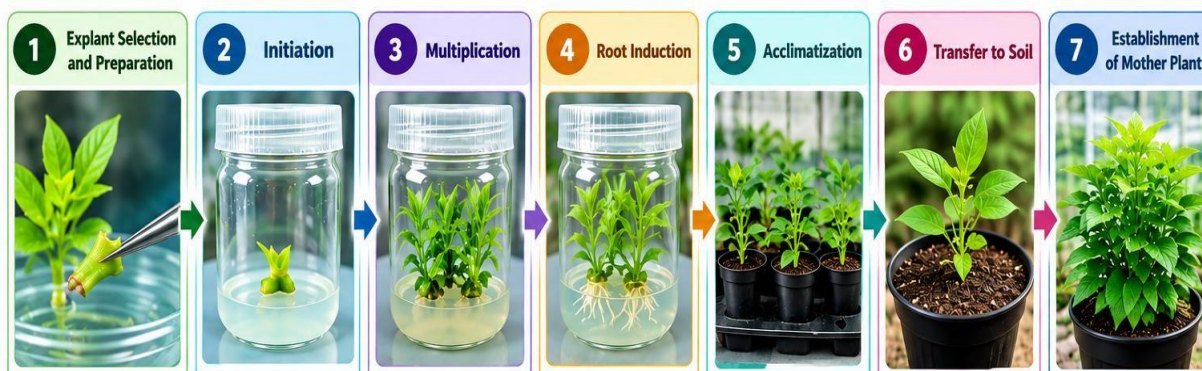
3.5 Rooting

Well-formed shoots are then transferred to the rooting medium or treated with rooting treatments. Auxins such as indole-3-butyric acid, indole-3-acetic acid, and naphthalene acetic acid can be used alone or in combination. Root quality is more important than root number alone because plants with a well-developed root system generally have better chances of surviving the transition from in vitro to ex vitro conditions, (Bhojwani, and Dantu, 2013).

3.6 Acclimatization

Plantlets grown in vitro develop in an environment which is high in relative humidity, low in irradiation and supplied with an artificial carbon source. Therefore, their cuticle development, stomata control and photosynthesis activity could be different from those of field plants. Acclimatization is a gradual adaptation of plantlets to low humidity, increased light and independent photosynthesis. This process is regarded as a critical phase of some commercial micropropagation systems, (Kumar et al., 2023).

Stages of Micropropagation



4. Genetic Fidelity and Clonal Uniformity

Genetic fidelity describes the conservation of the genetic composition of micropropagated plants compared to the donor plant genotype. In a genetically-faithful clonal propagation technique, regenerated plants should have identical genotypes as the mother plant except for the variations present in the donor tissue, (Bairu et al., 2011). The term is important in fruit and ornamental crops as these crops get economic importance based on certain morphological, physiological and biochemical features. Genetic alteration in some cases can give rise to phenotypic abnormalities in plantlets whereas others may not reveal any differences until blooming or fruiting, (Krishna, et al., 2011).

The evaluation of genetic fidelity is therefore not simply an academic exercise. It is a component of nursery quality assurance, germplasm conservation and commercial planting-material production, (George et al., 2008).

(Debnath et al., 2011) reviewed the use of molecular markers for assessing genetic fidelity, uniformity, stability and true-to-type characteristics in micropropagated horticultural crops and emphasized their value in overcoming limitations associated with conventional phenotypic assessment. Genetic fidelity evaluation is thus not just an academic undertaking. It is one of the elements that go into nursery quality assurance, germplasm conservation, and production of planting materials. Debnath considered the application of molecular markers for the assessment of genetic fidelity, uniformity, stability and true-to-type nature of micropropagated horticultural crops and underscored the importance of such applications in overcoming limitations of phenotypic assessments, (Krishna et al., 2016).

5. Somaclonal Variation

Somaclonal variation is one of the key biological problems arising during tissue culture. This term can be defined in general as the occurrence of genetic or epigenetic differences in plants obtained through tissue culture, (Majumder et al., 2025).

This phenomenon was first noted by Larkin and Scowcroft; they proved that cell culture could produce genetic variability and that the resulting plants were different from the donor genotype. Somaclonal variation can be divided into genetic and epigenetic variations in general (Sato, et al., 2011). The former can occur due to point mutation, insertion, deletion, chromosomal rearrangement, changes in chromosome number and inactivation of transposable elements. The latter can occur due to changes in DNA methylation, histone modification and chromatin structure, (Kumar et al., 2018).

The rate of occurrence of somaclonal variation is not stable. It largely depends on the genotype, explants used, method of regeneration, duration of culture, concentration of phytohormones, number of subculture and environment.

6. Mechanisms underlying the occurrence of somaclonal variation

6.1 Genomic mutations

During tissue culture, cells repeatedly divide. As a result, mutations in DNA sequences occur because of DNA replication errors or stress in cells. The mutations that have occurred in cells may get fixed in regenerated plants, (Bhojwani, and Dantu, 2013).

6.2 Changes in the number of chromosomes

Chromosomes can be broken during tissue culture. There might be chromosome rearrangements, aneuploidy and change in the ploidy level. These events may lead to visible abnormalities in phenotypes, (Manchanda, et al., 2025).

6.3 Transposable elements

Transposable elements are sequences of DNA that can change their positions in the genome. Some specific stress in tissue culture may activate these elements and cause genetic variation. For example, Sato et al. described activation of a transposon during tissue culture in *Saintpaulia*. It led to excision of a transposon from the genome with the subsequent change in flower colour. (Sato et al 2011). observed that the development of flower-colour variants could be caused by the changes in the promoter region of the flavonoid 3',5'-hydroxylase gene.

6.4 Epigenetic alterations

Alterations to epigenetics do not necessarily involve changes to the DNA code but can affect the expression of genes through other means (Dorani, et al., 2024). The alteration of DNA methylation is important in this context since tissue culture can subject the cell to an environment that does not normally exist due to hormonal and nutritional factors. Therefore, molecular fidelity experiments relying only on DNA sequences may overlook certain variations, (Chen, and Henny, 2006).

7. Factors Influencing Genetic Stability During Micropropagation

There are many factors that contribute to the genetic stability of micropropagated plants, which interact with each other. Genotype of the donor plant is one of the most significant factors, since various genotypes can have very different responses to tissue culture procedures.

The type of explant is another factor. Meristematic explants represent a faster way of multiplication of clonal material than highly differentiated explants, which need to undergo dedifferentiation and form calluses before further development. Direct axillary shoot multiplication is often used in those cases, when the main purpose is genetic homogeneity, (Alizadeh et al., 2015).

Callus-induced regeneration represents one more possibility of introducing genetic variation into plants. Since cell has to go through a lot of changes before forming a new plant, there is a possibility of variation. However, callus-induced plants do not necessarily have genetic instability; it is just a possibility that is increased under certain culture conditions (Debnath et al., 2012). Concentration of phytohormones can also affect genetic stability of plants. Cytokinins and auxins regulate cell division and differentiation, and thus, their excessive use can cause stress to the plant cells.

The number of subcultures represents another important factor. Long-term maintenance and repeated multiplication expose cells to multiple rounds of cell division and artificial culture conditions, potentially increasing the opportunity for genetic and epigenetic changes. (Bairu et al 2011). reviewed the causes and detection of somaclonal variation. They emphasized the importance of culture conditions and biological factors in determining the frequency and nature of tissue-culture-induced variation.

8. Traditional Approaches to Assessing Genetic Fidelity

Prior to the use of DNA-based molecular markers, genetic fidelity was mainly determined using morphological, biochemical and cytological approaches. Morphological approach is easy and cheap and allows detection of gross off-types. Height of plant, morphology of leaves, morphology of stem, blooming period, flower morphology, fruit morphology, and growth habit can be compared in donor plants and in the regenerated ones (Bhatia et al. (2009). However, many morphological characteristics are subject to strong environmental influence, which means that the similarity in morphology does not mean genetic identity. Biochemical markers such as isozyme analyses give additional information regarding genetic variability. This type of analyses is easy to perform, but the number of loci analyzed is limited and enzyme expression is affected by both tissue and developmental stage, (Collard and Mackill, 2009).

The cytological approaches, including chromosome counting and flow cytometry, can reveal differences in chromosome number and nuclear DNA amount. The latter is especially helpful when the determination of ploidy level is required. However, these techniques might fail to detect small changes in DNA sequences which do not affect the chromosome number or genome size. For this reason, the DNA-based molecular markers became an important part of genetic fidelity studies.

9. Molecular Markers for Genetic Fidelity Testing

DNA markers are segments or sequences in the DNA that can be identified and compared between organisms (Rani and Raina 2000). DNA markers have been increasingly used for the analysis of plant genetics since the advent of PCR-based markers. Molecular markers are used for the purposes of genotyping, analysis of diversity, cultivar fingerprinting, and evaluation of genetic stability, (Salutgi, and Singh, 2026).

In the context of micropropagation, the concept is quite simple. DNA is isolated from the mother plant and from a sample of the regenerated plants. A consistent set of molecular markers is employed for all samples and compared (Mohan Jain, 2001). If the regenerated plants possess exactly the same or very similar reproducible profiles in comparison with the mother plant, this serves as the proof of genetic fidelity. The presence of reproducible polymorphic bands/alleles means that there may be some differences in the genotype, (Debnath, 2010).

10. Random Amplified Polymorphic DNA (RAPD)

RAPD markers were devised as a PCR-based technique for the identification of DNA polymorphism through the use of arbitrary primers. The method was first explained by Williams et al. 1990).

The major advantage of RAPD analysis is the lack of need for prior sequence data, which makes this method favorable for species with insufficient genomic data. Only a relatively small amount of DNA is needed, and the analysis can be done with the help of regular PCR and agarose gel electrophoresis. Genetic fidelity experiments involve the comparison of the DNA profile of the initial plant with those of the regenerated plantlets. Consistent amplification profiles on a sufficiently large number of primers will indicate genetic uniformity, (Gupta et al., 1999).

The biggest drawback of RAPD is its dependence on PCR conditions. Even small variations in DNA quality, Mg⁺⁺ levels, annealing or thermal cycling conditions may occasionally have some influence on amplification. In addition, RAPD markers are dominant: the presence of a band cannot distinguish between homozygous and

heterozygous states. Thus, the RAPD technique is helpful for preliminary screenings, but preferably should be used in combination with more reproducible marker systems, (Ray, et al., 2006).

11. Inter Simple Sequence Repeats (ISSR)

ISSRs make use of the high frequency of the simple sequence repeats within the genomes of plants. This method involves amplification of the region found between the microsatellite repeats, thus generating many fragments from a single primer. The basis of the use of microsatellite-anchored fingerprinting was established by Zietkiewicz et al. 1994), proving that SSR-anchored PCR can be used to produce complex fingerprints of the genome, which can reveal polymorphism.

There are several advantages of ISSR markers for micropropagation studies. First, they require no information about genome sequence in advance (Pathak and Dhawan (2008). Moreover, they can produce a comparatively large number of scorable fragments and are usually more reproducible than RAPDs.

As ISSR markers amplify microsatellite associated regions, they may detect certain variations that cannot be seen with the help of a few RAPD primers. This makes ISSR one of the common marker systems for clonal fidelity. However, ISSR is a generally dominant system, which does not allow the use of codominant information provided by SSRs. Thus, the information can only be interpreted based on presence or absence of DNA fragments, (Alizadeh, et al., 2015).

12. Simple Sequence Repeat (SSR) Markers

The SSR markers are alternatively called microsatellites. These markers comprise short sequences that repeat multiple times in the genome of the plant.

One of the key benefits of using the SSR markers is the codominance of these loci. It is important to note that these markers differ from other dominant markers since they allow recognizing different alleles (Adamek, et al., 2023). The SSR markers are useful for cultivar identification, genotyping, and even clonal characterization. When the species-specific SSR primers are available, the profiles can be quite reliable. At the same time, the creation of the species-specific SSR markers may require a significant amount of genomics data. In crops that have a lack of genomics information, the transfer between the related species can be used; however, this process depends on evolution, (Zietkiewicz, et al., 1994).

13. Amplified Fragment Length Polymorphism (AFLP)

It involves both restriction enzyme digestion and selective amplification by PCR of genomic DNA. It has the ability to produce numerous fragments throughout the whole genome. The major strength of AFLP is its ability to cover a large part of the genome with relatively high reproducibility. Therefore, AFLP can be used if the main goal is to identify small differences between very similar genotypes (Liu, 2005).

However, it is a more complicated process compared to RAPD and ISSR because it involves restriction enzyme digestion, adapter ligation, and selective amplification. Thus, AFLP is relatively costly and time-consuming. Simpler PCR-based markers may be preferable for commercial testing of fidelity, while AFLP can be useful for scientific studies.

14. Start Codon Targeted (SCoT) Markers

SCoT markers belong to a more recent group of gene-targeted markers, and the technique was developed by (Collard and Mackill, 2009). It takes into account conserved sequences located around the ATG translation initiation codon of plant genes.

The gene-targeted property of SCoT markers makes the technique advantageous as compared to the totally random markers since the amplified regions target gene sequences. The use of single primers and conventional PCR reaction conditions makes SCoT markers applicable through the equipment available in molecular biology laboratories, (Azamatov et al., 2026).

Subsequent applications of SCoT markers have included genetic diversity, cultivar identification and stability analysis in many plant species. The use of SCoT markers in micropropagation is especially attractive because of their ability to detect variations in gene-associated sequences that might have some biological importance. However, SCoT is still largely dominant, and its discriminating power largely depends on the number of primers used.

15. Sequence Characterized Amplified Region (SCAR)

SCAR markers are based on the polymorphic DNA fragments already identified. After identification of a polymorphic fragment, it can be sequenced, and primers for amplifying that region can be synthesized. The main advantages of SCAR markers include high specificity and reproducibility. Such markers are very useful in the identification of a particular cultivar or mutant or molecularly characterized variation, (Debnath, et al., 2012).

16. Single Nucleotide Polymorphism (SNP) Markers

SNP markers are the variations at individual bases within genomic DNA. Due to their abundant distribution in the plant genomes, SNP markers are very useful for high resolution genetic studies. SNP markers can provide reproducible and codominant data compared to the dominant marker systems. The advancement in the sequencing technology has enabled the identification of large amounts of SNPs, (Sharma and Jain 2024).

For genetic fidelity studies, SNP marker-based strategies can detect the mutations which could be missed using a few RAPD, ISSR or SCoT primers. Their major limitations are the requirement for appropriate genomic resources, sequencing or genotyping platforms, bioinformatics capacity and higher initial costs.

17. Next-Generation Sequencing and Genome-Wide Analyses

The emergence of next-generation sequencing technologies has brought about an essential change in the scale at which plant genomes can be investigated. Instead of comparing dozens of molecular markers, one can theoretically

investigate thousands and millions of genome positions in the comparison of donor and regenerated plants (Krishna, et al., 2016). Whole-genome sequencing allows detecting single nucleotide substitutions, small indels and structural rearrangements. Genotyping-by-sequencing and similar reduced representation methods can offer a cheaper solution for large populations, (Kumar, 1999).

This approach is particularly valuable for economically significant crops when even a tiny genetic polymorphism can result in considerable economic losses. However, the principal problem associated with genome-wide techniques is the generation of enormous amounts of data which require specific bioinformatic processing and analysis. As a result, the traditional ISSR, SSR, SCoT analyses retain their value for laboratories which lack budget and simplicity of technology, (Semagn, et al., 20006).

18. Fidelity of Epigenetics

The concepts of genetic fidelity and epigenetic fidelity cannot be used interchangeably. The plant could have an identical DNA profile, but at the same time be different in terms of DNA methylation and expression. Epigenetic diversity might develop due to tissue culturing in vitro, which is characterized by unusual nutrition, hormonal and environmental factors. These factors could affect plant morphology, growth, flowering and adaptation to stress. Recently, a lot of review articles highlight the importance of taking into account both genetic and epigenetic diversity in studying clonal stability. Modern techniques such as bisulfite sequencing, methylation-sensitive markers and transcriptome analysis allow one to examine these processes more thoroughly. It is an interesting field for further studies as traditional methods of DNA profiling will not be enough to understand the issue.

Table-1: Comparative Evaluation of Molecular Marker Systems

Marker system	Marker nature	Prior sequence information	Reproducibility	Polymorphism	Main advantage	Major limitation
RAPD	Dominant	Not required	Moderate	Moderate	Simple and inexpensive	PCR sensitivity
ISSR	Dominant	Not required	Generally high	Moderate–high	Good fingerprinting ability	Dominant nature
SSR	Codominant	Usually required	High	High	Precise genotype discrimination	Primer development
AFLP	Dominant	Not required	High	High	Broad genome coverage	Technically demanding
SCoT	Dominant	Not required	Generally good	Moderate–high	Gene-targeted	Limited codominant information
SCAR	Usually locus-specific	Required	Very high	Variable	Specific diagnosis	Requires prior marker development
SNP	Codominant	Usually required	Very high	Very high	Genome-wide potential	Higher cost and bioinformatics
NGS	Genome-wide	Genome resources useful	Very high	Very high	High-resolution detection	Data analysis

19. Micropropagation in Fruit Crops

Micropropagation assumes special significance in fruit crops where many of the best varieties happen to be heterozygous and cannot be preserved by conventional seed propagation.

A good case in point would be that of banana, where commercial cultivation often involves vegetative propagation, and tissue culture serves as a fast way of propagating the chosen planting material. But the problem of off-types makes genotypic fidelity analysis necessary. In grapes, citrus fruits, strawberries, apples, pineapples, papaya, and many other fruit crops, molecular markers too can be employed for verifying micropropagated material and determining if the regenerants retain the identity of the parent genotype, (Lal, et al., 2023).

The significance of molecular tests increases significantly in those cases when the planting material is to be raised in large-scale commercial plantations, since even a small fraction of genetically distinct plants can result in non-uniformity which manifests itself only after a few months or years of cultivation in the field.

Banana

Bananas represent an extremely valuable commodity for micropropagation because the traditional sucker propagation takes time and can result in pest/pathogen spread. Ray et al. performed the analysis of three

economically important cultivars of bananas employing RAPD and ISSR markers and observed somaclonal variants among regenerants.

Another long-term experiment involved the assessment of approximately 4000 micropropagated plants of banana by using RAPD and ISSR markers to determine the genetic diversity of morphologically different plants. In general, the molecular fingerprints showed great uniformity in relation to the mother plant, thus indicating that proper development of axillary shoot multiplication system guarantees genetic stability during extended periods of time. These experiments prove that banana can serve as both a valuable target for micropropagation and a useful model for genetic studies, (Ray, et al., 2006).

Guava

Guava is one of the vegetatively propagated fruits in which somatic embryogenesis is used to rapidly propagate the material.

Rai et al. (2012) evaluated somatic-embryogenesis-derived guavas with respect to genetic homogeneity using SSR and ISSR markers. Molecular analyses proved to be monomorphic for all the tested regenerants as well as the mother plant, confirming the genetic homogeneity of the material. This example shows that the application of different marker systems to prove conclusions is helpful.

Apple

Apple rootstocks are important micropropagation targets because the rootstock uniformity is crucial for the orchard performance.

Pathak and Dhawan (2008) analyzed the genetic stability of micropropagated apple rootstock MM111 after 22 passages using ISSR markers. It was shown that there were homogeneous amplification patterns for the mother plant and the tissue-culture-derived plants, confirming the clonal fidelity of axillary-bud-derived material. It proves that proper control of axillary multiplication allows maintaining the genetic stability through many passages, (Bisht et al., 2024).

Table 2. Representative studies of molecular assessment of clonal fidelity

Crop	Marker system	Major observation	Implication	Reference
Banana	RAPD + ISSR	Somaclonal variants detected in some populations	Molecular screening is necessary	(Rai et al., 2006)
Banana	RAPD + ISSR	High uniformity after long-term multiplication	Axillary propagation can maintain stability	Bhatia, et al (2009)
Gerbera	ISSR	44/45 clones grouped with mother at similarity 1.00	ISSR can detect rare variants	(Bhatia et al 2011)
Guava	SSR + ISSR	Monomorphic profiles among regenerants	Supports genetic homogeneity	(Rai, et al., 2012)
Apple MM111	ISSR	Homogeneous profiles after 22 passages	Axillary multiplication showed stability	(Pathak, and Dhawan, 2008)
<i>Dendrobium chrysotoxum</i>	RAPD + ISSR	96.30% monomorphism	High genetic fidelity	(Tikendra, et al 2019)
<i>Solanum khasianum</i>	ISSR + SCoT	Molecular confirmation of regenerants	Combined markers useful	(Chirumamilla, et al., 2021)

Table 3. Compilation of genetic variation reports, their causes, and detection methods in important agricultural and horticultural species

Plant species	Tissue culture method / Explants	Detection method / Molecular markers	Key findings	Reference
<i>Citrus jambhiri</i> (Rough lemon)	Somatic embryogenesis; nucellar and ovary tissues	RAPD	Plantlets regenerated from nucellar tissues showed no detectable variation, whereas those regenerated from ovary tissues exhibited variation in approximately 20–30% of plants.	(Savita, et al., 2015)
<i>Dendrobium fimbriatum</i> (Orchid)	Micropropagation	RAPD, ISSR and SCoT markers	Orchids regenerated on basal medium without phytohormones showed 100% monomorphism, whereas hormone-enriched media resulted in low levels of genetic polymorphism.	(Tikendra, et al., 2021)
<i>Vaccinium vitis-idaea</i> (Lingonberry)	Somatic embryogenesis; leaf explants	EST-SSR and GSSR markers	High genetic stability was observed among regenerated plants, indicating the suitability of the regeneration system for clonal propagation.	(Kundu, et al., 2025)
<i>Musa</i> spp.	Micropropagation	ISSR and	Somaclonal variation was detected among	(Shukla, et

(Banana)		RAPD markers	regenerants, emphasizing the importance of genetic-fidelity assessment during banana micropropagation.	al., 2025)
<i>Solanum tuberosum</i> (Potato)	Callus culture; internode explants	qPCR	Tissue culture-derived variation was associated with enhanced starch accumulation.	(Adly, et al., 2023)
<i>Malus domestica</i> (Apple)	Organogenesis	ISSR and SCoT markers	High genetic fidelity was observed, with approximately 98.43% of bands being monomorphic, indicating limited somaclonal variation.	(Bisht, et al., 2024)
<i>Citrus madurensis</i> (Calamondin)	Callus culture	SSR and RAPD markers	Diphenylurea derivatives enhanced somatic embryogenesis but compromised genetic stability. BAP or hormone-free media better maintained clonal fidelity.	(Siragusa, et al., 2007)
<i>Prunus persica</i> (Peach)	Callus culture; cotyledon explants	RAPD markers	High somaclonal variation was observed among regenerated plants.	(Hashmi, et al., 1997)
<i>Fragaria</i> × <i>ananassa</i> (Strawberry)	Micropropagation; shoot tips	qPCR	Somaclonal variation was detected among micropropagated plants.	(Zhang, et al., 2023)
<i>Ficus carica</i> (Fig)	Callus culture; leaf and stem explants	ISSR markers	High genetic variation was observed among tissue-culture-derived plants.	(Eissa et al., 2021)
<i>Elaeis guineensis</i> (African oil palm)	Callus culture	SSR and RAPD markers	Genetic variation was detected among oil-palm regenerants. SSR and RAPD analyses identified polymorphisms associated with culture conditions and subculturing practices.	(Ting, et al., 2013)
<i>Rubus idaeus</i> (Raspberry)	Organogenesis; bioreactor leaf segments	SSR markers	Regenerated plants were genetically stable, indicating high clonal fidelity of the regeneration system.	(Debnath, 2014).
<i>Aloe vera</i> (Aloe)	Organogenesis/axillary shoot buds; callus culture from inflorescence axis	ISSR and RAPD markers	Plants produced through indirect organogenesis exhibited considerable genetic variation, whereas those produced through direct organogenesis showed complete genetic uniformity.	(Rathore, et al., 2011)
<i>Coffea arabica</i> (Coffee)	Somatic embryogenesis	SRAP and SCoT markers	Regenerated and mother plants showed approximately 98% and 99% genetic similarity based on SRAP and SCoT analyses, respectively, indicating high genetic fidelity.	(Bobadilla et al., 2013)
<i>Phoenix dactylifera</i> (Date palm)	Callus culture; shoot tips and axillary shoot meristems	RAPD markers	No detectable genetic variation was observed among the regenerated plants.	(Mirani, et al., 2020)

(Source, Majumder, et al., 2025)

Table-4. Compilation of genetic variation reports, their causes, and detection methods in important agricultural and horticultural species

Plant species	Tissue culture method / Explants	Detection method / Molecular markers	Key findings	Reference
<i>Corylus avellana</i> (Hazel)	Embryogenesis	ISSR and RAPD markers	Genetic differences were identified between in vitro- and field-grown hazelnuts, with effects reported on nut quality and tree height.	(Martins, et al., 2009)
<i>Caladium</i> × <i>hortulanum</i> (Caladium)	Callus culture / leaf culture	SSR markers and flow cytometry	High somaclonal variation was observed in in vitro-cultured caladiums. Aneuploid plants, particularly tetraploid aneuploids, exhibited the greatest variability, indicating changes in genetic fidelity and ploidy.	(Yu, et al., 2022)
<i>Carica papaya</i> (Papaya)	Somatic embryogenesis	RAPD and ISSR markers	RAPD and ISSR markers were used to detect genetic variation and assess genetic fidelity among regenerated papaya plants.	(Vishal, et al., 2024)
<i>Tylophora indica</i> (Indian ipecac)	Indirect, direct and somatic embryogenesis	SCoT markers and flow cytometry	2C DNA content was determined by flow cytometry, while SCoT markers were used to verify the genetic uniformity of regenerated plants.	(Mamgain, et al., 2022)
<i>Cannabis sativa</i>	Callus culture	SSR markers	No detectable genetic variation was observed	(Ioannidis, et

(Hemp)			among the regenerated plants.	al., 2022)
<i>Musa rubra</i> (Bronze banana)	Micropropagation / shoot tips	ISSR markers	Genetic stability was observed among the micropropagated plants.	(Ploetz, 2015)
<i>Vaccinium vitis-idaea</i> (Lingonberry)	Micropropagation / shoot tips	EST and SSR primers	Genetic uniformity was observed among micropropagated plants, indicating high genetic fidelity.	(Sharma, et al., 2023)
<i>Hevea brasiliensis</i> (Rubber)	Somatic embryogenesis / flower and zygotic embryo explants	RAPD and SSR markers	No genetic variation was detected between the mother plants and in vitro-derived plantlets based on RAPD and SSR marker analysis.	(Tongtape, et al., 2023)
<i>Psidium guajava</i> (Guava)	Somatic embryogenesis / zygotic embryo	RAPD, ISSR and SSR markers	Approximately 99% of the analyzed bands were monomorphic, indicating high genetic similarity among regenerated plants.	(Kamle, et al., 2014)
<i>Vanilla planifolia</i> (Vanilla)	Callus culture / immature capsules	ISSR markers and phenotypic observation	Molecular analysis of regenerated plantlets revealed approximately 71.66% genetic polymorphism, indicating considerable variation among regenerants.	(Ramírez-Mosqueda, et al., 2011)
<i>Musa acuminata</i> (Banana)	Organogenesis / shoot apices	RAPD markers	Molecular analysis showed 23.46% polymorphism, whereas plants obtained through transverse shoot-apex sections showed genetic uniformity with the parent plants.	(Safarpour, et al., 2017)
<i>Hypericum gaitii</i> (St. John's wort)	Organogenesis / apical and axillary meristems	ISSR markers	No polymorphism was detected between micropropagated plants and mother plants, indicating genetic uniformity.	(Swain, et al., 2016)
<i>Rhaponticum carthamoides</i> (Maral root)	Direct organogenesis / leaf explants	Flow cytometry, RAPD and ISSR markers	Genetic stability was observed among regenerated plants based on cytological and molecular analyses.	(Skała, et al., 2022)
<i>Curcuma angustifolia</i> (Indian arrowroot)	Organogenesis / shoot tips and nodal explants	EST-ISSR markers	No detectable genetic variation was observed among regenerated plants, indicating high genetic fidelity.	(Sahoo, et al., 2021)
<i>Artemisia vulgaris</i> (Mugwort)	Organogenesis / nodal explants	SCoT, ISSR markers and DNA barcoding	Genetic stability was observed among the regenerated plants using molecular-marker and DNA-barcoding approaches.	(Jogam, et al., 2020)
<i>Cicer arietinum</i> (Chickpea)	Organogenesis / embryo axis, half-seed, axillary meristem and cotyledonary node explants	SCoT and ISSR markers	No polymorphism was detected, indicating genetic uniformity of the regenerated plants.	(Sadhu, et al., 2020)
<i>Magnolia dealbata</i> (Cloudforest magnolia)	Somatic embryogenesis from zygotic embryo	SSR markers	Approximately 90% genetic integrity was observed among regenerated plants.	(Chávez-Cortazar, et al., 2020)
<i>Cymbidium aloifolium</i> (Cymbidium orchid)	Organogenesis / seeds	SCoT, DAMD and ISSR markers	In vitro-derived plantlets showed 86.87% genetic monomorphism and 13.13% polymorphism, indicating generally high but not complete genetic fidelity.	(Monica, and Kumaria, 2025)
<i>Rhynchostylis retusa</i> (Punjab fig orchid)	Organogenesis / capsule	Molecular marker analysis*	High genetic uniformity was observed among regenerated plants and their mother plants.	(Oliya, et al., 2021)

(Source, Majumder, et al., 2025)

20. Applications in Vegetable Crops

In addition to field crops, micropropagation is also applied to vegetable crops whenever it is necessary to multiply disease-free plants, or maintain rapid multiplication and/or particular genotypes, (Chirumamilla, et al., 2021).

Potato is an important example because the technique is used for the rapid multiplication of pathogen-free plants and production of planting stocks. Molecular analysis may help to verify genetic identity in the process of multiplication.

Another potential application of micropropagation is the conservation of particular genotypes of crops such as onion, garlic, asparagus, tomato, cucumber and other vegetables. It is especially critical to use molecular markers in case when plants are regenerated using routes that involve callus formation since the occurrence of somaclonal variation is possible.

21. Applications in Ornamental Crops

Ornamental crops are one of the most sensitive groups in terms of genetic stability testing because the commercial value of particular genotype is often related to highly specific visual traits (Hârța, and Clapa, 2022). Color, shape, arrangement, architectural form, flowering period and fragrance of flowers may determine the genotype of a plant. Small genetic or epigenetic changes may have commercial implications, (Tikendra et al., 2019).

The Saintpaulia example provides a clear demonstration of how tissue culture can result in stable flower-colour variation associated with molecular changes. (Sato et al 2011). showed that tissue culture-induced transposon excision could alter expression of the flavonoid 3',5'-hydroxylase gene and consequently affect flower pigmentation. Molecular fidelity testing is consequently highly relevant to orchids, rose, chrysanthemum, carnation, gerbera and other high-value ornamental crops.

Gerbera

Gerbera represents a valuable ornamental crop where flower quality and uniformity play key role in economic success. Bhatia et al. (2009) proved that ISSR markers were able to differentiate a rare variant among micropropagated gerberas. It should be noted that in this study genetic stability was found to depend on the explant source. Shoot-tip and capitulum-derived plants showed high uniformity while one leaf-derived regenerant was genetically different. The findings support the general principle that regeneration pathway and explant choice should be considered during genetic-fidelity assessment.

Orchids

Orchids are one of the most significant ornamental plants for micropropagation because regular multiplication is quite time-consuming, and seedling development may require specific conditions.

(Tikendra et al. 2019) studied the use of RAPD and ISSR markers to analyze micropropagated Dendrobium chrysotoxum. It was found that 96.30% of monomorphism and 3.6% polymorphism was observed in all studied plants and mother plant with minimal Nei genetic distance and high regenerants grouping with the donor. The research proves the significance of using both RAPD and ISSR markers at once instead of a single marker system.

22. Applications in Medicinal and Aromatic Plants

Micropropagation technology has increased its relevance to medicinal and aromatic plants due to the slow growth rates and difficulties in propagation of certain species or their threat from over-harvesting. Genetic identity of planting material becomes particularly important for medicinal species where the activity depends on the concentration of certain biochemicals. Regenerants with the different genetics can have the changed concentrations of secondary metabolites. Consequently, molecular markers are also needed besides biochemical analyses in order to ensure genetic identity and consistency of phytochemical composition, (Mathe et al., 2015). The genetic stability of *in vitro*-propagated *A. vera* plants was evaluated using 10 inter-simple sequence repeat (ISSR), 10 start codon targeted (SCoT), and 10 simple sequence repeat (SSR) markers. Among these, 5 ISSR, 3 SCoT, and 6 SSR primers produced resolvable, reproducible, and scorable bands. The ISSR primers generated 32 amplification products ranging from 200 to 1040 bp, with a mean of 6.4 bands per primer, whereas the SCoT primers generated 33 amplicons ranging from 160 to 2410 bp, with an average of 11 bands per primer. The SSR primers produced a total of 6 bands, with each primer generating one band ranging from 410 to 720 bp. All the banding profiles were monomorphic, confirming the genetic stability of the *in vitro*-propagated *A. vera* plants. The study demonstrated the suitability of supplementing the *in vitro* culture medium with *Aloe vera* gel (AvG) to enhance direct regeneration from shoot-tip explants of *A. vera* and support its large-scale micropropagation, (Hamdeni et al., 2022).

23. Applications in Plantation and Woody Horticultural Crops

Micropropagation of woody horticultural crops is usually more difficult than that of herbaceous species due to problems with phenolic oxidation, browning of explants, recalcitrance and lengthy regeneration periods. However, tissue culture technology has practical importance for some genotypes of plantation and tree crops, (Aziz et al., 2020).

24. The Effect of Cultivation Technique on Genetic Stability

The regeneration route has an immense effect on genetic stability. Direct development of shoots from axillary buds usually maintains the integrity of the plant and is, hence, usually preferred as the route to use when propagating plants clonally, (Naing et al., 2019).

In indirect regeneration, dedifferentiation and production of callus take place before the growth of shoots. Callus cells can be genetically unstable due to extensive cell reprogramming that takes place; therefore, callus culture can be considered risky as the route of true-to-type propagation.

Nonetheless, the effect is not universal since the genetic stability varies between genotypes, species and culture techniques. Some callus-based systems have been observed to generate genetically stable plants. Thus, molecular assessment is always better than making assumptions based on the regeneration route, (Bhardwaj et al., 2025).

Table-5: Somaclonal Variation and Genetic Stability in Different Plant Species

Plant Species	Tissue Culture Method/Explanants	Culture Medium	Cultivar/Genotype	Cycles	Culture Conditions	Key Findings	Reference
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<i>Citrus jambhiri</i> (Orange)	Leaf, nodal, root segments, stigmas, styles, ovaries, nucellar tissues, cotyledons, juice vesicles	MS medium with BAP, KN, ME, 2,4-D for callus induction; BA, 2,4-D, ABA for somatic embryo induction, sucrose for germination	Nanjanagudu Rasabale	Not specified	Controlled Environment	Callus was induced from most explants except styles and juice vesicles; somatic embryos formed from ovary and nucellar tissues with BA and ME, and 20–30% variation in ovary-derived plantlets.	(Savita, et al., 2015)
<i>Vaccinium vitis-idaea</i> (Lingonberry)	Juvenile leaf explants from two genotypes	Berry basal medium + 5.5 μ M thidiazuron for callus induction; 4.0 μ M zeatin for plantlet regeneration	Not specified	Not specified	Standard in vitro conditions	92% embryogenic callus induction with thidiazuron, enhanced plantlet regeneration with 4.0 μ M zeatin, confirmed somatic embryo development, and demonstrated increased flavonoids, anthocyanins, antioxidants, stress resistance.	(Kundu, et al., 2025)
<i>Musa acuminata</i> (Banana)	Leaf base explants	MS medium with high levels of BAP (up to 53.28 μ M) and kinetin (55.80 μ M) for shoot proliferation	Not specified	Not specified	Standard in vitro conditions	High cytokinin levels (BAP and kinetin) produced up to 80 shoots per segment and ensuring genetic stability for conserving the endangered NR cultivar.	(Shukla, et al., 2025)
<i>Aloe vera</i> (Aloe vera)	Axillary buds (direct); Inflorescence via callus	Not specified	Sweet genotype	Not specified	In vitro regeneration with and without callus	Axillary buds showed 0% polymorphism (true to type), while indirect regeneration via callus from the inflorescence base exhibited 80%	(Rathore, et al., 2011)

						polymorphism	
<i>Ficus palmate</i> (fig)	Leaves and stems	MS basal medium, 2,4-D (2.0 mg/L) + Kin (0.2 mg/L)	Not specifically named	Not mentioned	Gamma radiation at 0, 20, 30, 40 Gy	Highest callus induction at 30–40 Gy. A total of 99.58% polymorphism due to gamma treatment, yet tissue culture plants remained genetically similar to mother plants. GC-MS revealed increased secondary metabolites at 30 and 40 Gy.	(Eissa, et al., 2021)
<i>Carica papaya</i> (Papaya)	Apical, nodal, petiole, leaf, and root segments	MS basal medium, BAP, Zeatin, NAA (direct); MS + BAP (1.0 mg/L), TDZ (0.3 mg/L), NAA (0.10 mg/L), 30 g/L sucrose (indirect)	Red Lady 786	Not specified	Standard in Vitro conditions	Direct regeneration with 65–88% success; indirect regeneration with 75–85% somatic embryogenesis ; efficient medium for somatic embryos with TDZ, BAP, NAA, and sucrose.	(Vishal, et al., 2024)
<i>Dendrobium fimbriatum</i> (Orchid)	Not specified	supplemented with KN (0.8–4.8 mg/L), IBA, NAA, or BAP for regeneration	Not specified	Not specified	Standard in Vitro conditions	High shoot multiplication; root development was achieved with KN, IBA, and BAP/KN combinations, while low genetic polymorphism was observed.	(Tikendra, et al., 2021)
<i>Rubus idaeus</i> (Red Raspberry)	Leaf segments	Liquid medium + 2.3–9.0 µM thidiazuron (TDZ) for regeneration, 4.4 µM BA for shoot elongation	‘Latham’, ‘Heritage’, ‘Festival’, ‘Nova’	Not specified	Bioreactor system, controlled Field conditions	Tissue culture plants of ‘Latham’ and ‘Festival’ outperformed root cutting (RC) plants in cane and berry yields, with clonal fidelity observed. In contrast, bioreactor	(Debnath, 2014).

		, PGRs for rooting				culture influenced juvenile branching, promoting growth and berry production.	
<i>Malus domestica</i> (Apple Rootstock MM 104)	Not specified	MS basal medium, 3.0% sucrose, 0.8% agar, BA (5.0 µM), NAA (1.0 µM) for bud establishment, PGRs BA (1.0 µM) + NAA (1.0 µM) for shoot multiplication, IBA (0.1 µM) for rooting	MM 104	Not specified	5-day dark period for rooting, acclimatization in vermiculite: perlite: sand: soil (2:2:1:1) Mix	In vitro culture (100%) with 9.8 shoots/explant and 76% survival under field conditions.	(Bisht, et al., 2024)
<i>Citrus mitis</i> (Calamondin)	Style-stigma explants	BAP (6 µM), 4-CPPU, PBU, 2,3-MDPU (12 µM each), hormone-free (HF) conditions	Not specified	Not specified	In vitro Culture	Phenylurea derivatives (4-CPPU, PBU, 2,3-MDPU) enhanced embryogenic potential, with 2,3-MDPU and PBU causing 3.7% somaclonal variation, while BAP/HF medium showed no variability.	(Siragusa, et al., 2007)
<i>Coffea arabica</i>	Not specified	MS + 0.4–0.6 mg/L	Not specified	Not specified	In vitro Culture followed by field testing	Efficient somatic embryogenesis was achieved. in both hybrids, with SRAP and SCoT marker analyses showing high genetic similarity to mother plants.	(Bychappa, et al., 2019)

(Source, Majumder, et al., 2025)

Table-6: Somaclonal Variation and Genetic Stability in Different Plant Species

Plant name	Explants	Media composition and PGRs	Genotype/ Cultivar	Subculture cycles	Environmental factors	Key findings	Reference
<i>Stevia rebaudiana</i> (Candy leaf)	Not specified	MS + BAP (4.0 mg/L), NAA (2.0 mg/L), 2,4-D (2.0 mg/L)	Not specified	6 subcultures	Standard in vitro conditions	Indirect organogenesis induced somaclonal variation, improving rebaudioside A content and the rebaudioside A/stevioside ratio. SCoT analysis showed genetic variation, whereas subculturing had no significant effect on genotype or glycoside profile.	(Dyduch-Siemińska, et al., 2024)
<i>Hevea brasiliensis</i> (Rubber tree)	Mixed floral explant	MS + BA (2.0 mg/L) + 2,4-D (1.5 mg/L); germination on MS + GA ₃ (0.25 mg/L)	Early introduced clone, white-root-disease resistant	3 passages (4 weeks each)	Standard in vitro conditions	Secondary somatic embryogenesis produced 39.84% somatic embryo induction and 3.25 cotyledonary embryos per callus. About 50% developed embryonic axes and 25% formed shoots, with genetic stability confirmed.	(Tongtape, et al., 2023)
<i>Musa acuminata</i> ‘Grand Naine’ (Banana)	Individual shoot apex; transverse sections	MS + BAP (10, 20 or 30 µM) + NAA (1.0 µM) for proliferation; MS + IBA (6 µM) for rooting	‘Grand Naine’	Monthly subcultures for 6 months	Standard in vitro conditions	Transverse sectioning of the shoot apex improved shoot production, reaching up to 32.77 shoots per section after 6 months. No genetic variation was detected in plants derived from transverse sections, whereas 23.46% polymorphism was detected in plants regenerated from whole shoot apices.	(Safarpour, et al., 2017)

<i>Vanilla planifolia</i> (Vanilla)	Immature seeds	MS + TDZ (2.27 μ M) for callus induction; MS + BA (8.88 μ M) for shoot regeneration; MS without PGRs for rooting	<i>Vanilla planifolia</i>	Not specified	Standard in vitro conditions	Indirect organogenesis was reported from immature seed-derived cultures, demonstrating the influence of PGR combinations on callus induction and shoot regeneration.	(Ramírez-Mosqueda, et al., 2015)
<i>Vaccinium vitis-idaea</i> (Lingonberry)	Leaf, node and cutting explants	NC1, NC2, NC3 and LC1 culture media	Not specified	Multiple subcultures, in vitro and ex vitro	Tissue culture conditions	Culture-derived plants showed higher methylation than cutting-propagated plants. NC1, NC2, NC3 and LC1 cultures exhibited 108 methylation bands compared with 79 bands in cutting-propagated plants. Some culture-derived plants also showed higher secondary metabolite production.	(Sikdar, et al., 2022)
<i>Coffea arabica</i> (Coffee)	Embryogenic suspensions	Low 2,4-D concentrations with short proliferation periods	Erntedank cultivar	6 months of culture	Tissue culture conditions	Somaclonal variation was very low (0.74%), with minimal genetic polymorphism. Chromosome loss occurred in rare variants, and no correlation was observed between phenotype and MSAP patterns.	(Bobadilla Landey, et al., 2013)
<i>Vaccinium</i> spp. (Lowbush blueberry)	Softwood cutting (SC), tissue culture (TC)	Standard tissue culture medium	Wild clone QB9C, cultivar Fundy	Not specified	Conventional propagation for SC; in vitro culture for TC	Tissue-culture-derived plants showed higher DNA methylation (29% in TC-QB9C and 20% in TC-Fundy) than softwood-cutting plants (25% and 19%, respectively). Polymorphism was detected only in TC plants.	(Goyali, et al., 2018)

<i>Fragaria × ananassa</i> (Strawberry)	Shoot tips	Standard tissue culture medium	<i>Fragaria nilgerrensis</i>	6 stages (from explants to acclimation)	Tissue culture conditions	Dedifferentiation and redifferentiation stages affected genes associated with hormone metabolism, development and stress responses, and were linked with epigenetic variation.	(Cao, et al., 2021)
<i>Ananas comosus</i> (Pineapple)	Callus culture-derived plants	Standard tissue culture medium	Somaclonal variation (SV) plants	Not specified	Controlled in vitro conditions	DNA methylation changes were observed in somaclonal-variation plants, with significant differences in methylation patterns between SV plants and cutting seedlings (CK).	(Lin, et al., 2019)
<i>Lactuca sativa</i> (Lettuce)	Somatic tissues	Not specified	Major lettuce cultivars and wild relatives	Not specified	Controlled environment	Lettuce domestication resulted in a significant increase in DNA methylation. Epigenetic variation was associated with leafy and stem types and influenced gene expression and chromatin accessibility, contributing to their divergence.	(Cao, et al., 2024)
<i>Coffea canephora</i> (Robusta coffee)	Somatic tissues for somatic embryo (SE) induction	Not specified; 5-azacytidine used as an epigenetic inhibitor	Various accessions	Not specified	Controlled environment during SE induction and maturation	Dynamic changes in DNA methylation and histone modifications accompanied somatic embryogenesis, indicating an important role of epigenetic regulation during SE progression.	(Nic-Can, et al., 2013)

(Source, Majumder, et al., 2025)

The first approach toward minimization of any adverse variation is the selection of genetically homogeneous and superior mother plant. If the initial material is heterogeneous, the variation cannot be automatically eliminated in tissue culture. The second approach is the use of proper explants. Meristematic tissues and axillary buds are normally preferred if clonal integrity is the priority concern.

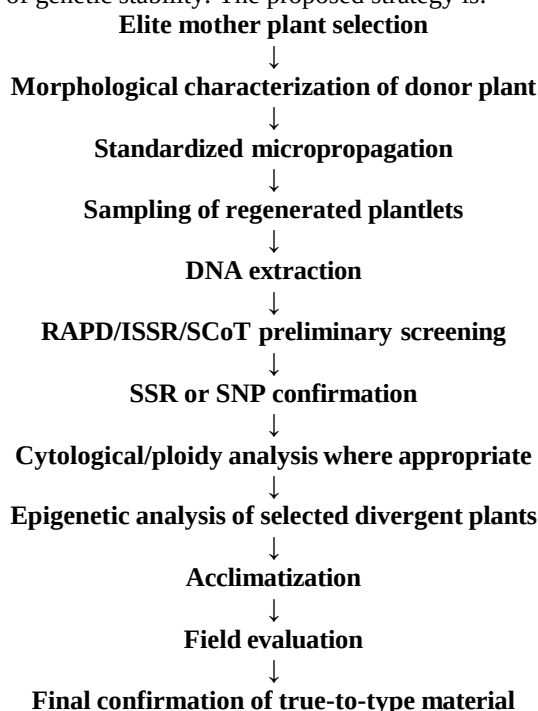
The third approach is the manipulation of plant growth regulators' concentration. Over stimulation by hormones must not occur if abnormal growth and extended dedifferentiation takes place.

The fourth approach is to limit the unnecessary production of callus and minimize the number of subcultures.

Prolonged culture must be avoided if a satisfactory multiplication rate can be accomplished within a shorter procedure. The fifth approach is the molecular analysis of regenerants at specific intervals. This enables laboratories to identify new variation before they distribute plants for commercial purposes.

25. Integrated Strategy for Genetic Fidelity Assessment

A reliable genetic-fidelity programme should not depend exclusively on one analytical method. An integrated approach can provide stronger evidence of genetic stability. The proposed strategy is:



26. Future Perspectives

In the future, the assessment of genetic fidelity will more and more tend towards high resolution genomics analysis. SNPs, genotyping-by-sequencing and whole genome sequencing can reveal rare mutations that cannot be detected by conventional marker assays.

Long reads sequencing might bring additional knowledge about structural changes and genome rearrangements. Methylation sequencing may clarify whether differently phenotyped plants have different epigenetics despite very similar DNA sequence.

Also, artificial intelligence and machine learning methods may find their applications in assessing plant genetic fidelity. High resolution photos of leaves, stems, flowers and fruits could be analyzed in order to detect possible morphological off-type plants. These systems could be eventually combined with molecular diagnosis.

Another prospective field for the future development is the creation of molecular marker panels for certain crops. Instead of checking tens of random primer pairs, a laboratory could test validated SSR or SNP markers chosen specially for the given cultivar or species. The integration of digital phenotyping, molecular markers, genomics and epigenomics may become a powerful quality control system for commercial micropropagation of plants.

27. Integrated Quality-Control Strategy

Genetic fidelity should ideally be treated as a quality-control process rather than a single laboratory test.

Table 3. Integrated quality-control system

Stage	Recommended assessment	Purpose
Mother plant	Morphological + molecular fingerprint	Establish reference genotype
Explant establishment	Health and contamination assessment	Ensure quality starting material
Early multiplication	Molecular screening of representative samples	Detect early variation
Repeated subculture	Molecular monitoring	Identify culture-associated changes
Rooting	Morphological assessment	Identify abnormal plantlets
Acclimatization	Morphology + molecular testing	Confirm stable regenerants
Nursery	Molecular confirmation	Prevent off-type distribution
Field establishment	Morphological evaluation	Confirm horticultural uniformity
Long-term conservation	Periodic molecular assessment	Monitor genetic stability

28. Research Gap

Despite significant advancements that have been made to date, a number of gaps in research are still remaining. Firstly, there is no universally standardized molecular protocol available for evaluation of genetic fidelity of horticultural crops. The number of primers used in different studies varies; so does the sample size and the

statistical approach.

Secondly, there are quite a lot of studies analysing only a few number of regenerated plants. As somaclonal variants can happen with a low frequency, larger sample size will be required for reliable estimation of genetic stability.

Thirdly, conventional DNA markers are not capable to detect all epigenetic modifications. Thus, future researches should consider increasing their usage in combination with sequence analysis.

Fourthly, many researches assess genetic stability of plants shortly after their regeneration, without long-term field cultivation for several years. Evaluation in the field is especially important for perennial horticultural crops. Lastly, many publications describe molecular polymorphism but fail to demonstrate whether the observed polymorphism results in any phenotypic or horticultural variation. Therefore, molecular profiling should be coupled with phenotypic and biochemical one in future researches.

29. CONCLUSION

Micropropagation is undoubtedly one of the most promising methods for the rapid propagation and conservation of elite genotypes of horticultural species. The successful use of the technique, however, requires not only obtaining the highest multiplication rates but also maintaining genetic identity of regenerants.

Somaclonal variation is a biological problem as it involves both genetic and epigenetic modifications that may occur during tissue culture. These modifications may be unwanted for commercial cloning of a cultivar but can prove beneficial in breeding programs.

Morphological, biochemical and cytological studies are important and provide valuable information, but the molecular markers are a more straightforward tool for analyzing genetic fidelity. The RAPD technique is still useful owing to its simplicity, while ISSR offers relatively reliable fingerprinting and does not require the prior knowledge of the DNA sequence. The SSR markers are the most reproducible and codominant, SCoT is the gene-targeted method. AFLP gives a possibility of covering the whole genome, and SNP and sequencing approaches allow genome-wide analysis.

Marker choice will depend upon the purpose of the research, the biology of the crop, availability of genomic resources and facilities, and resolution needed. In general terms, ISSR or SCoT will be useful for screening, while SSR or SNP markers would work better for cultivar authentication or reliable genetic identification. In the case of research that needs to detect rare genotypes genome wide, sequencing-based technology is needed.

The best approach would therefore be a combined strategy of micropropagation optimized, proper choice of mother plant, morphological analysis, suitable molecular marker analysis, and cytological and epigenetic analysis when necessary. This approach will help avoid distributing wrong plants for commercial use in horticulture.

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