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Biotechnological approaches for the conservation of old local plum and grapevine genotypes

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Abstract

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Conservation of plant biodiversity is of great importance in the face of changing climate conditions. The Balkan Peninsula is rich in valuable genetic resources of fruit species. At the Fruit Growing Institute – Plovdiv (Bulgaria), different biotechnological approaches have been applied to preserve and enrich the biodiversity. A new project to identify and protect local varieties and forms of fruit species and grapevines began three years ago. Two plum (*Prunus* spp.) and three grapevine (*Vitis* spp.) genotypes have been successfully introduced into *in vitro* culture. Reliable micropropagation protocols have been developed for these valuable genotypes, and they have been included in the *in vitro* gene bank. Plum and grapevine plants were successfully acclimatized to *ex vitro* conditions and stored in the *ex situ* collection at the Fruit Growing Institute – Plovdiv. This would facilitate their study, transportation, and further practical applications for germplasm conservation and genetic improvement of this species, as well as their inclusion in future breeding programs.

Keywords: biodiversity; micropropagation; plant genetic resources; rootstock; tissue culture
Abbreviations: 6-Benzylaminopurine (BAP); Indole-3-butyric acid (IBA); N6-(2-Isopentenyl) adenine (2iP); Kinetin (Kin); Thidiazuron (TDZ)

Introduction

Grape and plum are economically important temperate fruit crops. They have been cultivated for millennia in the Balkan Peninsula (Europe). Local varieties are an integral part of the national traditions, customs, heritage, and cultural identity of the local population. Additionally, they provide a genetic basis for breeding programs targeting new cultivars and rootstocks. Most of these genetic resources, however, are seriously endangered and are slowly disappearing from the orchards. The use of plant genetic resources in crop improvement is one of the most sustainable methods for conserving valuable genetic resources for the future, while simultaneously increasing agricultural production and food security (Haussmann et al., 2006). Breeding activities during the second half of the 20th century led to the commercial introduction of a large number of improved cultivars, which progressively replaced old, locally adapted, and traditionally grown cultivars (Sonnino, 2017). Other causes of genetic erosion are climate change and the long-term inoculum pressure of numerous pathogens (Vujović et al., 2020).

According to Shashidhara et al. (2023), the conservation and sustainable use of plant genetic resources are essential for achieving the Sustainable Development Goals (SDGs), including Zero Hunger (SDG 2), Climate Action (SDG 13), Life on Land (SDG 15), and Sustainable Production and

Consumption. Despite their importance, many PGRs face the risk of being lost due to various threats, including climate change, land-use changes, and the loss of traditional farming practices (FAO, 2019).

Field collections, where plum and grapevine genetic resources are typically stored, are convenient for year-round monitoring and the easy provision of available plant material for use in breeding and propagation programs. However, they are expensive and high-maintenance due to their intensive management requirements, and are at risk of losses from pest and disease attacks and environmental disasters (Elgelmann, 1997; Gisbert et al., 2018; Bettoni et al., 2021).

The conservation of plant genetic resources is essential, and modern biotechnologies, along with *in vitro* conservation techniques, provide valuable tools for protecting and preserving plant biodiversity. Nowadays, plant biotechnology offers important opportunities for the collection, propagation, conservation, molecular characterization, indexing, and elimination of pathogens, as well as the exchange of disease-free plant genetic resources (Agrawal et al., 2019; Bettoni et al., 2021; Wang et al., 2022). *In vitro* techniques can make a significant contribution to overcoming some of the problems associated with conserving plant genetic resources (Cheong, 2012; Kulak et al., 2022; Rai, 2022). *Ex situ* conservation of plant genetic resources using *in vitro* procedures is underway in many countries (Chokheli et al., 2020; Vujović et al., 2020; Nezami and Gallego, 2023).

The study aimed to establish an *in vitro* culture and develop a successful protocol for micropropagation of two plums and three grapevine genotypes, serving as an alternative for preserving the valuable genetic resources of Bulgarian old local fruit and grapevine genotypes. In addition, having a reliable micropropagation protocol is an important prerequisite for their inclusion in breeding programs and the development of modern germplasm conservation techniques, such as cryopreservation.

Materials and Methods

The study was conducted in the Laboratory of Plant Biotechnology at the Fruit Growing Institute in Plovdiv, Bulgaria. The experiments were performed with two plum genotypes (*Prunus domestica* Karadzheyka and local form from the village of Lovni dol) and three grapevine genotypes (*Vitis vinifera* Plovdivski rubin, Trakiya, and Chasselas Cioutat).

Karadzheyka (*Prunus* spp.). The tree is vigorous, late flowering, ripening time: end of July – beginning of August; Fruits are small (9 g), dark blue, meat – golden yellow, juicy, lovely, with pleasant acid and pleasant aroma and excellent taste (Figure 1A).

Local form from the village of Lovni dol. The tree is of medium to strong growth, with a large and wide canopy, and early to medium early flowering. Ripening time: August 20 – 30. The fruits are large, flattened globular, green-yellow,

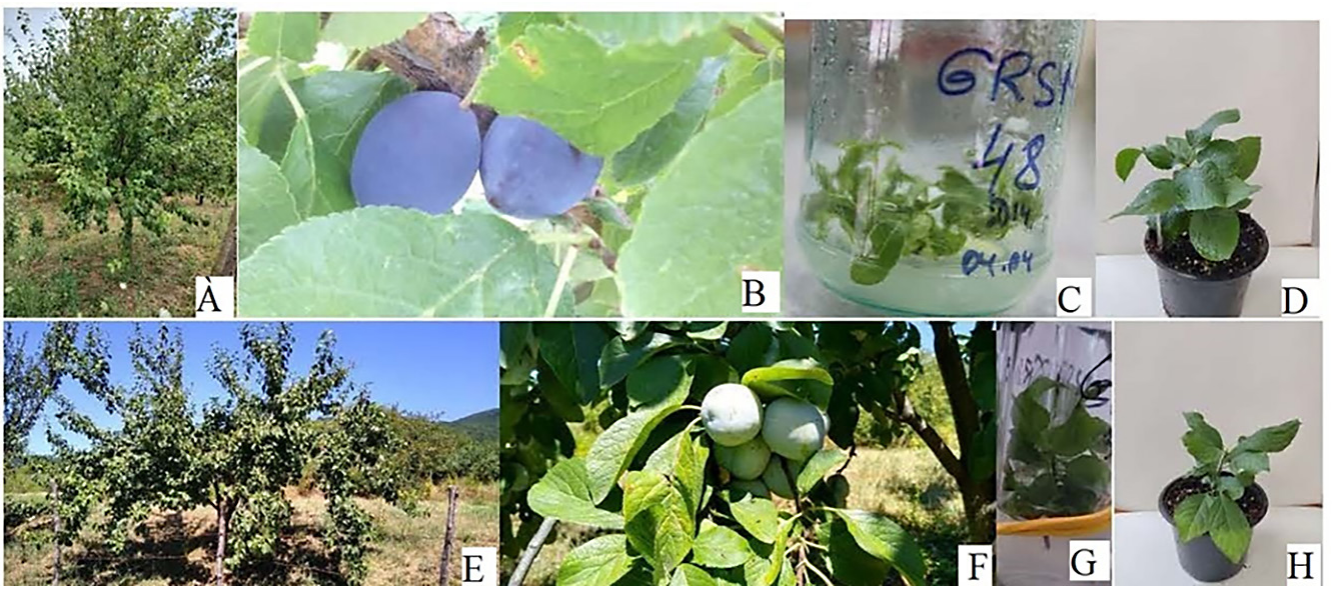

Fig. 1. Local plum (*Prunus* spp.) forms – Karadzheyka (A-D) and from the village of Lovni dol (E-H) – the habitus of the trees (A and E), the fruits (B and F), *in vitro* cultures (C and G) and *ex vitro* acclimatized plants in the *ex situ* collection at Fruit Growing Institute – Plovdiv (D and H)

lovely, juicy, with a pleasant aroma, detachable from the pit, with very good to excellent quality (Figure 1E).

Grapevine genotypes (*Vitis vinifera*): Plovdivski rubin is a red Bulgarian table variety (Figure 2A). It was obtained by crossing the varieties Chaouch × Muscat noir × Cardinal (Roychev, 2012). It is an early table variety that has delicious taste qualities. Bud fertility is high, resulting in high-quality yields. The grapes are resistant to rot. Grapes typically contain approximately 15% sugars and 6 g dm^{-3} of titratable acids. The leaf is five-lobed with hairs. The flowers are hermaphrodite. The average weight of a grape bunch is about 500 g. The grapes are harvested in mid-August. The variety is resistant to gray mold, but not resistant to powdery mildew.

The Trakia variety (Figure 2B) is a local white table variety obtained by crossing Misket Thracian and Bolgar. The variety Trakiya exhibits excellent recovery ability after frost damage to the buds. It also has good affinity with most rootstocks. The bunches are resistant to *Botrytis cinerea* Pers., which is one of the economically significant diseases in Bulgaria. Grapes typically contain approximately 19% sugars and 6 g dm^{-3} of titratable acids. The leaf is five-lobed without hairs. The bunch is up to 20 cm long, with a conical shape. The skin is thick and tough. The average weight of a grape is about 400 g, harvested towards the middle of August. The average mass of the grain is about 5 g. Not resistant to powdery mildew.

Chasselas cioutat (Figure 2C) is a French white table grape variety. However, due to its long cultivation in the specific habitat, it has become a local form adapted to the environmental conditions. It shows resistance to most diseases due to its thicker skin. The locals highly value it for its excellent taste. There are 90 more known synonyms. Grapes typically contain approximately 18% sugars and 6 g dm^{-3} of titratable acids. The flowers are hermaphrodite. The leaves are very characteristic, with numerous and deep lateral sinuses. The average weight of a grape is about 350 g. The grain is round and measures 1.9–2.0 cm in length. The skin of the grain is thin with a wax coating. The weight of 1 grain is 4–5 g. The grapes are ripening in late August. The variety is resistant to gray mold. Not resistant to powdery mildew.

The shoots were collected from old plants grown in the field at the beginning of June (in the spring) from the corresponding trees or shrubs. They were disinfected according to the standard for the laboratory procedure (Nacheva

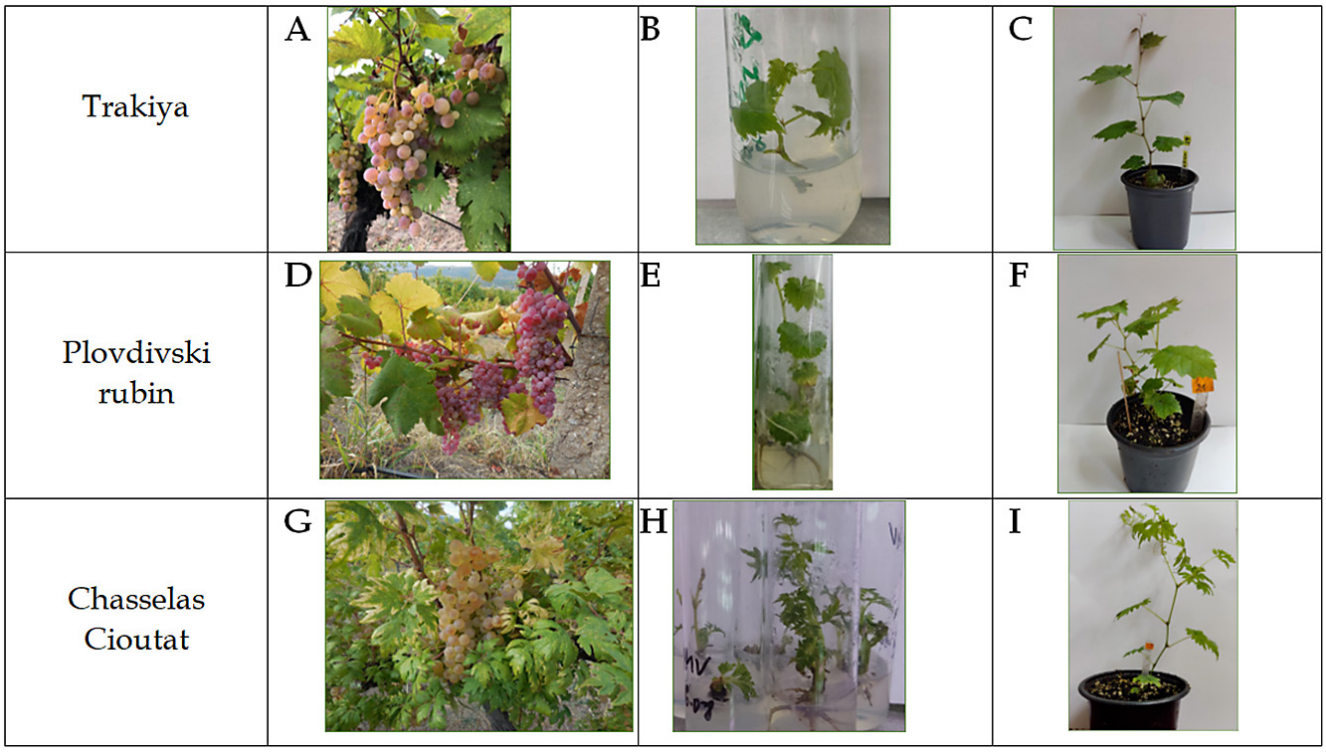

Fig. 2. Grapevines (*Vitis* spp.) genotypes Trakiya (A-C), Plovdivski rubin(D-F) and Chasselas Cioutat (G-I) – the appearance of grapes (A,D.G), *in vitro* culture of grapevine (B, E, H), *ex vitro* acclimatized plants (C, F, I) in the *ex situ* collection at Fruit Growing Institute – Plovdiv

and Ivanova, 2017). Briefly, the shoots, approximately 1–2 cm long and containing a single bud, were stripped of their leaves and surface sterilized by immersion in 70% ethanol for 30 s and then rinsed with distilled water. Then, they were treated with 5% solution of calcium hypochlorite containing 2–3 drops of Tween 20 for 7 min and rinsed four times (10 min each time) in distilled water. Apical and axillary segments (10 mm) were cut from the shoots and inoculated on the solid nutrient medium in test tubes. For plum genotypes, two different media were tested – MS medium (Murashige and Skoog, 1962) or DKW (Driver and Kuniyuki, 1984) medium, supplemented with 2.5 μM 6-benzylaminopurine (BAP), 0.05 μM indol-3-butyric acid (IBA), 30 g/l sucrose, 6.5 g/l Phyto agar (Duchefa, The Netherlands). For grapevine genotypes, the above-mentioned MS medium was tested (MS), and another MS medium (MS2) supplemented with 0.2 mg/L IBA (without cytokinin) was used. The pH of all media was adjusted to 5.6 before autoclaving at 121°C for 20 min. The clean and viable explants were transferred to fresh media every two weeks until they reached the required number of plants for the multiplication experiment. Then, *in vitro* shoot cultures were transferred to baby food jars (200 ml volume) containing the above-mentioned nutrient media (25 ml, five explants each). The cultures were cultivated in a growth room at 22±2°C under a 16/8 day/night photoperiod (cool-white fluorescent lamps, OSRAM, 40 W, 40 μmol m^{-2} s^{-1} photosynthetic photon flux density, PPFD).

After three 4-week passages, the Multiplication index (MI) was calculated as the number of proliferated shoots from the initial shoot at the end of eight passages. Additionally, the length (mm) of the shoots and the number of leaves were also recorded.

For the rooting phase, shoot apices approximately 20 mm in length were excised from four-week-old shoot clumps and transferred to the rooting medium composed of half-strength MS macronutrients, full-strength micronutrients and vitamins, 1.5 μM IBA, 20 g/l sucrose, 6.5 g/l Phytoagar (Duchefa, The Netherlands) at pH 5.6. The cultures were cultivated in a growth room under the above-mentioned conditions. The percentage of rooted plants and the number of roots per rooted plant were recorded after two weeks on the rooting medium. Well-developed rooted plantlets were removed from the agar medium, and their roots were washed in distilled water to remove excess culture medium. Then they were potted in a peat: perlite substrate (1:1, v/v) and covered with plastic wrap for two weeks to undergo *ex vitro* acclimatization. Plants were grown in a glass greenhouse with 50% shading, where they remained for 40 days. The photosynthetic photon flux density (PPFD) ranged from 300 to 700 μmol m^{-2} $s^{-1,}$ and the relative humidity ranged from 60 to 80%.

For the establishment of *in vitro* cultures, 50 explants (test tubes) were used for each genotype/nutrient medium combination. Three jars (with five explants each) were used for each genotype/nutrient medium in each multiplication experiment, and the experiment was repeated twice. Rooting was performed using 20 shoots (four jars × five explants) per treatment (genotype/medium), and the experiment was repeated twice. Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by comparison of group means (Tukey-b test) with the program SPSS ver. 26.0 (IBM).

Results

The disinfection protocol routinely used in our laboratory yielded a high percentage of clean and viable explants, exceeding 70%, regardless of the plant species.

After the establishment of aseptic culture, shoots of studied genotypes were multiplied on the corresponding media previously determined to be the most optimal for the multiplication of other plum and grapevine genotypes (Nacheva et al., 2002a,b; Rankova et al., 2006; Vujović et al., 2018; Nacheva et al., 2023). All tested plum (*Prunus* spp.) and grapevines (*Vitis* spp.) genotypes were successfully introduced into *in vitro* culture (Figures 1C, G, and 2B, E, H).

The multiplication index (MI) for both plum forms was within narrow limits (2.4–2.8), and no significant differences were noted between the two investigated nutritional media (Table 1). Shoot length and number of leaves of plum plantlets grown on both nutrient media were also similar (Table 1). The length of the shoots varied from 16 to 18.2 mm in Karadzeika and from 15.6 to 17.3 mm in Lovni dol genotype. The number of leaves at Karadzeika was 5.6–6.2, and at Lovni dol – 5.3–5.8. (Table 1).

All three studied grapevine genotypes exhibited different growth habits on the two nutrient media studied (MS and MS2) (Table 1). On the medium containing the cytokinin BAP (MS), the explants formed shoot clumps. On the medium containing the auxin IBA without cytokinin (MS2), the explants first formed roots and then grew in height, and the apical and nodal segments could be used as explants for the following passage. On the MS2 medium, a higher MI index, taller stems, and a greater number of leaves were reported in the varieties Trakia and Plovdivski rubin compared to the plants on MS medium (Table 1). In the cultivar Chasselas Cioutat, no statistically significant differences in MI and plant height were recorded between the two cultivation variants. A higher shoot length and number of leaves were reported on MS2 medium (Table 1).

Table 1. Growth parameters of studied plum and grapevine genotypes at the multiplication stage in cultures grown for 28 days

Genotype/medium	Multiplication index MI	Length of the shoots, mm	Number of leaves
Plum (*Prunus* spp.)			
Karadzheyka			
MS medium	2.4 a	16.0 a	5.6 a
DKW medium	2.7 a	18.2 a	6.2 a
Lovni dol			
MS medium	2.5 a	15.6 a	5.3 a
DKW medium	2.8 a	17.3 ab	5.8 a
Grapevine (*Vitis* spp.)			
Trakiya			
MS medium	2.2 b	30.1 b	5.1 b
MS2 medium	4.8 a	50.3 a	8.1 a
Plovdivski rubin			
MS medium	2.3 b	33.2 b	5.3 b
MS2 medium	3.6 a	57.3 a	8.2 a
Chasselas Cioutat			
MS medium	4.2 a	88.3 a	9.4 b
MS2 medium	5.1 a	95.2 a	13.1 a

*Means followed by the same letter were not different at $p \leq 0.05$, according to the Tukey-b test.

In our study, both plum genotypes exhibited a satisfactory rooting rate (62–67%) with 2.3–2.6 roots per plant (Table 2).

Using the protocol reported in this study, we found very high rooting percentages (between 87 and 93%) for the three studied grapevine genotypes (Table 2). No significant difference was reported in the number of roots per plant.

Plum and grapevine plants were successfully acclimatized to *ex vitro* conditions and grown in the *ex situ* collection in the field at the Fruit Growing Institute – Plovdiv (Figures 1D and H and 2C, F, I).

Table 2. Growth parameters of studied plum and grapevine genotypes at the rooting stage grown for 14 days in rooting media

Genotype/medium	Rooting, %	Number of roots per plant
Plum (*Prunus* spp.)		
Karadzheyka	62	2.3 ±0.3 a
Lovni dol	67	2.6 ±0.4 a
Grapevine (*Vitis* spp.)		
Trakiya	87	3.2±0.6 a
Plovdivski rubin	92	3.6±0.4 a
Chasselas Cioutat	93	3.6±0.3 a

*Means followed by the same letter were not different at $p \leq 0.05$

Discussion

Microbial contaminants, such as fungi and bacteria, pose a significant challenge in establishing *in vitro* culture (Strobel et al., 2001; Kulkarni, 2007). Most of the sterilizing agents used to initiate viable *in vitro* cultures are toxic to plant tissues. Therefore, it is necessary to refine the concentrations of the disinfectants and optimize the exposure time of the explants. The disinfection procedure routinely used in our laboratory was successfully applied to the studied plum and grapevine genotypes, yielding a high percentage, over 70%, of clean and viable explants.

The multiplication index (MI) for both plum forms reported in our experiments (2.4–2.8) is similar to that obtained by Vujović et al. (2020) with the Serbian plum variety Crvena Ranka (multiplication index 2.0–2.5). A similar positive response for shoot induction and shoot multiplication on medium containing 0.5 or 1 mg/l BAP, but in combination with a higher concentration of IBA (0.1 mg/l), was also obtained for plum cultivar Stanley (Wolella, 2017).

Similar to our study, MS medium has been widely used in the *in vitro* cultivation of many grapevine genotypes (Alizadeh et al., 2010; Mozafairi et al., 2016). El-Agamy et al. (2009) reported that MS medium improved the average weight of plants. Kinfe et al. (2017) also noted that culturing shoots on basal MS medium supplemented with 0.5 mg/L BAP was effective for shoot initiation. Mukherjee et al. (2011) found that BAP at 1.0 mg/l added to MS medium also showed the best proliferation level.

Ibáñez et al. (2003) reported that among the other cytokinins studied – N6-(2-Isopentenyl) adenine (2iP), Kinetin, and Thidiazuron (TDZ) – the culture medium supplemented with BAP produced the best results, especially at 6.67 and 8.9 μM. The results of other authors are similar (Singh et al., 2004; Kurmi et al., 2011). Kurmi et al. (2011) also noted that the nodal segment explants were excellent for shoot proliferation and other stages of micropropagation, which was consistent with the present findings.

Rooting is a crucial step in whole-plant formation during the micropropagation process, which is often a limiting factor in many species, especially woody ones (Díaz-Sala, 2014; Legué et al., 2014; Guan et al., 2015). The rooting ability is genetically determined, but also influenced by cultural environment factors, such as culture conditions, mineral content in the culture medium, auxin type, and concentration, among others (Hartman et al., 2002; Hazarika, 2006). European plums typically exhibit poor rooting ability *in vitro*-induced shoots, which can be a significant drawback in commercial micropropagation (Vujović et al., 2020). Both plum genotypes studied showed good rooting (over 60%). Close to our results,

Vujović et al. (2020) reported a rooting efficiency of 60% in the autochthonous plum cultivar Crvena Ranka (*Prunus domestica* L.), originating from Serbia, when shoots were cultured on a medium containing NAA (1 mg/l) instead of IBA (1 mg/l).

Using the protocol reported in this study, we observed a high multiplication rate (Table 1) and a very high rooting percentage (ranging from 87% to 93%) for the three studied grapevine genotypes (Table 2). Similar results for rooting percentage (70.1–87.7) in four grape (*Vitis* spp.) rootstocks were reported by Alizadeh et al. (2010), depending on the genotype. Many authors have reported that the *in vitro* performance of grapevine genotypes is strongly genotype-dependent (Barreto et al., 2008; Abido et al., 2013).

As many authors note, the transfer of plantlets *in vitro* to *ex vitro* conditions is a critical stage in the process of their micropropagation and is the main obstacle to a large-scale use of the clonal micropropagation method is the high mortality rate of plants during their acclimatization to the non-sterile conditions (Kumar and Rao, 2012; Dziedzic and Jagła, 2013). The good physiological quality of *in vitro* plantlets is crucial for successful acclimatization to *ex vitro* conditions. It has long been recognized that the good rooting of shoots regenerated is crucial for ensuring *ex vitro* plant survival, as it facilitates the uptake of nutrients and water from the soil (Kozai et al., 1992). The protocol for multiplication and rooting of the studied plum and grapevine genotypes presented in this study is a good prerequisite for their successful acclimatization to *ex vitro* conditions.

Conclusions

To prevent further genetic erosion, extensive efforts are needed to conserve the plant biodiversity of native fruit species and grapevines, followed by efforts to improve the genetic base of new varieties. The reliable protocols for the micropropagation of two plum and three grapevine genotypes presented in this study could serve as an alternative for the multiplication and conservation of the valuable genetic resources of Bulgarian old local plum and grapevine accessions. It would be helpful to study the phenology and economic value of the samples, their phenotyping, and their inclusion in breeding programs. In addition, it would facilitate the safe exchange of genetic material, as well as the development of new, modern germplasm conservation techniques, such as somatic regeneration, slow-growth cultures, and cryopreservation.

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Conflict of interest

The authors declare the absence of conflict of interest.

References

Abido, A., Aly, M., Hassanen, S. & Rayan, G. (2013). *In vitro* propagation of grapevine (*Vitis vinifera* L.). muscat of Alexandria cv. for conservation of endangerment. *Middle-East Journal of Scientific Research*, *13*(3), 328 – 337. DOI: http://dx.doi.org/10.5829/idosi.mejsr.2013.13.3.1926.

Agrawal, A., Singh, S., Malhotra, E. V., Meena, D. P. & Tyagi, R. K. (2019). *In vitro* Conservation and Cryopreservation of Clonally Propagated Horticultural Species. *In:* Rajasekharan, P., Rao, V. (eds), Conservation and Utilization of Horticultural Genetic Resources. *Springer,* Singapore, 529–578. DOI:http://dx.doi.org/10.1007/978-981-13-3669-0_18.

Alizadeh, M., Singh, S. K. & Patel, V. B. (2010). Comparative performance of *in vitro* multiplication in four grape (*Vitis* spp.) rootstock genotypes. *International Journal of Plant Production, 4*(1), 41 – 50. DOI: https://doi.org/10.22069/ijpp.2012.680.

Barreto, M., Nookaraju, A., Joglekar, A., Karibasappa, G. & Agrawal, D. (2008). Variability among *Vitis vinifera* cultivars to *in vitro* propagation. *In*: International Symposium of Grape Production and Processing, *Acta Horticulturae*, *785*, 127 – 140. DOI:https://doi.org/10.17660/ActaHortic.2008.785.15.

Bettoni, J.C., Markovic, Z., Bi, W., Volk, G. M., Matsumoto, T. & Wang, Q.-C. (2021). Grapevine shoot tip cryopreservation and cryotherapy: secure storage of disease-free plants. *Plants, 10*(10), 2190. DOI: https://doi.org/10.3390%2Fplants10102190.

Cheong, E. (2012). Biotechnological approaches for improvement and conservation of *Prunus* species. *Plant Biotechnol. Rep.*, *6*, 17 – 28. DOI: http://dx.doi.org/10.1007/s11816-011-0195-y.

Chokheli, V. A., Dmitriev, P. A., Rajput, V. D., Bakulin, S. D., Azarov, A. S., Varduni, T. V., Stepanenko, V. V., Tarigholizadeh, S., Singh, R. K., Verma, K. K. & Minkina, T. M. (2020). Recent development in micropropagation techniques for rare plant species. *Plants*, *9*(12), 1733. DOI: https://doi.org/10.3390%2Fplants9121733.

Díaz-Sala, C. (2014). Direct reprogramming of adult somatic cells toward adventitious root formation in forest tree species: The effect of the juvenile-adult transition. *Front. Plant Sci., 5*, 310. DOI: https://doi.org/10.3389/fpls.2014.00310.

Driver, J. & Kuniyuki, A. (1984). *In vitro* propagation of paradox walnut rootstock. *HortScience, 19*(4), 507–509. DOI: http://dx.doi.org/10.21273/HORTSCI.19.4.507.

Dziedzic, E. & Jagła, J. (2013). Micropropagation of *Rubus* and *Ribes* spp. *In:* Lambardi, M., Ozudogru, E. and Jain, S. (eds.)

Protocols for Micropropagation of Selected Economically Important Horticultural Plants, 149 – 160. Totowa, N.J.: *Humana Press*. DOI: http://dx.doi.org/10.1007/978-1-62703-074-8_11.

El-Agamy, S. Z., El-Mahdy, T. K. & Mohamed, A. A. (2009). *In vitro* propagation of some grape rootstocks. *In:* International Symposium on Biotechnology, *Acta Horticulturae*, *839*, 125–131. DOI: 10.17660/ActaHortic.2009.839.14.

Elgelmann, F. (1997). *In vitro* conservation methods. *In:* Callow, J.A., Ford-Lloye, B.V., Newbury, H.J., (eds), Biotechnology and Plant Genetic Resources, 119–161. *CAB International,* Wallingford, UK.

FAO (2019). The state of the world's biodiversity for food and agriculture. Retrieved from: http://www.fao.org/3/ca6030en/ca6030en.pdf.

Gisbert, C., Peiró, R., San Pedro, T., Olmos, A., Jiménez, C. & García, J. (2018). Recovering ancient grapevine varieties: From genetic variability to *in vitro* conservation, a case study. *In:* Jordão, A.M., Cosme, F., Eds.; Intech: London Grapes and Wines – Advances in Production, *Processing, Analysis and Valorization*, 3 – 21. UK. DOI: 10.5772/intechopen.71133.

Guan, L., Murphy, A., Peer, W., Gan, L., Li, Y. & Cheng, Z. (2015). Physiological and molecular regulation of adventitious root formation. *Critical Reviews in Plant Sciences*, *34* (5), 506–521. DOI: http://dx.doi.org/10.1080/07352689.2015.1090831.

Hartmann, H. T., Kester, D. E., Davies, J. R. & Geneve, R. L. (2002). Plant propagation: principles and practices. New Jersey: *Prentice Hall.*

Haussmann, B. I. G., Parzies, H. K., Presterl, T. & Miedaner, T. (2006). Plant genetic resources in crop improvement. *Plant Genet. Resour.*, *2*(1), 3–21. DOI: http://dx.doi.org/10.1079/PGR200430.

Hazarika, B. N. (2006). Morpho-physiological disorders *in vitro* culture of plants. *Scientia Horticulturae*, *108*(2), 105 – 120. DOI: https://doi.org/10.1016/j.scienta.2006.01.038.

Ibáñez, A., Valero, M. & Morte, A. (2003). Influence of cytokinins and subculturing on proliferation capacity of single-axillary-bud microcuttings of *Vitis vinifera* L. cv. Napoleó. *Anales de Biología, 25*, 81–90.

Kinfe, B., Feyssa, T. & Bedada, G. (2017). *In vitro* micropropagation of grapevine (*Vitis vinifera* L.) from nodal culture. *African Journal of Biotechnology, 16*(43), 2083–2091. DOI: http://dx.doi.org/10.5897/AJB2016.15803.

Kozai, T., Fujiwara, K., Hayashi, M. & Aitken-Christie, J. (1992). The *in vitro* environment and its control in micropropagation. *In:* Kurata, K. and Kozai, T. (eds.) *Transplant Production Systems*, 247–282. The Netherlands: *Kluwer Academic Publishers.* DOI: https://doi.org/10.1007/978-94-011-2785-1_14.

Kulak, V., Longboat, S., Brunet, N., Shukla, M. & Saxena, P. (2022). *In vitro* technology in plant conservation: relevance to biocultural diversity. *Plants, 11*(4), 503. DOI: https://doi.org/10.3390%2Fplants11040503.

Kulkarni, A. A., Kelkar, S. M., Watve, M. G. & Krishnamurthy, K. V. (2007). Characterization and control of endophytic bacterial contaminants in *in vitro* cultures of *Piper* spp., *Taxus baccata* subsp. *wallichiana* and *Withania somnifera*. *Can. J. Microbiol*., *53*(1), 63 – 74. DOI: 10.1139/w06-106.

Kumar, K. & Rao, U. (2012). Morphophysiological problems in acclimatization of micropropagated plants in *ex vitro* conditions – a reviews. *J. Ornam. Hortic. Plants*, *2*, 271–283.

Kurmi, U. S., Sharma, D. K., Tripathi, M. K., Tiwari, R., Baghel, B. S. & Tiwari, S. (2011). Plant regeneration of *Vitis vinifera* (L.) via direct and indirect organogenesis from cultured nodal segments. *International Journal of Agricultural Technology*, *7*(3), 721–737.

Legué, V., Rigal, A. & Bhalerao, R. (2014). Adventitious root formation in tree species: involvement of transcription factors. *Physiologia Plantarum*, *151*(2), 192–198. DOI: https://doi.org/10.1111/ppl.12197.

Mozafari, A., Ghoraishi, O., Ghaderi, N. & Javadi, T. (2016). Micropropagation of grape cultivars on different basal media supplemented with benzyl adenine. *Agriculturae Conspectus Scientificus*, *81*(3), 123–129.

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.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bio assays for tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473–497. DOI: https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Nacheva, L., Ivanova, K. & Milusheva, S. (2002a). Elimination of PPV in plum cvs Kyustendilska sinya and Valjevka through *in vitro* techniques. *Acta Horticulturae*, *577*, 289–291. DOI: 10.17660/ActaHortic.2002.577.49

Nacheva, L., Zlatev, Z., Ivanova, K. & Manolov, P. (2002b). Possibilities for application of photoautotrophy in micropropagation of Dzhanka 4 rootstock. *Acta Horticulturae*, *577*, 199 – 207. DOI: http://dx.doi.org/10.17660/ActaHortic.2002.577.33.

Nacheva, L. & Ivanova, V. (2017). Silver nitrate and chlorhexidine gluconate – effective surface sterilization agents in disinfection procedures at initiation of woody shoot tip and embryo culture. *Journal of BioScience and Biotechnology*, *6* (3), 187–190.

Nacheva, L., Dimitrova, N., Koleva-Valkova, L., Stefanova, M., Ganeva, T., Nesheva, M., Tarakanov, I. & Vassilev, A. (2023). *In vitro* multiplication and rooting of plum rootstock Saint Julien (*Prunus domestica* subsp. *insititia*) under fluorescent light and different LED spectra. *Plants*, *12*(11), 2125. DOI: https://doi.org/10.3390/plants12112125.

Nezami, E. & Gallego, P. (2023). History, phylogeny, biodiversity, and new computer-based tools for efficient micropropagation and conservation of pistachio (*Pistacia* spp.) germplasm. *Plants*, *12*(2), 323. DOI: https://doi.org/10.3390/plants12020323.

Rai, M. (2022). Plant tissue culture targeting germplasm conservation. *In:* Rai, A., Kumar, A., Modi, A., Singh, M. (eds.) Advances in Plant Tissue Culture: Current Developments and Future Trends, 205-221. *Academic Press, Elsevier Inc.*

Rankova, Z., Nacheva, L., Zapryanova, K., Gercheva, P. & Bozhkova, V. (2006). Vegetative habits of plum rootstock 'Wangenhaims' after treatment with terbacil under *in vitro* conditions. Proceedings of Sixth Science Technological Conference "Ecology and Health", Plovdiv, Bulgaria, 339–343 (Bg).

Roychev, V. (2012). Ampelography. Agricultural University Plovdiv,

ISBN 978-954-517-146-8., 193–194, Plovdiv, Bulgaria (Bg).

Shashidhara, N., Dhanalakshmi, T. & Santosh, D. (2023). Plant Genetic Resources in Sustainable Agriculture: Current State, Future Perspectives, and Challenges, 93–102. *In:* Biradar, N., Shah R., Ahmad, A. (eds.) Recent Advances in Agricultural Sciences and Technology. *Dilpreet Publishing House,* New Delhi, India.

Singh, S. K., Khawale, R. N. & Singh, S. P. (2004). Techniques for rapid *in vitro* multiplication of *Vitis vinifera* L. cultivars. *J. Hort. Sci. Biotech*., *79*(2), 267–272. DOI: http://dx.doi.org/10.1080/14620316.2004.11511759.

Sonnino, A. (2017). International instruments for conservation and sustainable use of plant genetic resources for food and agriculture: an historical appraisal. *Diversity, 9*(4), 50. DOI: https://doi.org/10.3390/d9040050.

Strobel, G. A., Dirkse, E., Sears, J. & Markworth, C. (2001). Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. *Microbiology*, *147*(11), 2943–2950. DOI: 10.1099/00221287-147-11-2943.

Vujović, T., Jevremović, D., Marjanović, T. & Glišić, I. (2020). *In vitro* propagation and medium-term conservation of autochthonous plum cultivar Crvena Ranka. *Acta Agriculturae Serbica, 25*(50), 141–147. DOI: http://dx.doi.org/10.5937/AASer2050141V.

Vujović, T., Marjanović, T., Ružić, Đ. & Glišić, I. (2018). *In vitro* propagation of plum rootstocks. *Journal of Pomology*, *52*(203/204), 91 – 97. DOI: 10.5937/AASer2050141V.

Wang, M., Bi, W., Bettoni, J., Zhang, D., Volk, G. & Wang, Q. (2022). Shoot tip cryotherapy for plant pathogen eradication. *Plant Pathology*, *71*(6), 1241–1254. DOI: https://doi.org/10.1111/ppa.13565.

Wolella, E. (2017). Surface sterilization and *in vitro* propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants. *Journal of Biotech Research*, *8*, 18 – 26.

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