Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TOWARDS *IN VITRO* CONSERVATION OF SEVERAL PLUM CULTIVARS (*PRUNUS DOMESTICA* L.)

Larisa-Daniela STRÎMBU (VLAȘIN)[1, 2], Luminița-Antonela ZAGRAI[1], Zsolt JAKAB-ILYEFALVI[1], Ioan ZAGRAI[1], Georgeta Maria GUZU[1, 2], Mirela Irina CORDEA[2]

[1]Fruit Research and Development Station Bistrița, 3 Drumul Dumitrei Nou Street, 420127, Bistrița, Romania
[2]Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Mănăștur Street, 400372, Cluj-Napoca, Romania

Corresponding authors emails: luminita.zagrai@scdp-bistrita.ro, mcordea@usamvcluj.ro

Abstract

Prunus domestica L. is one of the most economically important fruit species in the global plum industry, serving as a key species for both fresh consumption and processed products. In vitro micropropagation is one of the most suitable methods to preserve the valuable cultivars of species. Twenty nine plum cultivars certified at Prebasic category were initiated on Woody Plant Medium (WPM) with benzylaminopurine (BAP) or meta-topolin (mT), to assess their effect on initiation rate. Although the average of both variants showed a similar initiation rate, there were significant differences between some cultivars. During the multiplication stage, the explants from 19 plum cultivars were cultivated on two medium (WPM or Murashige & Skoog) with the same hormones, and the multiplication coefficient (number of proliferated shoots) and regeneration efficiency (proportion of explants developing into healthy plants) were assessed. Results revealed that both indicators proved to be highly variable dependending mainly on cultivar and less of the four culture variants.

Key words: *cultivars, cytokinins, initiation rate, multiplication coefficient, plum preservation.*

INTRODUCTION

Prunus domestica L., commonly known as European plum, is one of the two key pillars of the plum industry. Its adaptability for both fresh consumption and processing has made it valuable crop worldwide, especially in Europe and North America (Hartmann & Neumüller, 2009). Over the past two decades, Romania has ranked as the second - larger producer globally (FAOSTAT, 2025).

Micropropagation is a widely used technique in plant biotechnology for vegetative propagation and an effective tool for the conservation of plant genetic resources, providing several advantages over traditional methods (Bettoni et al., 2024; Sharma & Jain, 2024).

This technique facilitates the preservation of valuable cultivars in a controlled environment, protected from external stressors and pathogens (Nezami & Gallego, 2023). Organogenesis is an important pathway in tissue culture, extremely useful in micropropagation for plant regeneration and in germplasm conservation of horticultural crops (Canli & Tian, 2009; Yao et al., 2011; Romadanova et al., 2016).

Furthermore, it enables rapid multiplication of plant material, which is particularly advantageous for slow-growing woody species, such as plums (Vujović et al., 2022; Nacheva et al., 2023).

In vitro conservation of plum cultivars, together with *ex situ* conservation in an insect-proof house, forms an integrated strategy for genetic resources conservation (Zagrai & Zagrai, 2015; Zagrai et al., 2022). Preserving diverse plum cultivars is essential for maintaining genetic diversity and ensuring the availability of desirable traits for breeding programs and commercial production (Yuan et al., 2009). However, prolonged successive subculturing of *in vitro* plants on fresh medium, along with their maintenance under standard growth conditions, may lead to a decline or loss of the culture's morphogenetic potential (Chauhan et al., 2019).

Moreover, tissue culture technology facilitates the conservation of plant genetic resources for short-, medium-, or long-term, depending on specific requirements and the technique employed (Grout, 1990; Engelmann, 1997). Short-term conservation requires regular sub-culturing to maintain cultures (Sharma & Jain, 2024). Due to a great potential for preserving plant material via micropropagation, our study aims to evaluate the short-term *in vitro* conservation of several plum cultivars by assessing their responses to different culture media and growth regulators. Initiation rate, multiplication coefficient, and regeneration efficiency will be analyzed to identify the most effective strategies for preserving plum cultivars.

MATERIALS AND METHODS

This study was carried out at the Fruit Research and Development Station Bistrița, Romania, in the period of 2023-2025. Annual young shoots from 29 plum cultivars were collected in winter from trees grown in an insect-proof screen house. These included 23 Romanian cultivars (‘Agent’, ‘Andreea’, ‘Carpatin’, ‘Centenar’, ‘Dani’, ‘Delia’, ‘Diana’, ‘Doina’, ‘Elena’, ‘Flora’, ‘Geta’, ‘Gras ameliorat’, ‘Iulia’, ‘Ivan’, ‘Jubileu 50’, ‘Matilda’, ‘Minerva’, ‘Pescăruș’, ‘Romaner’, ‘Silvia’, ‘Tita’, ‘Tuleu dulce’ and ‘Zamfira’) and 6 foreign cultivars (‘Anna Späth’, ‘Blue free’, ‘d`Agen’, ‘President’, ‘Reine Claude d’Althan’ and ‘Stanley’).

Nodal segments excised and surface sterilized with a fungal solution containing copper hydroxide (50%) and metallic copper equivalent (50%). They were then washed in sterile distilled water on a plate with a magnetic stirrer for 15 minutes.

The following steps were performed in a horizontal laminar air flow hood: uninodal shoots were immersed in ethanol 70% for 1 minute, then in sodium hypochlorite 20% for 20 min with 2-3 drops of Tween 20. Subsequently, they were rinsed four times with sterile distilled water.

The cultures were incubated in a vegetative growth room at 23±1°C under artificial light conditions, providing 4000 lux and 16/8 hours photoperiod for initiation and multiplication stages.

Initiation stage

Apexes explants (1-3 mm) were excised and cultured on Woody Plant Medium (WPM) supplemented with salts and vitamins (Lloyd & McCown, 1980) in the initiation stage. Various growth hormones were added in two different variants (Table 1).

For the initiation and multiplication stages the culture medium was supplemented with PVP (polyvinylpyrrolidone) 0.05 g/L, acid ascorbic 0.05 g/L and sucrose 30 g/L, and the medium was solidified using Phytoagar 5.8 g/L. Hormones were prepared as stock solutions. The medium was adjusted to pH 5.8 with NaOH and HCl, prior to dispensing and autoclaving at 121°C, 1.1 atm for 20 min. All chemicals were from Duchefa Biochemie B. V.

Table 1. Culture variants for initiation and multiplication stages

Stage	Culture variants	Medium culture	Growth hormones (mg/L)		
			BAP	mT	GA_3
Initiation	VI-1	WPM	0.3	-	-
	VI-2		-	0.3	-
Multiplication	VM-1	WPM	0.5	-	0.5
	VM-2		-	0.5	0.5
	VM-3	MS	0.5	-	0.5
	VM-4		-	0.5	0.5

The apexes were initially cultured in test tubes containing 10 mL of medium with one explant per tube. After four weeks of incubation in the growth room, the initiation rate was assessed based on the cytokinins used. Initiation rate was defined as the percentage of explants actively growing from initial cultured explants, after four weeks on the initiation medium.

Multiplication stage

Media used for the multiplication stage was WPM or MS medium (Murashige and Skoog, 1962), both supplemented with salts and vitamins, supplemented with growth hormones in different combinations (Table 1).

Explants obtained during the initiation stage were transferred to multiplication medium in 200 ml jar containing 50 ml solid medium. In this stage, 19 cultivars were selected for regeneration, including 17 Romanian cultivars (‘Andreea’, ‘Carpatin’, ‘Centenar’, ‘Delia’, ‘Diana’, ‘Doina’, ‘Elena’, ‘Geta’, ‘Gras ameliorat’, ‘Matilda’, ‘Minerva’, ‘Pescăruș’, ‘Romaner’, ‘Silvia’, ‘Tita’, ‘Tuleu dulce’, ‘Zamfira’) and 2 foreign cultivars (‘Anna Späth’ and ‘Blue free’) in different culture media.

After subcultivation, at 4 weeks on each variant, the multiplication coefficient was determined. The multiplication coefficient was defined as the number of shoots that had proliferated from the initial *in vitro* plant for all replications. In this stage was determined also the regeneration efficiency, which represents the percentage of explants that successfully developed into new and healthy plants after a subcultivation cycle on each variant of culture.

The research involves a comparative evaluation of Woody Plant Medium (WPM) and Murashige & Skoog (MS) medium, along with the plant growth regulators benzylaminopurine (BAP) and meta-topolin (mT), to determine the optimal composition for each cultivar.

Statistical analysis

The data were organised as followed: three replications per each variant and cultivar in initiation phase and four replications for every variant and cultivar in multiplication stage. All data were subjected to an analysis of variance (ANOVA). In the initiation stage, the differences between the variants were assessed using the Student's t-test ($p<0.05$), while in the multiplication stage, mean values were analysed with the Fisher post hoc test ($p<0.05$, $p<0.01$). Data are expressed as means ± standard deviation (SD). Principal component analysis (PCA) was performed using the average values of the variants in the multiplication stage. Research data was analysed with the aim of XLSTAT statistical software (Addinsoft, France) ver. 2019 1.1, embedded in MS Excel.

RESULTS AND DISCUSSIONS

Initiation stage

The average values of the initiation rate of the 29 cultivars studied on two cultures media was quite similar, 59.05% for VI-1 and 60.14% for VI-2 with no statistical differences (Table 2). This indicates that both cytokinin-type hormones incorporated into the culture media facilitated optimal regeneration and development of the explants during the initial stage for studied plum cultivars.

When take into account the two cultures media for each cultivar, the situation is a bit different. A comparative analysis using the *t-test* indicates that the differences in initiation rates between VI-1 and VI-2 are statistically significant in most cases (cultivars marked with an asterisk).

Table 2. Initiation rate depending the variants (%)

Origin of cultivars	Cultivars	Culture variants	
		VI-1 (%)	VI-2 (%)
Romanian	Agent*	18.17±0.82	**54.5±4.55**
	Andreea	70.58±4.41	66.28±1.75
	Carpatin	85.71±1.29	80.0±5.0
	Centenar*	**50.0±5.0**	27.27±1.72
	Dani*	**37.50±2.5**	8.33±7.23
	Delia*	25.0±21.73	**100.0±0.0**
	Diana	73.68±1.32	73.68±2.32
	Doina	50.0±0.0	57.13±22.86
	Elena	66.67±16.67	42.86±7.13
	Flora*	30.0±3.33	**38.75±1.25**
	Geta	64.70±5.29	64.28±1.71
	Gras ameliorat*	**55.55±2.44**	33.32±1.67
	Iulia*	**78.57±1.43**	54.54±4.55
	Ivan*	50.0±1.0	**55.56±2.43**
	Jubileu 50	72.85±12.85	71.25±8.75
	Matilda	33.49±34.66	44.58±25.35
	Minerva	77.30±7.31	68.89±8.89
	Pescăruș*	**88.89±1.11**	81.82±3.17
	Romaner	58.82±1.07	58.82±2.17
	Silvia*	**77.78±0.21**	62.5±2.5
	Tita*	63.63±3.36	**84.62±3.37**
	Tuleu dulce*	40.0±3.0	**80.0±4.0**
	Zamfira	62.22±8.37	63.07±16.92
Foreign	Anna Späth	53.12±21.87	48.21±23.21
	Blue free*	**84.61±3.38**	60.0±5.0
	d`Agen	38.46±1.54	41.76±3.32
	President	80.0±5.0 0	83.33±3.67
	Reine Claude d’Althan*	50.0±1.00	**63.63±4.26**
	Stanley	75.0±5.0	75.0±5.0
Average		**59.05±20.94**	**60.14±20.78**

*$p<0.05$. Cultivars followed by the * are significantly different according to Student’s t-test.

The highest average initiation rate was found on medium with mT (VI-2) for ‘Delia’ cultivar (100%). The use of BAP (VI-1) resulted in obtaining average values of the initiation rate in the range from 18.17±0.82 (‘Agent’) to 88.89±1.11 (‘Pescăruș’), while on medium with mT (VI-2) from 8.33±7.23 (‘Dani’) to 100.0±0.0 (‘Delia’). The statistical difference in the means of initiation rates was observed for several cultivars, the highest values on medium with BAP (VI-1) for ‘Centenar’, ‘Dani’, ‘Gras ameliorat’, ‘Iulia’, ‘Pescăruş’, ‘Silvia’ and ‘Bluefree’, or the highest values on medium with mT (VI-2) for ‘Agent’, ‘Delia’, ‘Flora’, ‘Ivan’, ‘Tita’, ‘Tuleu dulce’ and ‘Reine Claude d’Althan’.

These results confirmed the importance of selecting an appropriate cytokinin type to optimize the initiation phase of micropropagation, particularly for specific cultivars.

In a previous study, similar establishment rates were obtained for ‘Delia’ (20.6%) and ‘Tita’ (46%) cultivars on MS medium supplemented with 0.2 mg/L BAP (Vescan et al., 2011). Another study revealed that the medium containing BAP in concentration of 0.5 or 1 mg/l showed significant positive response for shoot induction and shoot multiplication than kinetin hormone for ‘Stanley’ cultivar (Wolella, 2017).

Multiplication stage

Multiplication coefficient

The average values of the multiplication coefficient for the 19 cultivars studied on four cultures variants (Table 3) showed that plants propagated on WPM with each hormone (VM-1 and VM-2) had the highest significant values than those on medium MS medium with each hormone (VM-3 and VM-4).

The highest average value of the multiplication coefficient of plum cultivars was found on nutrient medium WPM supplemented with BAP for ‘Doina’ cultivar (6.10±4.14). For all cultivars on nutrient medium WPM supplemented with BAP (VM-1), relatively high values of the multiplication coefficient are observed, ranging from 2.05±0.66 (‘Anna Späth’) to 6.10±4.14 (‘Doina’) shoots per explants. On nutrient medium WPM supplemented with mT (VM-2), the range of multiplication coefficient was from 1.48±0.97 (‘Gras ameliorat’) to 5.22±4.26 (‘Blue free’) shoots per explants. In contrast, the range of the multiplication coefficient was narrower when nutrient medium MS supplemented with BAP (VM-3) was used, more specifically from 1.21±0.26 (‘Blue free’) to 3.09±1.39 (‘Pescăruș’) shoots per explants. Regarding the nutrient medium MS supplemented with mT (VM-4), the multiplication coefficient ranged from 1.45±0.15 (‘Anna Späth’) to 3.27±1.21 (‘Geta’) shoots per explants.

Table 3. Multiplication coefficient depending the culture variants

Origin of cultivars	Cultivars	Culture variants			
		VM-1	VM-2	VM-3	VM-4
Romanian	Andreea*	**3.12±1.45 a**	2.49±0.69 ab	1.63±0.42 b	1.74±0.98 ab
	Carpatin**	3.06±2.54 a	4.37±2.23 a	2.51±2.38 a	2.41±0.85 a
	Centenar**	**3.91±1.47 a**	2.42±0.58 ab	1.78±0.67 b	2.21±0.80 ab
	Delia*	2.81±1.39 ab	**4.80±1.98 a**	2.18±0.74 b	3.00±2.02 ab
	Diana**	2.32±1.30 a	3.30±0.96 a	2.06±0.63 a	1.98±0.43 a
	Doina*	**6.10±4.14 a**	3.37±1.11 ab	2.89±0.53 ab	2.15±0.16 b
	Elena**	2.43±0.87 a	3.06±1.71 a	2.65±0.75 a	2.58±0.71 a
	Geta**	2.37±0.43 a	2.25±1.89 a	3.00±1.81 a	3.27±1.21 a
	Gras ameliorat**	2.74±1.31 a	1.48±0.97 a	2.18±0.83 a	2.83±1.00 a
	Matilda**	**3.73±1.87 a**	1.39±0.49 b	2.31±0.75 ab	2.33±0.48 ab
	Minerva*	3.02±1.24 ab	**3.14±0.82 a**	2.45±1.32 ab	1.49±0.19 b
	Pescăruș*	3.81±1.59 ab	**4.91±2.00 a**	3.09±1.39 ab	2.31±1.21 b
	Romaner*	**5.12±2.63 a**	3.22±1.52 ab	2.75±0.80 ab	2.05±0.59 b
	Zamfira**	**2.21±0.54 a**	1.81±0.60 ab	1.40±0.08 ab	1.12±0.08 b
	Silvia**	**4.42±1.45 a**	2.66±0.65 ab	2.10±1.19 ab	2.01±0.84 b
	Tita*	**4.75±2.09 a**	4.04±1.29ab	2.29±1.00 b	2.23±0.29 b
	Tuleu dulce*	**5.83±2.87 a**	3.76±2.61 ab	1.45±0.48 b	3.00±2.34 ab
	Zamfira**	**2.21±0.54 a**	1.81±0.60 ab	1.40±0.08 ab	1.12±0.08 b
Foreign	Anna Späth**	2.05±0.66 a	2.81±1.31 a	2.56±1.56 a	1.45±0.15 a
	Bluefree*	2.89±1.93 ab	**5.22±4.26 a**	1.21±0.26 b	1.52±0.45 b
Average**		**3.51±2.04 A**	**3.18±1.85 A**	**2.24±1.08 B**	**2.19±1.02 B**

*p<0.05, ** p<0.01. Means followed by the different letters are significantly different according to Fisher test.

Based on the comparative analysis, significant differences of multiplication coefficients were observed between VM-1 and VM-4 for the cultivars 'Doina', 'Romaner', 'Zamfira' and 'Silvia', and between VM-1 and VM-3 for the cultivars 'Andreea', 'Centenar' and 'Tuleu dulce'. Similarly, for the 'Matilda' cultivar, VM-1 showed higher multiplication coefficients compared to VM-2 while for 'Tita' cultivar in contrast with VM-3 and VM-4.

On nutrient medium WPM supplemented with mT (VM-2), the highest significant multiplication coefficients were observed for 'Delia' cultivar in comparison to VM-3, for 'Minerva' and 'Pescăruș' cultivars in contrast with VM-4, and for 'Blue free' cultivar in comparison to VM-3 and VM-4.

These findings suggest that WPM, particularly when supplemented with BAP or mT, provides optimal conditions for micropropagation. However, cultivar-specific responses must be considered when selecting the most suitable medium for efficient shoot multiplication.

The composition of the culture medium plays an essential role in *in vitro* micropropagation, as it directly affects plant growth, development, and regeneration (Figure 1). Medium nutritive culture most used for *Prunus domestica* are MS (Alam & Barua, 2015; Gago et al., 2020), WPM (Yao et al., 2011) or another such as Quoirin & Lepoivre Medium - QL (Plopa et al., 2010), DKW/Junglans medium and Westvaco WV5 medium (Jakab et al., 2009).

Similar results were obtained for cultivars 'Anna Späth', 'Carpatin',' Dani', 'Ivan', 'Jubileu 50', 'Reine Claude d'Althan' and 'Stanley' proliferated on MS medium and BAP (Clapa et al., 2013). Another previous study showed the lowest proliferation rate on medium with mT compared to BAP, but was observed a positive effect on the growth and quality for the shoots with mT for *Prunus domestica* 'Torinel' and *P. domestica x insititia* 'Ferdor' (Gentile et al., 2014).

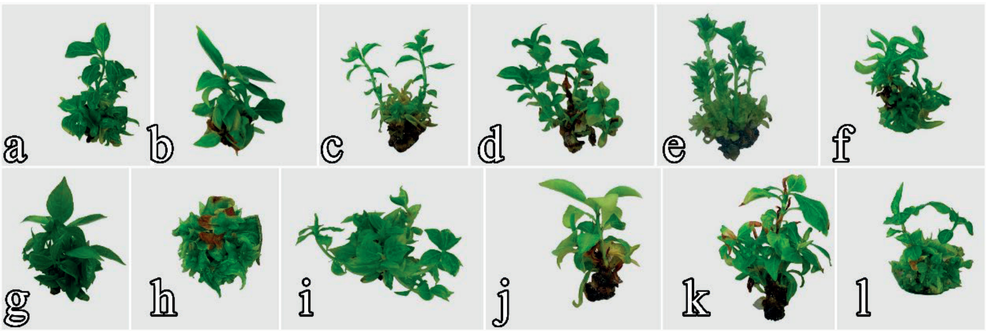


Figure 1. Micropropagation of high regenerated cultivars: 'Andreea' (a), 'Bluefree' (b), 'Centenar' (c), 'Delia' (d), 'Doina' (e), 'Matilda' (f), 'Minerva' (g), 'Pescăruș' (h), 'Romaner' (i), 'Silvia' (j), 'Tita' (k), 'Tuleu dulce' (l)

Principal Component Analysis (PCA), which was performed in order to explore the relationships between cultivars and culture multiplication variants (Figure 2), revealed a total variability of 63.13%, explained by the two components (F1 and F2**)** of the total variance. Regarding the culture variants, VM-1 and VM-2 are positively correlated and contribute strongly to F2, and VM-3 and VM-4 are negatively correlated with F2 and positively with F1. A cultivar high in VM-1 and VM-2 is likely to be low in VM-3 and VM-4 (and vice versa).

'Tuleu Dulce' and 'Tita' cultivars have high values for VM-2, likely have moderate values for VM-1 and lower values for VM-3 and VM-4.

'Doina', 'Pescăruș', 'Carpatin', 'Romaner' and 'Delia' cultivars have strong correlations with VM-1 and tend to have high values for this trait, moderate influence from VM-2 but lower values in VM-3 and VM-4.

'Elena' and 'Geta' cultivars show high values for VM-3 and VM-4 and conversely, they have low values for VM-1 and VM-2.

'Blue free' cultivar was strongly influenced by VM-2. However, it is far from VM-1, VM-3, and VM-4, suggesting it has lower values for these variants.

'Zamfira', 'Andreea', 'Centenar', 'Silvia' 'Anna Späth', 'Minerva', 'Diana', 'Matilda' and 'Gras ameliorat' cultivars cluster near the centre or slightly to the negative side, suggesting moderate to low values in all variants.

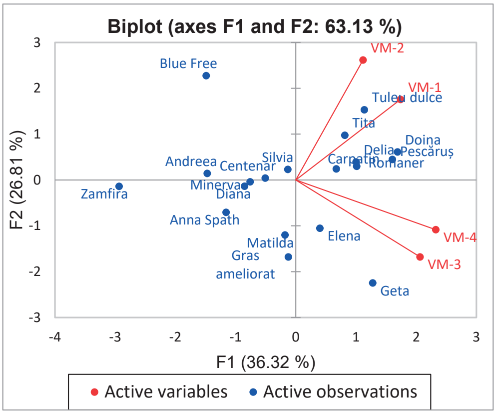


Figure 2. Principal Component Analysis (PCA) of 19 cultivars on four culture variants of multiplication medium

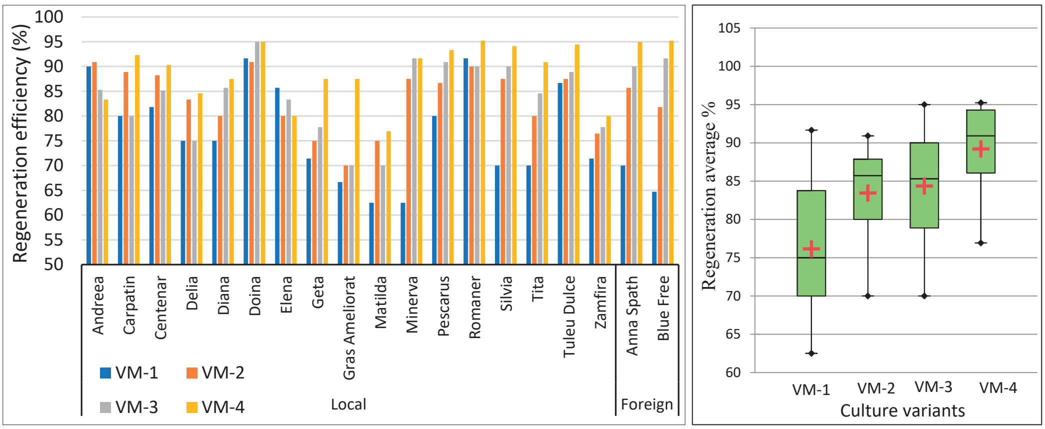
Regeneration efficiency

Regeneration efficiency varied from 63% ('Matilda' and 'Minerva') on variants VM-1 to 95% ('Doina', 'Romaner', 'Anna Späth' and 'Blue free') on variants VM-4 and on variants VM-3 for 'Doina' cultivar (Figure 3a). Some cultivars, with regeneration efficiency between 65-80% ('Delia', 'Geta', 'Gras ameliorat', 'Matilda' and 'Zamfira') showed a high level of necrotic plants, unable to regenerate. In this case medium composition or hormonal balance should be adjust for each genotype.

The highest average regeneration efficiency was obtained for VM-4 (Figure 3b). Regarding of culture variants, all lead to good regeneration efficiency.

Regeneration efficiency is mostly influenced by the cultivars and also by the medium composition (Long et al., 2022). Our results are in accordance with other report regarding successfully regenerated microshoots for three plum cultivars and on three different medium culture (Batukaev et al., 2018).

Short term conservation of plum cultivars was successfully achieved using micropropagation techniques through tissue culture. This approach proved effective for eleven selected cultivars that demonstrated a high multiplication coefficient and superior regeneration efficiency ('Doina', 'Blue free', 'Carpatin', 'Centenar', 'Elena', 'Minerva', 'Pescăruș', 'Romaner', 'Silvia', 'Tita', 'Tuleu dulce'). These cultivars exhibited strong *in vitro* adaptability, ensuring their rapid propagation and maintenance under controlled conditions. The high regeneration efficiency of these cultivars highlights the potential of micropropagation as a valuable tool for the preservation.



a b

Figure 3. Regeneration efficiency: a - for 19 cultivars, b - average regeneration rate for variants

The findings of this study contribute to the development of tailored *in vitro* conservation protocols for *Prunus domestica* L. cultivars, providing valuable material for the medium- and long-term conservation of plum genetic resources.

CONCLUSIONS

In the initiation stage both cytokinin-type hormones (BAP and mT) incorporated into the culture media effectively supported the initiation stage of plum cultivars. However, cultivar-specific variations were evident and revealed statistically significant differences in initiation rates for 14 cultivars, suggesting that certain genotypes respond more favorably to one cytokinin over the other.

The choice of nutrient medium and cytokinin type significantly influences the multiplication coefficient of plum cultivars, WPM medium supplemented with BAP (VM-1) highlighting its effectiveness in promoting shoot proliferation. Similarly, WPM medium with mT (VM-2) supported superior multiplication rates for specific cultivars.

Regeneration efficiency varied significantly among cultivars, with the highest success observed on VM-4. To optimize propagation, medium composition and hormonal balance should be adjusted for each genotype, particularly for those with higher necrosis rates.

All these findings showed that micropropagated plum cultivars are suitable for short-term conservation using tissue culture techniques, providing an effective method for preserving and propagating valuable cultivars.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development through ADER 2026 Program, financed from Project no. 6.3.4/2023, and also by Fruit Research and Development Station Bistrita, which we are grateful for the generous help and assistance.

REFERENCES

Alam, M. J., & Barua, R. (2015). *In vitro* regeneration and antibacterial activity of *Prunus domestica* L. *Journal of BioScience & Biotechnology*, *4*(1).

Batukaev, A. A., Bamatov, I. M., & Vinter, M. A. (2018). Studying tolerance of prune (*Prunus domestica*) to the plum pox virus (PPV) by criterion" Efficiency of microshoots' regeneration" in controlled *in vitro* conditions. *Journal of Pharmaceutical sciences and research*, *10*(1), 59-64.

Bettoni, J. C., Wang, M. R., & Wang, Q. C. (2024). *In Vitro* Regeneration, Micropropagation and Germplasm Conservation of Horticultural Plants. *Horticulturae*, *10*(1), 45.

Canli, F. A., & Tian, L. (2009). Regeneration of adventitious shoots from mature stored cotyledons of Japanese plum (*Prunus salicina* Lindl). *Scientia Horticulturae*, *120*(1), 64-69.

Chauhan, R., Singh, V., & Quraishi, A. (2019). *In vitro* conservation through slow-growth storage. *Synthetic Seeds: Germplasm Regeneration, Preservation and Prospects,* 397-416.

Clapa, D., Fira, A. L., Vescan, A., Zagrai, I., Zagrai, L., Jakab, Z. S., & Plopa, C. (2013). The micropropagation and *in vitro* maintenance of some plum cultivars. *Acta Hortic*., 981, 437-442

Engelmann, F. (1997). *In vitro* conservation methods. *Biotechnology in Agriculture Series*, 119-162.

(FAO) Food and Agricultural Organization. www.fao.org. Accesed in 25 february 2025.

Gago, D., Sánchez, C., Aldrey, A., Christie, C. B., Bernal, M. Á., & Vidal, N. (2022). Micropropagation of plum (*Prunus domestica* l.) in bioreactors using photomixotrophic and photoautotrophic conditions. *Horticulturae*, *8*(4), 286.

Gentile, A., Jàquez Gutiérrez, M., Martinez, J., Frattarelli, A., Nota, P., & Caboni, E. (2014). Effect of meta-Topolin on micropropagation and adventitious shoot regeneration in *Prunus* rootstocks. *Plant Cell, Tissue and Organ Culture (PCTOC),* 118, 373-381.

Grout, B. W. (1990). *In vitro* conservation of germplasm. *Plant Tissue Culture. Application and Limitations*, 394-411.

Hartmann, W., & Neumüller, M. (2009). Plum breeding. *Breeding plantation tree crops: temperate species*, 161-231.

Jakab, Z. I., Clapa, D., & Fira, A. (2009). Contributions concerning the *in vitro* multiplication in *Prunus domestica*. *Annals of the Romanian Society for Cell Biology*, *14*(1), 246-252.

Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. *Combined Proceedings, International Plant Propagators' Society*, 30, 421-427.

Long, Y., Yang, Y., Pan, G., & Shen, Y. (2022). New insights into tissue culture plant-regeneration mechanisms. *Frontiers in plant science*, 13, 926752.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473-497.

Nacheva, L., Dimitrova, N., Koleva-Valkova, L., Stefanova, M., Ganeva, T., Nesheva, M., ... & 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.

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

Plopa, C., Milusheva S., Butuc, M. M., Presa, S. A. & Isac, V. (2010). Obtaining virus free planting material of plum cultivars by *in vitro* method. *Scientific Paper of the RIFG Pitești*, *XXVI*.

Romadanova, N. V., Mishustina, S. A., Matakova, G. N., Kushnarenko, S. V., Rakhimbaev, I. R., & Reed, B. M. (2016). *In vitro* collection methods for Malus shoot cultures used for developing a cryogenic bank in Kazakhstan. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1113* (pp. 271-277).

Sharma, P., & Jain, S. M. (2024). Micropropagation Applications in Conservation of Horticultural Crops. In *Sustainable Utilization and Conservation of Plant Genetic Diversity* (pp. 683-710). Singapore: Springer Nature Singapore.

Vescan, L. A., Pamfil, D., Zagrai, I., Ioana, V. B., Clapa, D., Ciuzan, O., & Iulia, F. (2011). *In vitro* techniques for Plum Pox Virus elimination from two infected Romanian plum cultivars. *Bulletin UASVM Animal Science and Biotechnologies*, *68*, 1-2.

Vujović, T., Jevremović, D., Anđelić, T., & Vasilijević, B. (2022). Optimization of the protocol for *in vitro* propagation of autochthonous plum genotype 'Metlaš'. *Book of Proceedings, XIII International Scientific Agriculture Symposium "AGROSYM 2022*", 166-172.

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

Yao, Y. X., Sun, Y. W., Li, G. G., & Li, G. H. (2011). Regeneration of plants from *in vitro* culture of petioles in *Prunus domestica* Lindl (European plum). *Biotechnology & Biotechnological Equipment*, *25*(3), 2458-2463.

Yuan, H.Y., Wu, Y.X., Liao, K., Geng, W.J., Li, J., Xu, Z. and Wang, T. (2009). *In Vitro* Propagation Of Wild European Plum (*Prunus ×domestica* L.), A Rare And Endangered Species. *Acta Hortic*., 839, 99-104

Zagrai, I., & Zagrai, L. A. (2015). A nuclear-stock plum collection established in Romania. *Bulletin UASMV Cluj-Napoca, Horticulture*, *72(*1): 205-208.

Zagrai, L. A., Zagrai, I., Guzu, G. M., & Moldovan, C. (2022). Towards a widening of Romanian nuclear-stock plum varieties. *Bulletin UASMV Cluj-Napoca, Horticulture, 79*(1), 89-95