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CAPACITEATEA DE DIFERENȚIERE *IN VITRO* A UNOR PORTALTOI LA UNELE SPECII SÂMBUROASE

IN VITRO DIFFERENTIATION CAPACITY OF SOME ROOTSTOCKS OF STONE FRUIT SPECIES

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Abstract

***In vitro* culture media play a central role in the culture initiation phase. The ratio between the components determines the success of organogenesis. The hormonal balance decisively influences *in vitro* morphogenesis. At RIFG Pitesti, Romania, an experiment was conducted to evaluate the influence of different combinations of cytokinins (BAP), auxins (IBA), and gibberellic acid (GA3) on the initiation of *in vitro* cultures on peach rootstocks ('Adaptabil' and 'Miroper') and apricot rootstocks ('Apricor' and 'Baroc'). In the experiment the effect of the addition of gibberellic acid was followed, which can significantly improve the success of the process by stimulating elongation and differentiation, which can lead to more vigorous and better developed plantlets. The explants used were cultivated on Murashige and Skoog medium (MS, 1965) for apricot rootstocks and on Querin and Lepoivre medium (QL, 1977) for peach rootstocks, supplemented with various combinations of hormones, resulting in 27 combinations for each base medium. The best results regarding shoot development for 'Apricor' were obtained on MS medium with 1.0 mg/l GA3 + 0.1 mg/l IBA+ BAP 5 mg/l (86.67%). For 'Baroc', a combination of 0.0 mg/l GA3+0.1 mg/l IBA + 1.0 mg/l BAP resulted in a good regeneration rate of 73.33%. For 'Adaptabil', the combination of 0.5 mg/l GA3 + 0.5 mg/l IBA, in the absence of BAP cytokinin, resulted in a regeneration rate of 93.33%, while 'Miroper' had a regeneration rate of 60% under the same conditions. The responses varied depending on the genotype, confirming that each rootstock has an optimal hormonal pattern for initiation.**

Cuvinte cheie: diferențiere, portaltoi, *in vitro*, specii sâmburoase.
Key words: differentiation**,** rootstocks, *in vitro*, stone fruit species.

1. Introduction

Stone fruit crops of the *Prunus* genus, including peach (*P. persica*), apricot (*P. armeniaca*), and plum (*P. domestica*), represent a major segment of global horticultural production due to their high economic value and nutritional importance. Modern cultivation of these species increasingly relies on clonal rootstocks, which ensure genetic uniformity, high productivity, and adaptability to diverse environmental conditions. An effective rootstock is expected to exhibit broad compatibility with multiple cultivars, promote balanced tree vigor, induce early fruiting, and confer resistance to both biotic and abiotic stress factors (Yaremko et al., 2023).

Clonal propagation techniques have significantly advanced orchard establishment practices by enabling the large-scale production of uniform and pathogen-free planting material. Among these, micropropagation has become a key biotechnological tool, providing rapid multiplication of elite genotypes while maintaining genetic stability and high physiological quality (Ancu et al., 2016; Eliwa et al., 2025). Compared with traditional vegetative propagation, micropropagation allows for precise control of growth conditions and has proven highly efficient for *Prunus* rootstock production.

The success of micropropagation depends on several interrelated factors, including genotype, explant type, culture medium composition, and the use of specific plant growth regulators (Anđelić et al., 2008). However, plant responses *in vitro* are often genotype-dependent, emphasizing the need for optimization of nutrient formulations to improve morphogenic performance and overall propagation efficiency (Ramage and Williams, 2002; Greenway et al., 2012).

The positive effect of BAP used on the shootlets number obtained has been described in several studies (Falllahpour et al., 2015; Borkheyli et al., 2021), reflecting the role of cytokinin (BAP) in cell differentiation and division (Borkheyli et al., 2021).

Studies carried out by Clapa et al. (2013) on the Gisela 5 rootstock highlighted the determining role of BAP in bud activation and initial multiplication, while Plopa (2012) demonstrated that the addition of a small amount of GA_3 can improve shoot elongation and uniformity. The influence of GA_3 on plant growth has been demonstrated by the increase in cell division and elongation of the shootlets (Vernous et al., 2010; Paiva et al., 2023).

In a complementary way, IBA was included to balance the effects of cytokinin and subsequently stimulate root formation (Shaban et al., 2018).

The present studies aims to provide the effect of different combinations of growth hormones at the time of culture initiation and establishing *in vitro* differentiation capacity.

2. Material and methods

The study was conducted at the Plant Tissue Culture Laboratory of the Research Institute for Fruit Growing (ICDP Pitesti, Romania) and the biological material was represented by peach rootstocks: 'Adaptabil' and 'Miroper' and apricot rootstocks: 'Apricor' and 'Baroc'.

Explant source was represented by annual branches that were harvested in December. The explants inoculated on the culture media were obtained from meristems excised from the axillary buds on the annual branches. The explants were washed with tap water 3-5 times followed by liquid soap for 30 min with agitation to physically remove most microorganisms, and treated in 70% alcohol for 10 minutes and then in 6% calcium hypochlorite solution for 20 minutes for surface sterilization. Sterilization was followed by washing with 3 rinses with distilled water.

Sterilized explants of 'Apricor' and 'Baroc' were cultured in test tubes containing MS (Murashige and Skoog, 1962) medium fortified with 20 g/l sucrose, 9 g/l agar (Plant Agar) and varying level of BAP (0; 1.0; 5.0 mg/l) in combination with IBA (0; 0.1; 0.5 mg/l) and GA3 (0; 0.5; 1.0)(Table 1).

Sterilized explants of 'Adaptabil' and 'Miroper' were cultured in test tubes containing macroelements and microelements QL (Quoirin and Lepoivre, 1977) and LS (Linsmaier and Skoog, 1965) vitamins with the same combinations of hormones for the initiation experiment (Table 1).

All culture media contained NaFeEDTA 32mg/l. The test tubes were sterilized by autoclaving at 121° for 20 minutes.

The test tubes with cultured explants were properly sealed and maintained in the growth room equipped with metal shelves, illuminated with LED tubes, providing a light intensity of 2400-3000 LUX and a temperature of 23±3ºC, with a photoperiod of 16 hours of light and 8 hours of darkness

Three repetitions were used per variant with ten explants for each repetition. Results were taken after 28 days of *in vitro* culture and the following data were recorded: the number of viable explants, the number of contaminated explants (fungi and bacteria) and the number of necrotic explants. Data were analyze using Microsoft Excel 2010 facility.

3. Results and discussions

This study presents and discusses the results obtained regarding the effects of different combinations of growth regulators (BAP (0; 1.0; 5.0 mg/l) in combination with IBA (0; 0.1; 0.5 mg/l) and GA3 (0; 0.5; 1.0)) on *in vitro* micropropagation of the analyzed rootstocks.

The choice of the BAP–GA_3–IBA combination in the initiation stage was based on physiological reasoning and previous results reported for various rootstocks.

The study showed that the best results regarding the regeneration capacity for the 'Apricor' rootstock were obtained on MS medium supplemented with 1.0 mg/l GA_3+0.1 mg/l IBA+ 5.0 mg/l BAP (Table 2), with a regeneration rate of 86.67% (variant V24). These results are slightly better than what we found in the specilized literature, Șarpe et al., 2025 used QL medium with 0.01 mg/l IBA with 0.1 mg/l GA_3 and obtained a percentage of 80% of explants that survived.

The effectiveness of the treatment was evidenced by both the low level of contamination (with a mean of 6.67% and a standard deviation of 11.55) and the low necrosis rate. Studying the variability of the mean number of necrotic explants, the coefficient of variation (CV%) was found to be very high. This significant variability is mainly due to the large differences in the mean number of necrotic explants (Table 2). Overall, the treatment represented by V24 is recommended for *in vitro* explant culture due to its high efficiency and consistency of biological response.

For 'Baroc', a combination of 0.0 mg/l GA_3+0.1 mg/l IBA+ 1.0 mg/l BAP on MS basal medium led to a regeneration rate of 73.33% (variant V5) (Table 3). Even for this rootstock slightly better results were obtained than in the case of the research published by Şarpe et al.,2025, who obtained a 60% regeneration rate, when was it used QL medium with 0.01 mg/l IBA with 0.1 mg/l GA_3.

Regarding treatment efficacy for the 'Baroc' rootstock, this was also evidenced by a low contamination rate (with a mean of 6.67% and a standard deviation of 11.55), but in this case the mean number of necrotic explants - 20%, was higher than for the 'Apricor' rootstock (Table 2 and 3).

For 'Adaptabil', the combination of 0.5 mg/l GA_3+0.5 mg/l IBA, in the absence of cytokinin BAP on QL basal medium with LS vitamins led to a regeneration rate of 93.33 (variant V16) (Table 4). This result is similar to the study of Plopa et al., 2012 who reported a 95% regeneration rate when it was used QL medium with 0.01 mg/l IBA with 0.1 mg/l GA3, for 'Adaptabil'. 'Miroper' had a rate of 60% survived explants in the same conditions as 'Adaptabil' (variant V16) (Table 5).

For 'Adaptabil', the efficacy of the treatment was evidenced by both the low level of contamination (with a mean of 6.67% and a standard deviation of 11.55) and the fact that the mean number of necrotic explants was zero. Overall, the V16 treatment is recommended for *in vitro* explant culture due to its high efficacy and consistency of biological response.

For 'Miroper', the mean rate of explant contamination for the most effective treatment in terms of number of viable explants was 20%, indicating either insufficient sterilization efficiency or increased explant sensitivity. For the same treatment, the mean rate of explant necrosis was 20%, suggesting a lower tolerance of explants to the treatment.

4. Conclusions

The responses varied depending on the genotype, confirming that each rootstock has an optimal hormonal balance for initiation.

The experimental MS culture medium supplemented with 1.0 mg/l GA3+0.1 mg/l IBA+ 5.0 mg/l BAP was found to have the best shoot regeneration rate (86.67%).

According to the recorded data, it was found that the highest percentage regarding the regeneration rate of viable plantlets from meristems of the 'Baroc' was obtained on the MS culture medium supplemented with 0.1 mg/l IBA+ 1.0 mg/l BAP, in the absence of GA_3 (73.33%)(variant V5).

The best results in terms of the regeneration rate of axillary shoots from peach rootstock meristems ('Adaptabil') were obtained in the case of the experimental variant V16 - 93.33% (QL culture medium, with Linsmaier and Skoog vitamins). Considering the relatively low percentage of regeneration in the case of the 'Miroper', further investigations are needed to determine an optimal hormonal balance in the case of crop initiation.

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Tables and Figures

Table 1. **Composition of culture media used for initiating cultures for 'Apricor', 'Baroc', 'Adaptabil' and 'Miroper' rootstocks (regeneration phase)**

Variants	Basal medium MS and vitamins MS			Basal medium QL and vitamins LS		
	Apricor and Baroc			Adaptabil and Miroper		
	Growth regulators (mg/l)			Growth regulators (mg/l)		
	GA_3	IBA	BAP	GA_3	IBA	BAP
V1	0	0	0	0	0	0
V2	0	0	1	0	0	1
V3	0	0	5	0	0	5
V4	0	0,1	0	0	0,1	0
V5	0	0,1	1	0	0,1	1
V6	0	0,1	5	0	0,1	5
V7	0	0,5	0	0	0,5	0
V8	0	0,5	1	0	0,5	1
V9	0	0,5	5	0	0,5	5
V10	0,5	0	0	0,5	0	0
V11	0,5	0	1	0,5	0	1
V12	0,5	0	5	0,5	0	5
V13	0,5	0,1	0	0,5	0,1	0
V14	0,5	0,1	1	0,5	0,1	1
V15	0,5	0,1	5	0,5	0,1	5
V16	0,5	0,5	0	0,5	0,5	0
V17	0,5	0,5	1	0,5	0,5	1
V18	0,5	0,5	5	0,5	0,5	5
V19	1	0	0	1	0	0
V20	1	0	1	1	0	1
V21	1	0	5	1	0	5
V22	1	0,1	0	1	0,1	0
V23	1	0,1	1	1	0,1	1
V24	1	0,1	5	1	0,1	5
V25	1	0,5	0	1	0,5	0
V26	1	0,5	1	1	0,5	1
V27	1	0,5	5	1	0,5	5

Table 2. Results on the influence of hormonal balance on the regeneration of 'Apricor' rootstock regarding survival, contamination and necrosis of explants(%)

Basal medium MS	Surviving %		Contaminating %		Necroting %	
Variant	**Mean±SD**	**CV%**	**Mean±SD**	**CV%**	**Mean±SD**	**CV%**
V1	60.00±20.00	33.33	13.33±11.55	0.00	26.67±30.55	114.56
V2	80.00±0.00	0.00	13.33±11.55	86.60	6.67±11.55	173.21
V3	53.33±30.55	57.28	40.00±20.00	50.00	6.67±11.55	173.21
V4	53.33±23.09	43.30	20.00±20.00	100.00	26.67±11.55	43.30
V5	60.00±20.00	33.33	26.67±30.55	114.56	13.33±23.09	173.21
V6	26.67±11.55	43.30	73.33±11.55	15.75	0.00±0.00	-
V7	46.67±23.09	49.49	33.33±23.09	69.28	20.00±0.00	0.00
V8	33.33±11.55	34.64	46.67±11.55	24.74	20.00±0.00	0.00
V9	80.00±0.00	0.00	0.00±0.00	-	20.00±0.00	0.00
V10	73.33±23.09	31.49	6.67±11.55	173.21	20.00±0.00	100.00
V11	73.33±23.09	31.49	0.00±0.00	-	26.67±23.09	86.60
V12	66.67±23.09	34.64	6.67±11.55	173.21	26.67±30.55	114.56
V13	66.67±11.55	17.32	20.00±20.00	100.00	13.33±11.55	86.60
V14	60.00±20.00	33.33	6.67±11.55	173.21	33.33±23.09	69.28
V15	66.67±30.55	45.83	6.67±11.55	173.21	26.67±30.55	114.56
V16	73.33±11.55	15.75	13.33±11.55	86.60	13.33±11.55	86.60
V17	66.67±11.55	17.32	0.00±0.00	-	33.33±11.55	34.64
V18	33.33±11.55	34.64	6.67±11.55	173.21	60.00±0.00	0.00
V19	53.33±11.55	21.65	6.67±11.55	173.21	33.33±11.55	34.64
V20	13.33±11.55	86.60	20.00±0.00	0.00	66.67±11.55	17.32
V21	46.67±11.55	24.74	20.00±0.00	0.00	33.33±11.55	34.64
V22	60.00±20.00	33.33	6.67±11.55	173.21	33.33±11.55	34.64
V23	46.67±11.55	24.74	20.00±34.64	173.21	33.33±30.55	91.65
V24	86.67±11.55	13.32	6.67±11.55	173.21	6.67±11.55	173.21
V25	66.67±11.55	17.32	26.67±11.55	43.30	6.67±11.55	173.21
V26	73.33±11.55	15.75	13.33±11.55	86.60	13.33±11.55	86.60
V27	73.33±23.09	31.49	6.67±11.55	173.21	20.00±20.00	100.00

*SD-Standard Deviation; CV- coefficient of variation.

Table 3. Results on the influence of hormonal balance on the regeneration of 'Baroc' rootstock regarding survival, contamination and necrosis of explants(%)

Basal medium MS	Surviving %		Contaminating %		Necroting %	
Variant	**Mean±SD**	**CV%**	**Mean±SD**	**CV%**	**Mean±SD**	**CV%**
V1	33.33±23.09	69.28	6.67±11.55	11.55	60.00±20.00	33.33
V2	60.00±0.00	0.00	13.33±11.55	11.55	26.67±11.55	43.30
V3	26.67±23.09	86,60	20.00±34.64	34.64	53.33±50.33	94.37
V4	53.33±11.55	21.65	26.67±23.09	23.09	20.00±20.00	100.00
V5	73.33±30.55	41.66	6.67±11.55	11.55	20.00±20.00	100.00
V6	20.00±20.00	100.00	46.67±23.09	23.09	33.33±11.55	34.64
V7	6.67±11.55	173.21	66.67±30.55	30.55	33.33±30.55	91.65
V8	20.00±20.00	100.00	46.67±11.55	11.55	26.67±11.55	43.30
V9	26.67±30.55	114.56	13.33±11.55	11.55	60.00±34.64	57.74
V10	20.00±0.00	0.00	40.00±0.00	0.00	40.00±0.00	0.00
V11	53.33±11.55	21.65	6.67±11.55	11.55	40.00±20.00	50.00
V12	20.00±0.00	0.00	40.00±34.64	34.64	40.00±34.64	86.60
V13	6.67±11.55	173.21	33.33±11.55	11.55	60.00±20.00	33.33
V14	13.33±11.55	86,60	46.67±11.55	11.55	40.00±0.00	0.00
V15	0.00±0.00	-	20.00±0.00	0.00	80.00±0.00	0.00
V16	33.33±30.55	91.65	0.00±0.00	0.00	66.67±30.55	45.83
V17	46.67±11.55	24.74	13.33±11.55	11.55	40.00±0.00	0.00
V18	6.67±11.55	173.21	40.00±0.00	0.00	53.33±11.55	21.65
V19	6.67±11.55	173.21	26.67±11.55	11.55	53.33±11.55	21.65
V20	0.00±0.00	-	46.67±23.09	23.09	53.33±23.09	43.30
V21	33.33±23.09	69.28	26.67±11.55	11.55	40.00±20.00	50.00
V22	0.00±0.00	-	53.33±11.55	11.55	46.67±11.55	24.74
V23	20,00±0.00	0.00	46.67±11.55	11.55	33.33±11.55	34.64
V24	46.67±11.55	24.74	20.00±0.00	0.00	33.33±11.55	34.64
V25	53.33±11.55	21.65	20.00±20.00	20.00	26.67±11.55	43.30
V26	40.00±0.00	0.00	26.67±11.55	11.55	33.33±11.55	34.64
V27	40.00±20.00	50.00	20.00±0,00	0.00	33.33±30.55	91.65

*SD-Standard Deviation; CV- coefficient of variation.

Table 4. Results on the influence of hormonal balance on the regeneration of 'Adaptabil' rootstock regarding survival, contamination and necrosis of explants(%)

Basal medium QL	Surviving %		Contaminating %		Necroting %	
Variant	Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%
V1	33.33±30.55	91,65	46.67±30.55	65.47	20.00±0.00	0.00
V2	46.67±11.55	24,74	33.33±11.55	34.64	20.00±20.00	100.00
V3	66.67±30.55	45,83	13.33±11.55	86.60	20.00±20.00	100.00
V4	46.67±11.55	24,74	40.00±20.00	50.00	13.33±11.55	86.60
V5	26.67±11.55	43,30	60.00±0.00	0.00	13.33±11.55	86.60
V6	46.67±23.09	49,49	40.00±0.00	0.00	13.33±23.09	173.21
V7	53.33±11.55	21,65	46.67±11.55	24.74	0.00±0.00	-
V8	73.33±30.55	41,66	20.00±20.00	100.00	6.67±11.55	173.21
V9	53.33±23.09	43,30	26.67±30.55	114.56	20.00±20.00	100.00
V10	53.33±23.09	43,30	20.00±20.00	100.00	26.67±11.55	43.30
V11	33.33±11.55	34,64	46.67±11.55	24.74	20.00±0.00	0.00
V12	46.67±23.09	49,49	33.33±23.09	69.28	20.00±0.00	0.00
V13	6.67±11.55	173,21	46.67±23.09	49.49	46.67±11.55	24.74
V14	6.67±11.55	173,21	60.00±0.00	0.00	33.33±11.55	34.64
V15	13.33±23.09	173,21	60.00±0.00	33.33	26.67±11.55	43.30
V16	93.33±11.55	12,37	6.67±11.55	173.21	0.00±0.00	-
V17	13.33±11.55	86,60	40.00±0.00	0.00	46.67±11.55	24.74
V18	60.00±20.00	33,33	40.00±20.00	50.00	0.00±0.00	-
V19	40.00±34.64	86,60	20.00±20.00	100.00	40.00±20.00	50.00
V20	20.00±20.00	100,00	26.67±11.55	43.30	53.33±30.55	57.28
V21	33.33±23.09	69,28	46.67±11.55	24.74	20.00±20.00	100.00
V22	86.67±23.09	26,65	13.33±23.09	173.21	0.00±0.00	-
V23	33.33±11.05	34,64	33.33±11.55	34.64	33.33±11.55	34.64
V24	40.00±20.00	50,00	40.00±40.00	100.00	20.00±20.00	100.00
V25	26.67±11.55	43,30	33.33±41.63	124.90	40.00±34.64	86.60
V26	46.67±30.55	65,47	20.00±0.00	0.00	33.33±30.55	91.65
V27	40.00±0.00	0,00	26.67±11.55	43.30	33.33±11.55	34.64

*SD-Standard Deviation; CV- coefficient of variation.

Table 5. Results on the influence of hormonal balance on the regeneration of 'Miroper' rootstock regarding survival, contamination and necrosis of explants(%)

Basal medium QL	Surviving %		Contaminating %		Necroting %	
Variant	Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%
V1	0.00±0.00	-	66.67±30.55	45.83	33.33±30.55	91.65
V2	40.00±0.00	0.00	53.33±11.55	21.65	6.67±11.55	173.21
V3	53.33±11.55	21.65	26.67±23.09	86.60	20.00±20.00	100.00
V4	33.33±11.55	34.64	26.67±23.09	86.60	40.00±20.00	50.00
V5	53.33±41.63	78.06	40.00±34.64	86.60	6.67±11.55	173.21
V6	53.33±30.55	57.28	40.00±40.00	100.00	6.67±11.55	173.21
V7	26.67±23.09	86.60	46.67±23.09	49.49	26.67±23.09	86.60
V8	33.33±30.55	91.65	33.33±11.55	34.64	33.33±23.09	69.28
V9	26.67±30.55	114.56	53.33±30.55	57.28	20.00±0.00	0.00
V10	33.33±11.55	34.64	46.67±11.55	24.74	20.00±20.00	100.00
V11	53.33±30.55	57.28	33.33±41.63	124.90	13.33±11.55	86.60
V12	53.33±23.09	43.30	20.00±0.00	0.00	26.67±23.09	86.60
V13	40.00±20.00	50.00	60.00±20.00	33.33	0.00±0.00	-
V14	13.33±11.55	86.60	46.67±23.09	49.49	40.00±20.00	50.00
V15	6.67±11.55	173.21	60.00±20.00	33.33	33.33±23.09	69.28
V16	60.00±20.00	33.33	20.00±20.00	100.00	20.00±34.64	173.21
V17	20.00±0.00	0.00	40.00±0.00	0.00	40.00±0.00	0.00
V18	0.00±0.00	-	60.00±20.00	33.33	40.00±20.00	50.00
V19	0.00±0.00	-	46.67±30.55	65.47	53.33±30.55	57.28
V20	6.67±11.55	173.21	26.67±11.55	43.30	66.67±23.09	34.64
V21	26.67±30.55	114.56	40.00±0.00	0.00	33.33±30.55	91.65
V22	53.33±30.55	57.28	33.33±30.55	91.65	13.33±11.55	86.60
V23	46.67±11.55	24.74	46.67±11.55	24.74	6.67±11.55	173.21
V24	40.00±34.64	86.60	33.33±23.09	69.28	26.67±30.55	114.56
V25	40.00±20.00	50.00	33.33±11.55	34.64	26.67±11.55	43.30
V26	13.33±23.09	173.21	40.00±20.00	50.00	46.67±11.55	24.74
V27	13,33	173.21	20.00±20.00	100.00	66.67±11.55	17.32

*SD-Standard Deviation; CV- coefficient of variation.

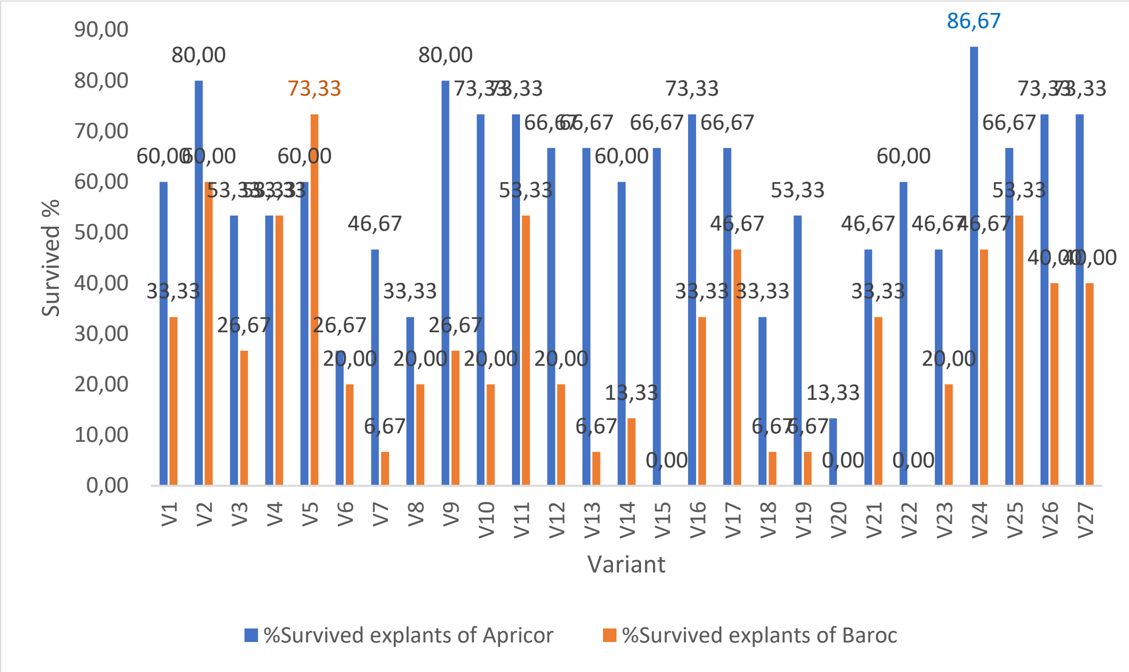

Fig. 1. Differentiation capacity for the 'Apricor' and 'Baroc' rootstocks

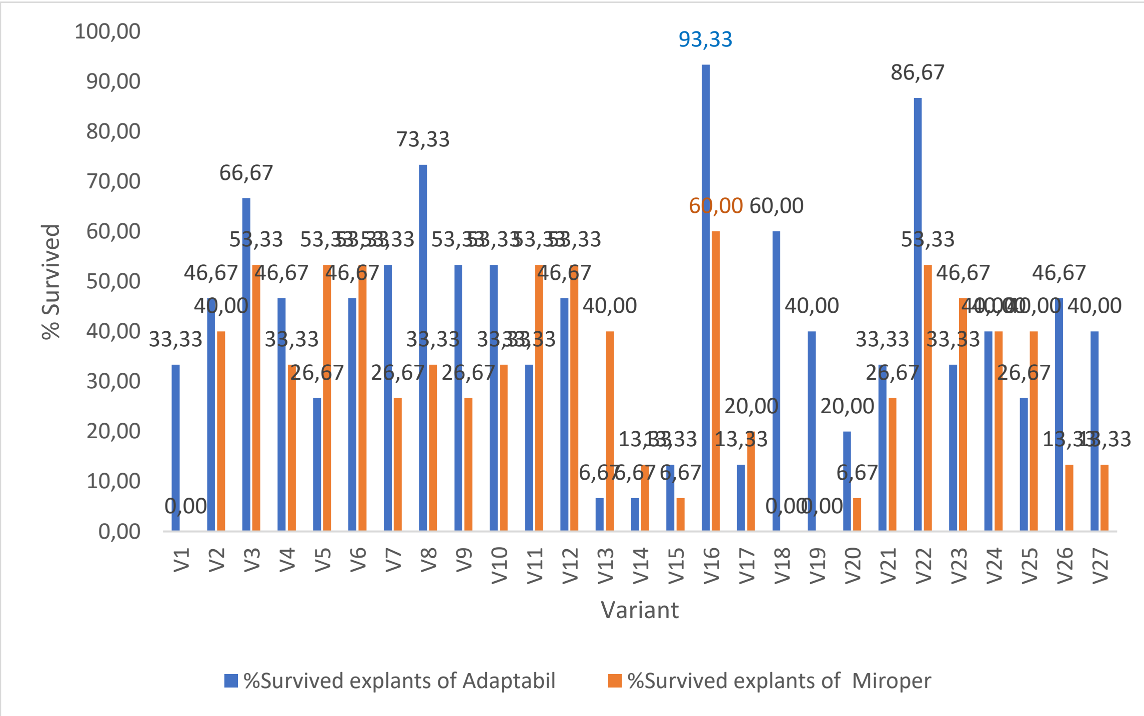

Fig. 2. Differentiation capacity for the 'Adaptabil' și 'Miroper' rootstocks

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Fig. 3. 'Apricor' - differentiation

Fig. 4. 'Baroc' - differentiation

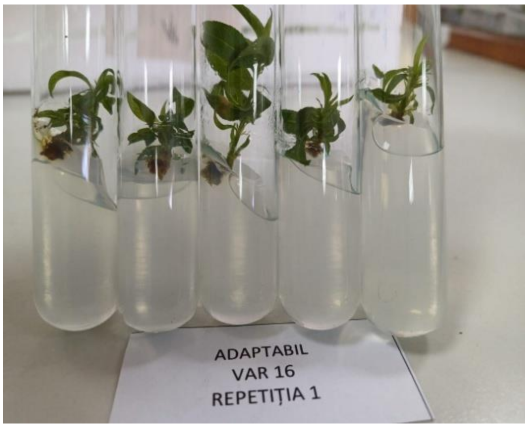

Fig. 5. 'Adaptabil' – differentiation

Fig. 6. 'Miroper' - differentiation