JOURNAL of Horticulture, Forestry and Biotechnology
Volume 29(2), 2025, 58-65
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

In vitro culture of Grasa de Cotnari cv. and evaluation of the genetic fidelity using SCoT markers

Monica HÂRŢA[1], Steliana Paula BARBU[2*], Doina CLAPA[1*], Doru PAMFIL[3]

[1] Faculty of Horticulture and Business in Rural Development, BIOCERA Research Centre, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, e-mail: monica.harta@usamvcluj.ro; doina.clapa@usamvcluj.ro
[2]Valea Călugărească Research, Development Institute for Viticulture and Vinification, e-mail: stelianap.barbu@gmail.com
[3] Romanian Academy, Cluj-Napoca Branch, 9 Republicii Street, 400015, Cluj-Napoca, Romania, e-mail: d.pamfil@gmail.com

* Corresponding author: doina.clapa@usamvcluj; stelianap.barbu@gmail.com

Manuscript received: 7 June 2025; revised: 20 June 2025; accepted: 21 June 2025

Abstract
In this study, eight variants (V1-V8) of Murashige and Skoog (MS) medium were utilized, supplemented with cytokinins such as kinetin (Kin), meta-topolin (mT), and 6-benzyladenine (BA), along with indole-3-butyric acid (IBA) as an auxin source: MS+0.5 mg/L Kin (V1), MS+0.75 mg/L Kin (V2), MS+0.5 mg/L mT (V3), MS+1 mg/L mT (V4), MS+0.2 mg/L BA (V5), MS+0.5 mg/L BA (V6), MS+0.2 mg/L BA+0.2 mg/L IBA (V7), and MS+0.5 mg/L BA+0.2 mg/L IBA (V8). The results indicated that the presence of Kin and mT led to the formation of the most elongated shoots in the following MS medium variants: 4.98 cm (V3), 4.81 cm (V2), 4.74 cm (V1), and 4.72 cm (V4), although there were no statistically significant differences among them. The highest number of shoots per explant was achieved on the MS+0.5 mg/L BA+0.2 mg/L IBA (V8) medium with 7.33 shoots. A genetic fidelity assessment of the regenerated plants was conducted using Start Codon Targeted (SCoT) markers, which confirmed their genetic stability. Optimizing the micropropagation protocol for Grasa de Cotnari is crucial for producing uniform and high-quality planting material.

Keywords: micropropagation, PGRs, Start Codon Targeted markers, *Vitis vinifera* L.

Introduction

Vitis vinifera L. is one of the most economically significant crops worldwide, both in terms of cultivated area and market value. Consequently, evaluating the potential of different genotypes for in vitro propagation is crucial [9]. This study investigated the interactions among various plant growth regulators (PGRs) during the in vitro multiplication phase of grapevine using Murashige and Skoog (MS) [17] medium, primarily focusing on the Grasa de Cotnari variety, a traditional cultivar recognized for its unique characteristics [26]. MS culture medium is the most widely used basal medium for *V. vinifera* micropropagation due to its balanced nutrient composition, which supports efficient shoot proliferation and rooting [29]. The cytokinins such as 6-benzyladenine (BA) and kinetin (Kin) are particularly relevant for promoting shoot multiplication. According to Kumsa (2020) [13] these PGRs enhance the rate of shoot proliferation and overall growth in various grapevine cultivars. The mechanism by which different cytokinins influence in vitro shoot proliferation and elongation has been highlighted in several studies, reinforcing the ongoing investigation of hormonal interactions in the propagation of grapevine species [16,19,13].

Kim et al. [12] emphasize the crucial role of in vitro culture in establishing virus-free and disease-free grapevine cultivation systems. They highlight the significance of optimizing the composition of culture media alongside combinations and concentrations of plant growth regulators (PGRs). Their findings indicate that the efficiency of in vitro propagation is significantly affected by genotype-specific responses to different types and concentrations of PGRs, reinforcing the necessity of selecting the most appropriate conditions for each variety.

Selecting appropriate culture media and hormonal balances not only helps optimize micropropagation protocols, but also ensures genetic fidelity of regenerated plants, which is essential for maintaining the integrity of the variety being propagated by vegetative multiplication [25,32].

Due to their simplicity, cost-effectiveness, and high polymorphism, SCoT (Start Codon Targeted) markers are valuable molecular tools in plant genetics and breeding. By targeting the conserved region

JOURNAL of Horticulture, Forestry and Biotechnology
Volume 29(2), 2025, 58-65
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

flanking the start codon, these markers enable the identification of genetic variations linked to specific traits without requiring prior sequence information. They have been widely employed in studies of genetic diversity, species identification, and the assessment of genetic fidelity in in vitro-propagated plants [14,3,24,4,31,1].

Consequently, the aim of this study was to determine the optimal type and concentration of plant growth regulators for mass propagation using in vitro culture, as well as to assess the genetic stability of in vitro-grown plants using SCoT markers in the grapevine variety Grasa de Cotnari.

Material and Method

In vitro shoots proliferation

The explants used in this experiment were excised from a six-week-old in vitro culture of the grapevine cultivar Grasa de Cotnari, which was maintained on MS supplemented with 0.5 mg/L BA, 3% (g/v) sucrose, and 5 g/L Plant agar, with the pH adjusted to 5.8.

For culture vessels, 720 mL glass jars (13.5 × 9 cm ø) with screw caps and ventilation holes (4 mm) were used, each containing 100 mL of culture medium. The basal medium was MS medium supplemented with various combinations and concentrations of cytokinins and auxins, resulting in eight culture variants as follows: MS + 0.5 mg/L meta-topolin (mT) (V1); MS + 1 mg/L mT (V2); MS + 0.5 mg/L Kin (V3); MS + 0.75 mg/L Kin (V4); MS + 0.2 mg/L BA (V5); MS + 0.5 mg/L BA (V6); MS + 0.2 mg/L BA + 0.2 mg/L indole-3-butyric acid (IBA) (V7); MS + 0.5 mg/L BA + 0.2 mg/L IBA (V8).

All components were added to the culture medium before autoclaving and adjusting the pH to 5.8. The culture media were autoclaved at 120°C for 20 minutes. Five explants, measuring 2–2.5 cm each, were inoculated into culture vessel of each variant of the culture. After inoculation, the in vitro cultures were incubated in a growth chamber under a 16-hour photoperiod, with a light intensity of 32.4 μmol $m^{-2}s^{-1}$ provided by Philips Core Pro LED tubes (1200 mm, 16 W, 865 CG, 1600 lm Cool Daylight). The environmental conditions were maintained at 23 ± 3°C and 50 ± 2% humidity.

After six weeks of in vitro culture, the average number of shoots per initial explant and the average shoot length were recorded.

The non-rooted shoots obtained from the eight variants of the culture media were subsequently subjected to ex vitro rooting and acclimatization in perlite, using mini-greenhouses (Versay, T1, dimensions 39 × 25 × 7.5 cm, PVC). After four weeks, the percentage of ex vitro rooting and simultaneous acclimatization was determined. All culture media components were purchased from Duchefa Biochemie BV (Haarlem, Netherlands).

Genetic Stability Assessment

Seven random plants from each culture media variant (V1-V8) were analyzed from the acclimatized plants to evaluate genetic uniformity and clonal fidelity compared to the mother plant. One gram of fresh leaves from each plant was collected to isolate DNA for SCoT genetic analysis. The extraction of total genomic DNA was performed using an Isolate II Plant DNA kit (Bioline, Germany) following the protocol described by the supplier company.

Seven SCoT primers were employed in this study, and PCR amplification reactions were conducted following the protocol outlined by Collard and Mackill [5]. To assess the reproducibility of the SCoT-PCR amplification results, the experiment was repeated twice for each SCoT primer used. The PCR-amplified products were separated according to the technical conditions specified by Hârţa et al. [10].

Data Analysis

Statistical analysis was performed using OriginPro (2021, OriginLab, Northampton, MA, USA). One-way ANOVA followed by Tukey's HSD posthoc test ($P \leq 0.05$) was performed to determine the statistically significant differences between the mean values of the analyzed morphological characteristics of vitro-plants. Values shown are means ± SE.

For the analysis of molecular markers, the electrophoretic gel images were evaluated using TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to determine the molecular weight (bp) range of the amplified SCoT monomorphic bands. Additionally, the number of amplified SCoT monomorphic bands was assessed in this study.

Results and Discussion

In vitro shoot proliferation

The results of this study indicated that the Grasa de Cotnari grapevine cultivar exhibited varying responses to in vitro cultivation under different PGR treatments (Fig. 1).

JOURNAL of Horticulture, Forestry and Biotechnology
Volume 29(2), 2025, 58-65
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

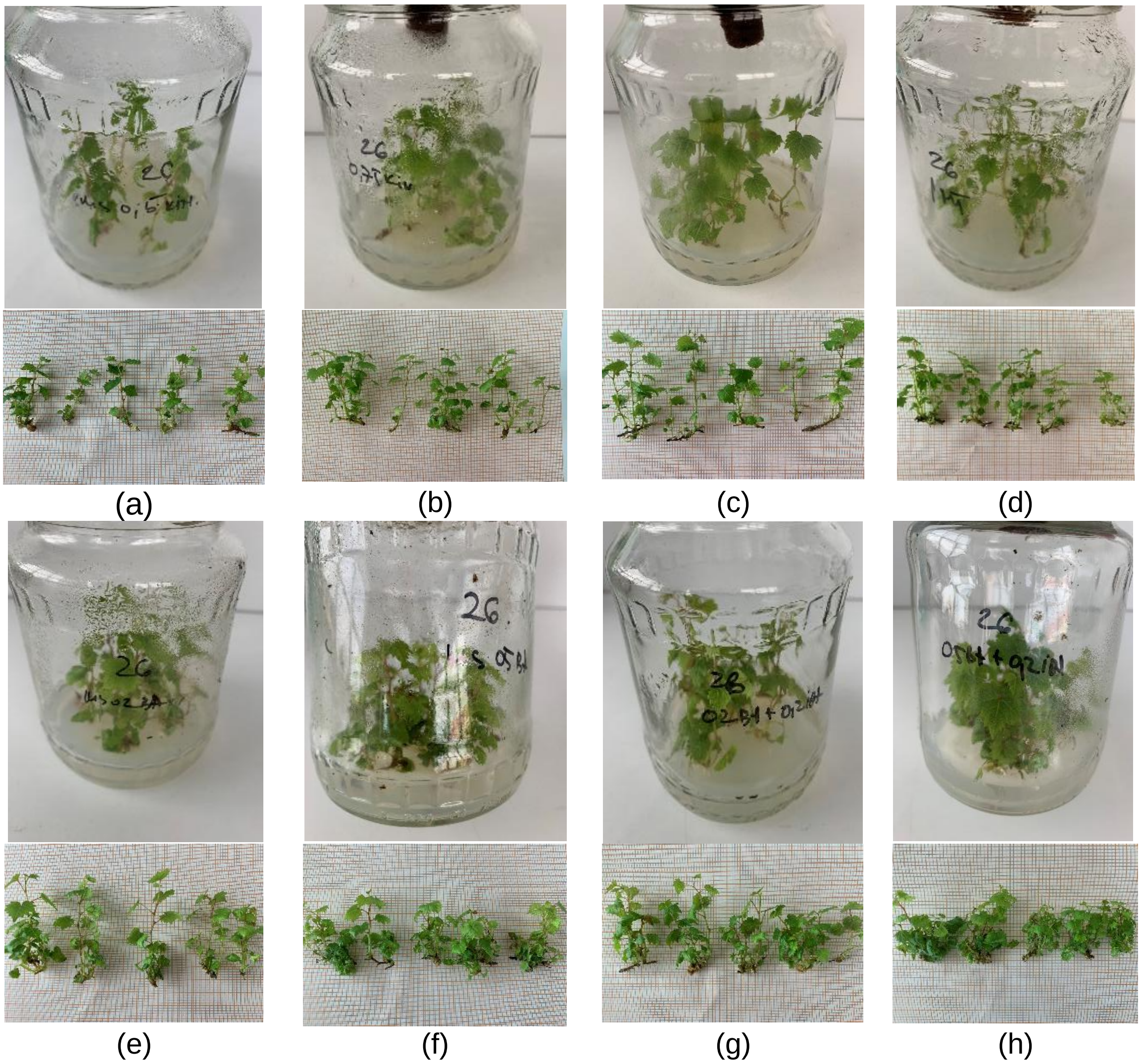


Figure 1. In vitro shoots proliferation of Grasa de Cotnari grapevine on MS medium supplemented with different auxins and cytokinins: (a) 0.5 mg/L Kin; (b) 0.75 mg/L Kin; (c) 0.5 mg/L mT; (d) 0.1 mg/L mT; (e) 0.2 mg/L BA; (f) 0.5 mg/L BA; (g) 0.2 mg/L BA + 0.2 mg/L IBA; (h) 0.5 mg/L BA + 0.2 mg/L IBA.

The highest number of shoots per explant was 7.33 ± 0.31, attained on MS medium supplemented with 0.5 mg/L BA + 0.2 mg/L IBA (V8), as illustrated in Fig. 2a.

Conversely, the lowest number of shoots per initial explant was 1.80 ± 0.14, recorded on MS medium supplemented with 0.5 mg/L Kin (V3). Furthermore, both kinetin (Kin) and meta-topolin (mT) were found to be less favorable cytokinins for shoot proliferation in grapevine (Fig. 1; Fig. 2a).

However, on the media supplemented with mT and Kin, the longest shoots were obtained, and there were no statistically significant differences among the four treatments containing varying concentrations of mT and Kin (Fig. 2b). The shoot lengths ranged from 4.98 ± 0.39 cm on MS + 0.5 mg/L mT (V1) to 4.72 ± 0.36 cm on MS + 1 mg/L mT (V2). Our results align with other studies indicating that while mT may not promote as many shoots as BA, the shoots produced are often healthier and demonstrate superior growth characteristics, including increased shoot length and improved root architecture [18, 21, 30]. Specifically, research has shown that mT can enhance the success of in vitro propagation by reducing stress responses that typically hinder growth, allowing for elongated shoot development without a corresponding increase in shoot number [27]. It was also found that Kin influences the dynamics of shoot proliferation in different ways. Sometimes, raising the concentration of Kin might result in fewer shoots produced per explant, even though it could

JOURNAL of Horticulture, Forestry and Biotechnology
Volume 29(2), 2025, 58-65
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

enhance shoot elongation [23]. The increase in shoot length when Kin is present can be partially linked to its influence on cell division and elongation, though this can occasionally reduce shoot multiplication [22, 6].

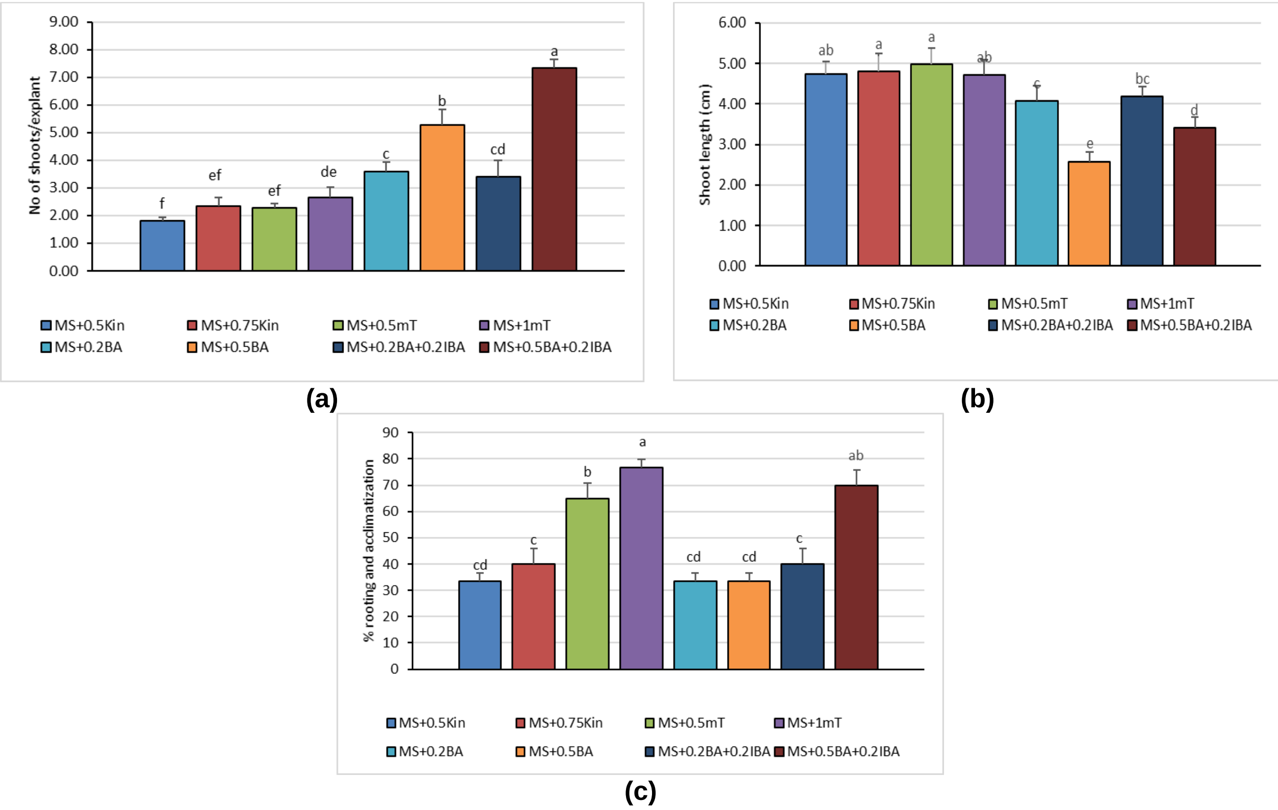


Figure 2. In vitro shoots proliferation of the Grasa de Cotnari grape variety cultured under eight different treatments of cytokines and auxins: MS+0.5 mg/L Kin, MS+0.75 mg/L Kin, MS+0.5 mg/L mT, MS+1 mg/L mT, MS+0.2 mg/L BA, MS+0.5 mg/L BA, MS+0.2 mg/L BA+0.2 mg/L IBA, and MS+0.5 mg/L BA+0.2 mg/L IBA: (a) Number of shoots obtained per initial explant; (b) Shoot length; (c) Percentage of rooting and acclimatization ex vitro in perlite

In line with our findings, Osama [22] reported that the grapevine varieties Superior and Red Globe, when cultivated in vitro on culture media supplemented with BA, 2iP, Kin, and TDZ, produced the highest number of shoots in the presence of BA. Furthermore, the longest shoots were observed in media supplemented with kinetin (Kin). Our results indicate that the grapevine variety Grasa de Cotnari generates a greater number of shoots in the presence of BA compared to Superior cv., which yielded an average of 2.02 shoots per explant, and Globul roşu cv., which yielded 2.57 shoots per explant. In terms of shoot length, Grasa de Cotnari cv. produced longer shoots on Kin-supplemented media, measuring 4.98 cm, than Superior cv. (3.80 cm) and Red Globe cv. (3.34 cm). Similarly, the grapevine cultivar Sev Khardji, when cultured in vitro on MS medium supplemented with 0.5 mg/L BA or 0.5 mg/L Kin, produced fewer shoots with shorter lengths compared to Grasa de Cotnari cv. under the same culture conditions [15].

The blue cells in the heat map (Fig. 3) indicate a positive correlation between the presence of BA in the culture media and the number of shoots per explant. In contrast, the length of the shoots shows a positive correlation with the presence of Kin and mT in the culture media. Figure 2c illustrates that the highest percentages of rooting and simultaneous acclimatization in perlite were achieved for shoots grown on culture media enriched with two concentrations of mT (65% and 76.66%), as well as the combination of 0.5 mg/L BA and 0.2 mg/L IBA (70%).

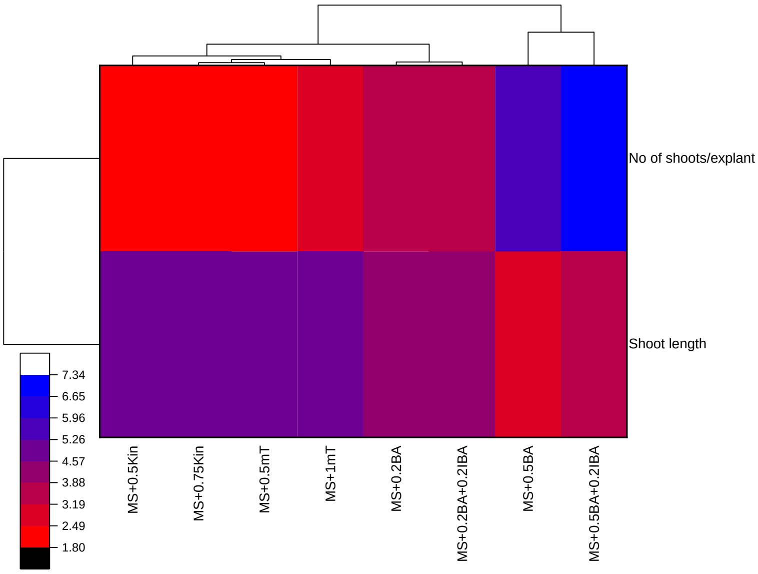


Figure 3. Heatmap based on the analysis of all variables and the Euclidean distance index of the grape variety Grasa de Cotnari grown under eight different cytokine and auxin treatments: MS+0.5 mg/L Kin, MS+0.75 mg/L Kin, MS+0.5 mg/L mT, MS+1 mg/L mT, MS+0.2 mg/L BA, MS+0.5 mg/L BA, MS+0.2 mg/L BA+0.2 mg/L IBA, and MS+0.5 mg/L BA+0.2 mg/L IBA. Columns indicate the eight PGR treatments, and rows indicate morphological parameters.

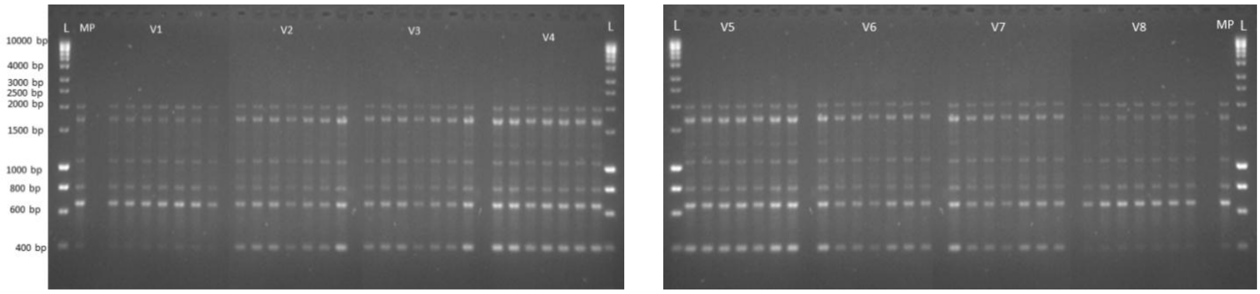
Genetic stability assessment by SCoT molecular markers

The seven SCoT primers used to analyse the genetic identity between mother plants and the acclimatized in vitro grown plants from each variant of culture media of the Grasa de Cotnari variety produced clear and reproducible DNA-PCR amplified bands. Table 1 presents the number and size range (bp) of total PCR amplified bands using SCoT primers in the analysed Grasa de Cotnari variety. The amplified PCR bands ranged in size from 400 bp (SCoT-3; SCoT-25) to 2200 bp (SCoT-1). The number of monomorphic amplified SCoT bands varied between 6 (SCoT-10) and 13 (SCoT-3) as shows in Table 1.

Table 1. Number and size range of monomorphic amplified SCoT bands detected in the analysed variety Grasa de Cotnari

Primer name	Primer sequence (5'-3')	Size range of bands (bp)	No. of scorable monomorphic bands
SCoT-1	CAACAATGGCTACCACCA	600-2200	10
SCoT-3	CAACAATGGCTACCACCG	400-1800	13
SCoT-4	CAACAATGGCTACCACCT	600-1900	10
SCoT-9	CAACAATGGCTACCAGCA	600-2100	12
SCoT-10	CAACAATGGCTACCAGCC	800-1800	6
SCoT-11	AAGCAATGGCTACCACCA	800-1900	8
SCoT-25	ACCATGGCTACCACCGGG	400-2000	7

In this study, we analysed the level of polymorphism between the in vitro-raised plants and their mother plants, and no polymorphic DNA bands were detected. For example, Figures 4a and 4 b show the electrophoretic profile generated with primer SCoT-25. As illustrated in Figure 4, there is no variation in the number of PCR-amplified bands between the mother plants and in vitro-acclimatized plants from each variant of culture media (V1-V8). Our result is consistent with other findings showing no genetic variations from in vitro raised plants of grapevine [2,20,11].



(a) (b)

Figure 4. The genetic profile generated with the SCoT 25 primer for the Grasa de Cotnari variety illustrates the genetic identity among the mother plant (MP) and the seven selected in vitro grown plants from each variant of culture media (V1- V8). L – molecular marker, 1 kb Ladder (Fermentas, Leon-Rot, Germany).

In large- scale production, verifying the clonal fidelity or genetic uniformity of micropropagated plants is essential [2]. In this context, DNA-based molecular marker techniques are valuable tools for confirming the genetic fidelity of in vitro-grown plants with their mother plants and ensuring their uniformity for commercial purposes [7,8]. The present study utilized SCoT markers to confirm the genetic fidelity of the micropropagated plants. Compared to other molecular marker systems such as RAPD or ISSR markers, which can amplify DNA fragments from non-coding regions of the plant genome, SCoT markers are associated with functional genes and their corresponding traits, making them more efficient for genetic identity studies [28].

Conclusions

Given the economic and agricultural importance of the Grasa de Cotnari grapevine, this study offers valuable insights into micropropagation techniques, aiding in the development of a reliable and disease-free source of planting material for growers. By connecting traditional viticulture with modern biotechnological methods, this research presents innovative strategies to enhance the efficiency of grapevine propagation.

Acknowledgements

This work was supported by a grant from the Romanian Ministry of Agriculture and Rural Development, under contract ADER 6.1.7. /2023 and project 6472/23.03.2022.

References

[1] AlGarawi, A. M., & Abd-Elgawad, M. E. (2025), *Genetic diversity of closely related Calligounum species collected from Saudi habitats by analyzing the mat K and rpoC1 genes, and SCoT and IRAP markers*. Plant Biotechnology Reports, 1-12.

[2] Alizadeh, M., & Singh, S. K. (2009), *Molecular assessment of clonal fidelity in micropropagated grape (Vitis spp.) rootstock genotypes using RAPD and ISSR markers*. Iranian Journal of Biotechnology, 7, pp. 37-44.

[3] Al-Khayri, J. M., Mahdy, E. M., Taha, H. S., Eldomiaty, A. S., Abd-Elfattah, M. A., Abdel Latef, A. A. H., ... & Hassanin, A. A. (2022), *Genetic and morphological diversity assessment of five kalanchoe genotypes by SCoT, ISSR and RAPD-PCR markers*. Plants, 11(13), 1722.

[4] Altaf, M.T., Nadeem, M.A., Ali, A., Liaqat, W., Bedir, M., Baran, N., ... & Baloch, F.S. (2024), *Applicability of Start Codon Targeted (SCoT) markers for the assessment of genetic diversity in bread wheat germplasm*. Genetic Resources and Crop Evolution, 1-14.

[5] Collard, B.C.Y., Mackill, D.J. (2009), *Start Codon Targeted (SCoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants*. Plant molecular biology reporter, 27, 86–93.

[6] Costa, A.D.O., Silva, L.A.S., Duarte, I.M., Sampaio, V.F., Machado, M., Silva, G.Z.D., ... & Rocha, D.I. (2019), *Kinetin and 6-benzyladenine induce different morphogenetic responses in cotyledonary segments of royal poinciana*. Ornamental Horticulture, 25(3), pp. 270-275.

[7] Clapa, D., & Hârța, M. (2021), *Establishment of an efficient micropropagation system for Humulus lupulus L. cv. cascade and confirmation of genetic uniformity of the regenerated plants through dna markers*. Agronomy, 11(11), 2268.

[8] Clapa, D., Hârţa, M., & Cordea, M.I. (2024), *Propagation of blackberry cultivars in three in vitro culture systems and evaluation of genetic uniformity.* Journal of Central European Agriculture, 25(4), 1076-1087.
[9] García, Y. S., GZapico, M., Ruiz, O. M., & Pedranzani, H. E. (2023), *Micro Propagation of Vitis Vinifera, Pinot Noir Genotype: Characteristics and Possibilities of Cultivation.*, Stechnolock Journal of Biology, Volume 1, Issue 1.
[10] Hârţa, M., Clapa, D., Cornea-Cipcigan, M., Borsai, O., Pop, R., Cordea, M.I. (2023), *Multivariate Assessment of Genetic Relationships between Two Streptocarpus Cultivars and Their F1 Progenies Using Morphological Characteristics and SCoT Molecular Markers*. Horticulturae, 9 (4), 497.
[11] Joon-Ho, K.W.O.N., Young-Sik, P.A.R.K., Si-Hong, K.I.M., & Jae-Yun, H.E.O. (2019), *Evaluation of genetic stability and effects of plant growth regulators for in vitro propagation of underutilized Vitis amurensis 'Cheongsan'*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47(3), 987-994.
[12] Kim, S.H., Zebro, M., Jang, D.C., Sim, J.E., Park, H.K., Kim, K.Y., ... & Park, S.M. (2023), *Optimization of plant growth regulators for in vitro mass propagation of a disease-free 'Shine Muscat'grapevine cultivar*. Current issues in molecular biology, 45(10), pp. 7721-7733.
[13] Kumsa, F. (2020), *Factors affecting in vitro cultivation of grape (Vitis vinifera L.): a review*. International Journal of Agricultural Research, Innovation and Technology, 10(1), pp. 1-5.
[14] Mamgain, J., Mujib, A., Ejaz, B., Gulzar, B., Malik, M.Q., & Syeed, R. (2022), *Flow cytometry and start codon targeted (SCoT) genetic fidelity assessment of regenerated plantlets in Tylophora indica (Burm. f.) Merrill*. Plant Cell, Tissue and Organ Culture (PCTOC), 150(1), pp. 129-140.
[15] Melyan, G., Sahakyan, A., Barsegyan, A., Dangyan, K., Sahakyan, N., Sargsyan, K., & Martirosyan, Y. (2024), *Micropropagation of (Vitis vinifera L.) cultivar'Sev Khardji'using biotechnological approaches and its impact on leaf quality*. Functional Food Science-Online ISSN: 2767-3146, 4(7), pp. 277-291.
[16] Mukherjee, P., Husain, N., Misra, S.C., & Rao, V.S. (2010), *In vitro propagation of a grape rootstock, deGrasset (Vitis champinii Planch.): Effects of medium compositions and plant growth regulators*. Scientia horticulturae, 126(1), 13-19.
[17] Murashige, T., & Skoog, F. (1962), *A revised medium for rapid growth and bioassay with tobacco tissue cultures*. Physiol Plant, 15: pp. 473-497.
[18] Naaz, A., Hussain, S. A., Anis, M., & Alatar, A.A. (2019), *Meta-topolin improved micropropagation in Syzygium cumini and acclimatization to ex vitro conditions*. Biol Plant, 63(1), pp. 174-182.
[19] Nascimento, D.C., Dini, M., Sampaio, N.V., & Schuch, M.W. (2019), *In vitro multiplication and rooting of grapevine: Culture media and plant growth regulators*. Plant Cell Culture & Micropropagation-ISSN 1808-9909, 15(1), pp. 1-7.
[20] Nookaraju, A., & Agrawal, D.C. (2012), *Genetic homogeneity of in vitro raised plants of grapevine cv. Crimson Seedless revealed by ISSR and microsatellite markers*. South African Journal of Botany, 78, 302-306.
[21] Nowakowska, K., & Pacholczak, A. (2020), *Comparison of the effect of meta-Topolin and benzyladenine during Daphne mezereum L. micropropagation*. Agronomy, 10(12), 1994.
[22] Osama, S.S. (2022), *Micropropagation of grapevine (Vitis Vinifera L.) Cvs. red globe and Superior*. The Iraqi Journal of Agricultural Science, 53(4), pp. 833-849.
[23] Papafotiou, M., Vlachou, G., & Martini, A.N. (2023), *Investigation of the effects of the explant type and different plant growth regulators on micropropagation of five mediterranean Salvia spp. native to Greece*. Horticulturae, 9(1), 96.
[24] Rai, M.K. (2023), *Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospect*s. Planta, 257(2), 34.
[25] Rohela, G.K., Jogam, P., Saini, P., Sandhya, D., Peddaboina, V., & Shekhawat, M.S. (2022), *Assessing the genetic stability of in vitro raised plants. In Commercial scale tissue culture for horticulture and plantation crops* Singapore: Springer Nature Singapore., pp. 245-276.
[26] Rotaru, L.G., Filipov, F., Mustea, M., & Stoleru, V. (2010), *Influence of some terroir viticole factors on quantity and quality of grapes*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(1), pp. 176-181.
[27] Souza, L.M.D., Silva, M.M.D.A., Herculano, L., Ulisses, C., & Camara, T.R. (2019), *Meta-topolin: an alternative for the prevention of oxidative stress in sugarcane micropropagatio*n. Hoehnea, 46, e1072018.
[28] Thakur, M., Sharma, V., Chauhan, A. (2021), *Genetic fidelity assessment of long term in vitro shoot cultures and regenerated plants in Japanese plum cvs Santa Rosa and Frontier through RAPD, ISSR and SCoT markers*. South African Journal of Botany, 140, 428–433.
[29] Torregrosa, L., Bouquet, A., Goussard, P.G. (2001), *In Vitro Culture and Propagation of Grapevine*. In: Roubelakis-Angelakis, K.A. (eds) Molecular Biology & Biotechnology of the Grapevine. Springer, Dordrecht, pp. 281-326.

JOURNAL of Horticulture, Forestry and Biotechnology
Volume 29(2), 2025, 58-65
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

[30] Vijayakumar, J., Ponmanickam, P., Samuel, P., Sudarmani, D.N.P., & Pandiarajan, J. (2017), *Influence of meta-topolin on efficient plant regeneration via micropropagation and organogenesis of safflower (Carthamus tinctorius L.) cv. NARI-H-15*. American Journal of Plant Sciences, 8(4), pp. 688-705.
[31] Yalinkiliç, N. A., Başbağ, S., Altaf, M. T., Ali, A., Nadeem, M. A., & Baloch, F. S. (2024), *Applicability of SCoT markers in unraveling genetic variation and population structure among sugar beet (Beta vulgaris L.) germplasm*. Molecular Biology Reports, 51(1), 584.
[32] Yan, X., Zheng, K., Li, P., Zhong, X., Zhu, Z., Zhou, H., & Zhu, M. (2024), *An efficient in vitro organogenesis protocol for the endangered relic tree species Bretschneidera sinensis and genetic fidelity assessment using DNA markers*. Frontiers in Plant Science, 15, 1259925.