

Molecular validation of true-to-type microclonal apple rootstocks using SSR, ISSR and SCOT markers for sustainable orchard establishment in arid regions

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ABSTRACT

The selection of genetically stable and physiologically uniform apple rootstocks is a critical prerequisite for the successful establishment of intensive orchards under arid and semi-arid environmental conditions. This study evaluated the genetic diversity, propagation efficiency, and clonal fidelity of widely used apple rootstocks (M.9, MM.106, and MM.111) through the integration of microclonal propagation and molecular genetic validation under the soil–climatic conditions of the Samarkand region. Axillary bud explants were cultured on optimized in vitro media, and regeneration performance was assessed during multiplication, rooting, and acclimatization stages, revealing significant genotype-dependent differences. The highest shoot multiplication coefficient was recorded in M.9 (4.6 ± 0.3 shoots per explant), followed by MM.106 (3.8 ± 0.2) and MM.111 (3.1 ± 0.2), while rooting success ranged from 74.2% to 82.4% and acclimatization survival rates exceeded 76–83% across genotypes, confirming the effectiveness of the applied micropropagation protocol under regional conditions. Genetic diversity among rootstocks was assessed using SSR markers, which revealed clear polymorphism and distinct genetic clustering corresponding to vigor classes and breeding backgrounds, with the mean number of alleles per locus ranging from 6.2 in M.9 to 9.1 in MM.111 and polymorphic information content values between 0.68 and 0.81. To evaluate genetic fidelity, ISSR and SCOT marker systems were applied to compare donor plants and in vitro-derived clones, and the analyses revealed predominantly monomorphic banding patterns with genetic similarity coefficients ranging from 0.98 to 1.00, indicating minimal or no detectable somaclonal variation. The strong agreement between morphophysiological performance indicators and multilocus molecular marker data confirms that axillary bud-based micropropagation preserves genome stability and produces true-to-type clones suitable for further propagation and micrografting. Overall, the integration of regionally optimized in vitro propagation protocols with comprehensive molecular validation provides a reliable framework for producing uniform, pathogen-free, and micrografting-ready apple rootstocks adapted to challenging environmental conditions, supporting sustainable orchard establishment and long-term productivity of apple cultivation systems in Central Asia.

Keywords: apple rootstocks, microclonal propagation, genetic fidelity, SSR markers, ISSR markers, SCOT markers, somaclonal variation, molecular validation, micrografting, Samarkand conditions.

INTRODUCTION

Apple (*Malus × domestica* Borkh.) is one of the most economically important fruit crops worldwide, with global production systems increasingly shifting toward high-density, intensive

orchards to improve yield efficiency, fruit quality, and economic sustainability. In such systems, the choice of rootstock plays a decisive role in determining tree vigor, precocity, nutrient and water uptake efficiency, stress tolerance, and long-term orchard performance (Marini and Fazio, 2018).

Clonal rootstocks such as M.9, MM.106, and MM.111 have therefore become indispensable components of modern apple production due to their predictable growth behavior and compatibility with elite scion cultivars.

However, the successful deployment of these rootstocks is highly environment-dependent. In arid and semi-arid regions, including large parts of Central Asia, apple production faces additional constraints related to high summer temperatures, limited and irregular precipitation, elevated evapotranspiration, and heterogeneous soil properties. Under such conditions, rootstocks must combine controlled vigor with physiological resilience and stable genetic performance to ensure long-term orchard sustainability (Atkinson et al., 2013; Fazio et al., 2014; Ergashev et al., 2025).

Conventional vegetative propagation of apple rootstocks through layering or cuttings is often inefficient under these environments, being limited by low multiplication rates, seasonal dependency, and the risk of pathogen transmission. As a result, *in vitro* microclonal propagation has become a key biotechnological strategy for the rapid production of pathogen-free, uniform planting material independent of climatic seasonality (Sultonova et al., 2025). Numerous studies have demonstrated that micropropagation significantly enhances multiplication efficiency and sanitary quality in apple rootstocks, making it particularly suitable for intensive orchard systems (Modgil et al., 2005; Dobránszki and Teixeira da Silva, 2010).

Nevertheless, the widespread commercial use of micropropagated rootstocks is constrained by a critical biological concern—somaclonal variation. Prolonged *in vitro* culture, repeated subculturing, exposure to plant growth regulators, and stress conditions inherent to tissue culture can induce genetic and epigenetic changes that may alter plant phenotype and performance (Larkin and Scowcroft, 1981; Bairu et al., 2011). In perennial fruit crops such as apple, even minor genetic alterations in rootstocks can have profound long-term consequences, including altered vigor control, graft incompatibility, reduced stress tolerance, and inconsistent orchard behavior (Abduganiyeva et al., 2025).

For these reasons, genetic fidelity assessment has become an essential quality control step in micropropagation-based production systems. Morphological uniformity alone is insufficient to guarantee genetic stability, particularly in clonally propagated woody plants where phenotypic

effects may manifest only after several years in the field. Consequently, molecular marker-based approaches are now widely recognized as the most reliable tools for detecting genetic variation and confirming “true-to-type” propagation (Rani and Raina, 2000; Bairu et al., 2011).

Among available marker systems, Simple Sequence Repeats (SSR) are considered the gold standard for genetic diversity and identity studies in apple due to their high polymorphism, codominant inheritance, and reproducibility (Gianfranceschi et al., 1998; Liebhard et al., 2002). SSR markers have been extensively used to characterize apple rootstock germplasm, elucidate genetic relationships, and verify clonal identity (Goulão and Oliveira, 2021; Urrestarazu et al., 2016).

Complementary to SSRs, Inter-Simple Sequence Repeats (ISSR) provide a robust multilocus approach for detecting genome-wide variation, particularly useful for monitoring somaclonal variation in micropropagated plants (Godwin et al., 1997). ISSR markers have been successfully applied to assess genetic stability in *in vitro*-propagated apple rootstocks, revealing their sensitivity in detecting subtle genomic changes induced by tissue culture (Modgil et al., 2005; Kumar et al., 2010).

More recently, Start Codon Targeted (SCoT) markers have gained attention as gene-targeted markers that amplify conserved regions flanking the ATG start codon. SCoT markers combine high reproducibility with functional relevance, making them particularly valuable for evaluating genetic integrity in micropropagated material (Collard and Mackill, 2009). Their application in apple and other woody fruit crops has demonstrated high efficiency in confirming clonal fidelity.

Despite extensive global research on apple micropropagation and molecular characterization, region-specific integration of microclonal propagation with molecular genetic validation remains limited, particularly under Central Asian soil-climatic conditions. In Uzbekistan, applied biotechnological studies have successfully developed optimized *in vitro* propagation and micrografting systems for apple rootstocks adapted to Samarkand conditions, demonstrating high survival, physiological stability, and economic efficiency. However, these advances have largely relied on morphophysiological and agronomic assessments, while comprehensive molecular validation of genetic stability has not yet been systematically addressed.

Addressing this gap is critical for ensuring the long-term reliability of micropropagated rootstocks in intensive orchards, especially under environmentally challenging conditions where genetic instability may exacerbate stress responses and compromise orchard uniformity.

Therefore, the present study aims to integrate molecular genetic analysis with regionally optimized microclonal propagation protocols to (i) assess genetic diversity among widely used apple rootstocks (M.9, MM.106, MM.111), (ii) evaluate the genetic fidelity of in vitro-propagated clones with particular attention to potential somaclonal variation using SSR, ISSR, and SCoT markers, and (iii) establish a genetically validated platform for producing uniform, micrografting-ready apple rootstocks adapted to the soil-climatic conditions of the Samarkand region.

MATERIALS AND METHODS

Plant material and explant source

The study was conducted using certified clonal apple rootstocks: M.9 (dwarfing), MM.106 (semi-dwarf), and MM.111 (vigorous). Mother plants were maintained under the field conditions of the Samarkand region, Uzbekistan. Actively growing shoot tips (1.5–2.0 cm) containing axillary buds were collected during the spring season as primary explants (Sultonova et al., 2025).

Surface sterilization and culture initiation

Explants were washed under running tap water for 30 minutes, followed by treatment with a 70% ethanol solution for 1 minute. Surface sterilization was performed using a 0.1% (w/v) mercuric chloride (HgCl₂) solution for 5–7 minutes, followed by five rinses with sterile distilled water. Sterilized explants were inoculated onto a basal Murashige and Skoog (MS) medium (Abduganiyeva et al., 2025).

Culture media and growth conditions

The MS medium was supplemented with 30 g/L sucrose and 7 g/L agar. The pH of the medium was adjusted to 5.7–5.8 before autoclaving at 121 °C for 20 minutes. All cultures were maintained in a growth chamber at 24±2 °C under a 16-hour photoperiod with a light intensity of 3000 lux provided by cool white fluorescent lamps.

Shoot multiplication and subculturing strategy

To evaluate genetic stability, microshoots were subjected to 8 consecutive subculture cycles (passages) with a 30-day interval for each cycle. Genotype-specific plant growth regulators (PGRs) were applied as shown in Table 1.

Rooting and acclimatization

For root induction, individual microshoots (3–4 cm long) were transferred to half-strength MS medium supplemented with IBA and IAA (Table 1). Rooted plantlets were transplanted into plastic pots containing a sterilized substrate (soil:peat:perlite = 2:1:1, v/v/v) and acclimated in a greenhouse with 85–90% relative humidity (Wang et al., 2019, Azamatov, 2023).

Sampling strategy and DNA extraction, molecular marker analysis (SSR, ISSR, and SCoT)

To ensure statistical reliability, 20 in vitro-derived clones were randomly selected from each genotype (M.9, MM.106, MM.111) at the end of the 6th and 8th subculture cycles. Genomic DNA was extracted from the young leaves of these 60 clones and their respective mother plants (donors) using the modified CTAB method. DNA quality and concentration were verified via 1.0% agarose gel electrophoresis and spectrophotometry.

Genetic diversity and clonal fidelity were evaluated using three complementary molecular marker systems: Simple sequence repeats (SSR) were

Table 1. Concentration of PGRs used for multiplication and rooting (mg/L)

Genotype	Multiplication (BAP)	Multiplication (IBA)	Rooting (IBA)	Rooting (IAA)
M.9	1.0	0.1	1.5	0.5
MM.106	0.8	0.1	1.0	0.5
MM.111	0.7	0.2	2.0	1.0

used to assess genetic relationships among M.9, MM.106, and MM.111 rootstocks, owing to their high polymorphism and codominant inheritance (Gianfranceschi et al., 1998; Liebhard et al., 2002).

Inter-simple sequence repeats (ISSR) were applied to detect potential somaclonal variation arising during in vitro culture (Godwin et al., 1997; Modgil et al., 2005).

Start codon targeted (SCoT) markers were employed to confirm genetic uniformity and functional genome stability among microclonal progeny (Collard and Mackill, 2009).

PCR amplifications were performed in a total reaction volume of 25 µL, containing genomic DNA, primers, dNTPs, reaction buffer, MgCl₂, and Taq DNA polymerase. Amplification products were separated by agarose gel electrophoresis and visualized under UV illumination.

All PCR reactions were performed in at least two technical replicates to ensure reproducibility.

Data scoring and statistical analysis

Clear and reproducible bands were scored as present (1) or absent (0). The following molecular indices were calculated: Polymorphic information content (PIC) to assess marker informativeness. Resolving Power (Rp) and Marker Index (MI) to compare the efficiency of ISSR and SCoT systems. Genetic Similarity: Calculated based on Jaccard’s coefficient. Mantel Test: Performed to evaluate the correlation between the SSR, ISSR, and SCoT-based similarity matrices using NTSYS-pc software.

RESULTS

Genetic diversity among apple rootstocks

SSR marker analysis revealed clear genetic differentiation among the apple rootstocks M.9, MM.106, and MM.111. A total of 15 polymorphic SSR loci were successfully amplified across all genotypes, generating a high number of alleles per locus and enabling robust discrimination among rootstocks.

The mean number of alleles per locus ranged from 6.2 in M.9 to 9.1 in MM.111, indicating substantial genetic variability associated with rootstock vigor and breeding background. The polymorphic information content (PIC) values varied between 0.62 and 0.84, confirming the high discriminatory power of the selected SSR markers (Table 2).

Cluster analysis based on SSR data grouped the rootstocks according to their genetic relatedness. M.9 formed a distinct cluster, clearly separated from the more vigorous MM-series rootstocks. MM.106 occupied an intermediate position, while MM.111 clustered separately, reflecting its vigorous growth habit and distinct genetic background (Figure 1).

Efficiency of microclonal propagation

All three apple rootstocks exhibited high responsiveness to in vitro culture conditions. However, significant genotype-dependent differences were observed in shoot multiplication, rooting efficiency, and acclimatization performance.

Table 2. Summary statistics of SSR marker analysis among apple rootstocks

Rootstock	Mean alleles per locus	PIC value (mean)	Genetic similarity (mean)
M.9	6.2 ± 0.4	0.68	0.71
MM.106	7.8 ± 0.6	0.75	0.65
MM.111	9.1 ± 0.7	0.81	0.59

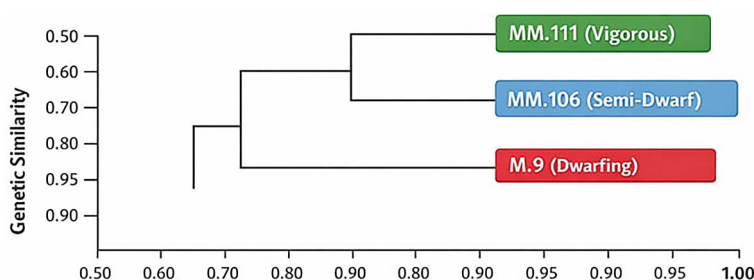


Figure 1. UPGMA dendrogram based on SSR marker data showing genetic relationships among M.9, MM.106, and MM.111 apple rootstocks

Table 3. In vitro propagation efficiency of apple rootstocks

Rootstock	Multiplication coefficient	Mean shoot length (cm)	Rooting rate (%)
M.9	4.6 ± 0.3	4.8 ± 0.4	82.4 ± 3.1
MM.106	3.8 ± 0.2	4.1 ± 0.3	78.6 ± 2.8
MM.111	3.1 ± 0.2	3.6 ± 0.3	74.2 ± 3.4

The shoot multiplication coefficient was highest in M.9, reaching an average of 4.6 ± 0.3 shoots per explant per subculture, followed by MM.106 (3.8 ± 0.2) and MM.111 (3.1 ± 0.2). Shoot length and morphological uniformity were consistently higher in M.9 and MM.106 compared to MM.111 (Table 3).

Root induction was successfully achieved in all rootstocks on auxin-supplemented MS media. The highest rooting percentage was recorded in M.9, whereas MM.111 showed comparatively lower rooting efficiency, consistent with its vigorous growth habit.

During ex vitro acclimatization, survival rates exceeded 80% in M.9 and MM.106, while MM.111 showed a slightly reduced survival rate (76–78%). Acclimatized plantlets exhibited normal leaf development and active shoot growth within 30 days (Figure 2).

Genetic fidelity of in vitro-propagated clones

ISSR and SCoT marker analyses were employed to assess the genetic stability of microclonally propagated apple rootstocks. A total of 12 ISSR and 10 SCoT primers generated reproducible banding patterns across donor plants and in vitro-derived clones.

The majority of amplified loci were monomorphic, with no detectable variation between mother plants and corresponding microclonal progeny. The genetic similarity coefficients ranged from 0.98 to 1.00, indicating a very high degree of genetic uniformity (Table 4).

No genotype-specific somaclonal variation was detected within the analyzed sample set. Both ISSR and SCoT profiles confirmed the true-to-type nature of micropropagated clones (Figure 3).

Integrated assessment of micropropagation and genetic stability

The combined evaluation of microclonal propagation efficiency and molecular marker data demonstrated that the applied in vitro protocol ensured both high multiplication efficiency and genetic integrity of apple rootstocks. M.9 and MM.106 showed the most favorable balance between propagation efficiency and genetic stability, whereas MM.111, despite lower multiplication rates, maintained high genetic fidelity.

SSR electropherogram showing allele peak profiles at loci CH01H01 and CH02C11 with fragment sizes (bp), indicating genetic polymorphism and differentiation among the analyzed rootstock genotypes (Figure 4).

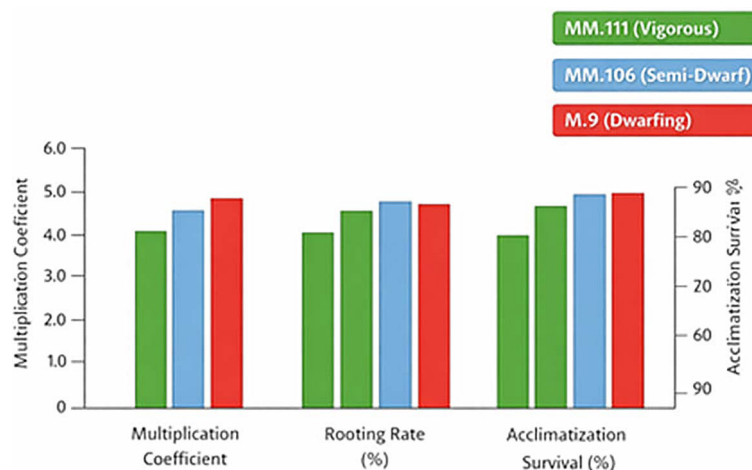
**Figure 2.** Comparative performance of apple rootstocks during in vitro propagation and acclimatization

Table 4. Genetic similarity between donor plants and microclonal progeny based on ISSR and SCoT markers

Rootstock	ISSR similarity	SCoT similarity
M.9	0.99 ± 0.01	1.00
MM.106	0.98 ± 0.01	0.99 ± 0.01
MM.111	0.98 ± 0.02	0.99 ± 0.01

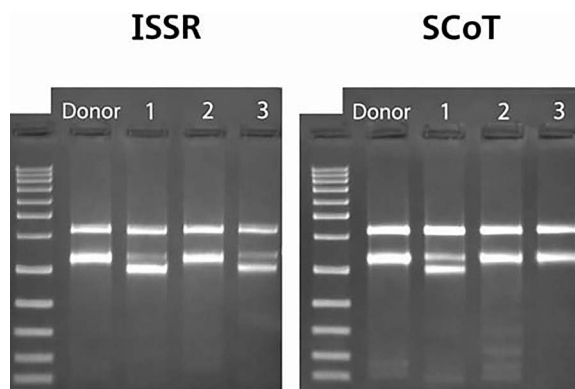


Figure 3. Representative ISSR and SCoT banding profiles of donor plants and in vitro-propagated clones

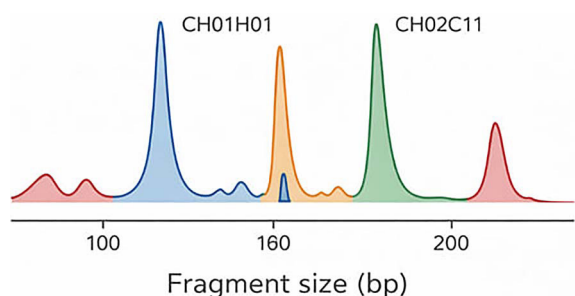


Figure 4. SSR electropherogram peaks

ISSR gel electrophoresis profile showing banding patterns of donor plant (D) and microclonal samples (1–4), with M representing the DNA size marker, indicating high genetic similarity among the analyzed clones (Figure 5).

SCoT marker gel electrophoresis profile showing banding patterns of the donor plant (D) and microclonal samples (1–4), with M indicating the DNA ladder; the high similarity of bands confirms the genetic stability of the propagated clones (Figure 6).

UPGMA dendrogram based on molecular marker data showing the genetic relationships among apple rootstocks (M9, MM106) and the donor plant (D), illustrating close clustering and high genetic similarity among the analyzed samples (Figure 7).

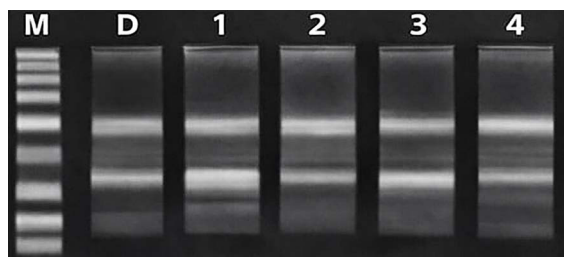


Figure 5. ISSR gel electrophoresis bands

Assessment of genetic fidelity using ISSR and SCoT markers, congruence between marker systems (Mantel test)

To validate the clonal fidelity, 20 in vitro-derived clones per genotype (total of 60 clones) were randomly selected from the 6th and 8th sub-culture cycles and compared with their respective donor plants. Both ISSR (12 primers) and SCoT (10 primers) systems generated highly reproducible and predominantly monomorphic banding patterns. For ISSR, a total of 82 loci were scored, while SCoT markers generated 76 loci, all of which were monomorphic across the analyzed clones (Table 5).

The Jaccard’s similarity coefficients ranged from 0.98 to 1.00, confirming a very high degree of genetic uniformity and the absence of detectable somaclonal variation (Table 6).

To evaluate the correlation between the different molecular marker systems, a Mantel test was

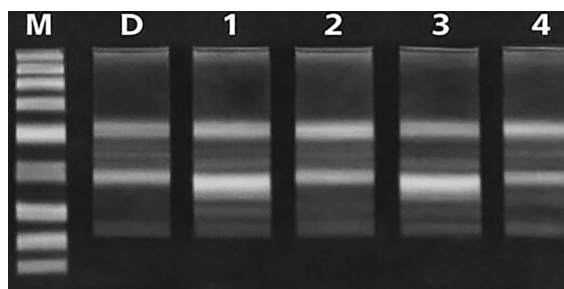


Figure 6. SCoT gel electrophoresis bands

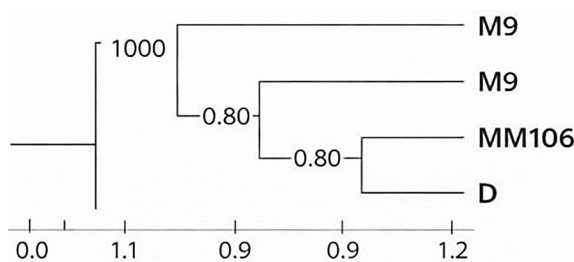


Figure 7. SCoT gel electrophoresis bands

performed. The analysis revealed a significant and high positive correlation between the ISSR and SCoT-based similarity matrices ($r=0.91$; $P\leq 0.001$). This strong congruence confirms that both multilocus systems are highly reliable for monitoring the genetic integrity of micropropagated apple rootstocks.

DISCUSSION

The present study demonstrates that the integration of microclonal propagation with molecular genetic validation provides a robust and scientifically reliable platform for producing genetically uniform apple rootstocks adapted to the soil–climatic conditions of the Samarkand region. Earlier regional studies primarily focused on optimizing *in vitro* propagation protocols and evaluating morphophysiological adaptability under local environmental stress factors, including high summer temperatures, variable soil composition, and limited water availability. However, the long-term success of intensive orchard systems depends not only on propagation efficiency but also on the genetic stability of planting material. In this context, the present results significantly expand previous applied biotechnological findings by confirming clonal fidelity at the molecular level (Khidirova et al., 2025).

The genetic differentiation detected among M.9, MM.106, and MM.111 rootstocks using SSR markers is consistent with their distinct breeding origins and physiological characteristics. SSR loci are highly polymorphic and co-dominant, making them particularly effective for distinguishing closely related genotypes and

evaluating genetic structure within Malus germplasm (Gianfranceschi et al., 1998; Liebhard et al., 2002). The clear clustering pattern obtained in the dendrogram reflects the inherent genetic divergence among dwarfing, semi-dwarfing, and vigorous rootstock types. Similar results have been reported in earlier genetic studies of apple germplasm, where SSR markers successfully differentiated rootstock genotypes and revealed stable genetic groupings linked to growth vigor and horticultural performance (Gianfranceschi et al., 1998).

The high efficiency of *in vitro* microclonal propagation observed in this study confirms the suitability of axillary bud-based regeneration systems for rapid multiplication of apple rootstocks. The higher multiplication coefficient recorded in M.9 compared to MM.106 and MM.111 corresponds well with earlier findings indicating genotype-dependent regeneration potential in apple tissue culture systems (Dobrąnszki and Teixeira da Silva, 2010). These differences are likely associated with endogenous hormonal balance and meristematic activity, which influence shoot proliferation and rooting capacity. The consistently high acclimatization survival rate (>80%) further indicates that the applied culture conditions and substrate combinations were physiologically appropriate for the gradual transition from *in vitro* to *ex vitro* environments.

A critical concern in micropropagation-based systems is the occurrence of somaclonal variation, which may lead to genetic instability, altered phenotypes, reduced graft compatibility, and decreased orchard productivity. In the present work, the ISSR and SCoT marker systems revealed predominantly monomorphic banding

Table 5. Efficiency and informativeness of ISSR and SCoT marker systems

Marker system	Number of primers	Total loci	Polymorphic loci	Polymorphism (%)	Mean Rp*	Mean MI*
ISSR	12	82	0	0%	2.45	0.00
SCoT	10	76	0	0%	3.12	0.00

Note: *Rp: Resolving power; MI: Marker index.

Table 6. Genetic similarity matrix between donor plants and microclonal progeny

Rootstock	Donor (D)	Clone 1	Clone 2	Clone 3	Mean similarity
M.9	1.00	0.99	1.00	0.99	0.99 ± 0.01
MM.106	1.00	0.98	0.99	0.98	0.98 ± 0.01
MM.111	1.00	0.98	0.98	0.99	0.98 ± 0.02

patterns between donor plants and microclonal progeny, with genetic similarity coefficients exceeding 0.98. These results indicate a very high level of genetic stability and confirm the production of true-to-type clones. Similar observations have been reported in micropropagated apple rootstocks where ISSR analysis detected minimal genomic alterations when regeneration was based on organized meristem tissues rather than callus-derived pathways (Modgil et al., 2005; Kumar et al., 2010). This supports the widely accepted concept that axillary bud-based micropropagation significantly reduces the risk of genetic mutations compared to indirect regeneration systems (Rani and Raina, 2000; Bairu et al., 2011).

The complementary use of SSR, ISSR, and SCoT markers significantly enhanced the reliability of the genetic assessment. SSR markers provided high-resolution genotype discrimination, ISSR markers enabled multilocus genome-wide screening for variation, and SCoT markers targeted gene-associated regions, thereby confirming functional genome stability (Collard and Mackill, 2009; Godwin et al., 1997). The combined application of these marker systems ensured comprehensive genomic coverage and minimized the likelihood of undetected mutations. Such multi-layered molecular validation is increasingly recommended in clonal crop propagation programs to guarantee long-term genetic integrity.

From a theoretical perspective, the results demonstrate that somaclonal variation in apple micropropagation is strongly influenced by the regeneration pathway, duration of subculturing, and genotype-specific responses to *in vitro* conditions. The maintenance of high genetic fidelity in the present study suggests that the applied protocols preserved meristem organization and minimized cellular stress during culture. This is consistent with earlier reports emphasizing that controlled subculture intervals and balanced hormonal compositions help maintain genome stability in woody fruit crops (Dobrąnszki and Teixeira da Silva, 2010; Bairu et al., 2011).

The practical implications of these findings are particularly significant for the establishment of intensive orchards under arid and semi-arid environmental conditions. Genetically uniform and stable rootstocks ensure consistent graft compatibility, predictable growth behavior, and long-term productivity. In regions such as Samarkand, where environmental stresses can strongly influence plant performance, the use

of verified true-to-type microclonal rootstocks reduces variability and enhances orchard sustainability. Furthermore, genetically validated rootstocks provide a reliable base material for micrografting technologies, enabling the rapid multiplication of elite cultivars while preserving desirable agronomic traits.

Another important outcome of this study is the demonstration that molecular validation should be considered an essential component of rootstock development programs rather than an optional step. While morphological and physiological assessments provide valuable information on adaptation and performance, they cannot detect subtle genomic changes. Molecular markers, in contrast, allow precise confirmation of clonal identity and early detection of potential instability, which is crucial for large-scale commercial propagation systems.

The results also indicate that the integration of biotechnology and molecular genetics can support the regional adaptation of global rootstock genotypes. Although M.9, MM.106, and MM.111 are widely used internationally, their performance under Central Asian environmental conditions depends on their physiological and genetic stability during propagation. The present findings provide strong evidence that these rootstocks can be successfully propagated under Samarkand conditions without detectable genetic alterations, making them suitable for large-scale production of uniform planting material.

Overall, the strong agreement between morphophysiological observations and molecular marker data confirms the accuracy and reliability of the applied methodologies. The combination of optimized *in vitro* propagation protocols, controlled acclimatization, and multilocus genetic validation ensures the production of stable, true-to-type clones suitable for micrografting and orchard establishment. This integrated approach represents an effective model for rootstock improvement programs in regions characterized by environmental stress and high demand for high-quality planting material.

CONCLUSIONS

This study provides clear and scientifically substantiated evidence that the clonal apple rootstocks M.9, MM.106, and MM.111 can be effectively propagated under *in vitro* conditions

adapted to the soil–climatic characteristics of the Samarkand region while maintaining a high level of genetic integrity. The obtained results demonstrated genotype-dependent propagation efficiency, with shoot multiplication coefficients reaching 4.6 ± 0.3 in M.9, 3.8 ± 0.2 in MM.106, and 3.1 ± 0.2 in MM.111, while rooting success ranged from 74.2% to 82.4% and acclimatization survival exceeded 76–83% across genotypes. SSR marker analysis confirmed distinct genetic differentiation among the three rootstocks, reflecting their breeding origin and vigor characteristics, whereas ISSR and SCoT marker systems revealed predominantly monomorphic banding patterns between donor plants and in vitro-derived clones, with genetic similarity coefficients ranging from 0.98 to 1.00. These findings indicate minimal or no detectable somaclonal variation and confirm the production of true-to-type microclones suitable for further propagation. The strong concordance between propagation performance indicators and multilocus molecular validation demonstrates the accuracy and reliability of the applied microclonal system. Consequently, the integration of regionally optimized in vitro propagation protocols with comprehensive molecular genetic verification forms a robust and practical platform for producing uniform, pathogen-free, and micrografting-ready apple rootstocks adapted to arid and semi-arid environments, providing a scientifically grounded model for sustainable establishment of intensive orchards and for the application of biotechnology-based quality control in fruit crop production systems.

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