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IN VITRO REGENERATIVE GENOTYPIC SPECIFITY OF MERISTEMS FROM VIRUS INFECTED GRAPEVINE CULTIVARS

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**SVETLANA SMEREA, LARISA ANDRONIC,
TATIANA GRIGOROV, VALERIU BUJOREANU**
Institute of Genetics and Plant Physiology of the Academy of Sciences of Moldova, 20 Padurii str.,Chisinau, MD 2002, Republic of Moldova,tel:+(373 22)66 03 89; fax: + (373 22)55 61 80; e-mail: smerea_svetlana@yahoo.com;

Abstract

In this paper were presented the results of the study of in vitro meristem regeneration of autochthonous grapevine cultivars of great interest for viticulture. The current study involved the reaction of 11 grapevine genotypes on three culture media with different hormonal combinations based on MS medium. Deviations registered in the regenerative capacity were dependent primordialy on genotype. The evaluation of meristem regenerative rate showed that the effect of genotype was statistically significant for all of the traits ((P≤0,001). For regenerative rate were recorded significant deviations in frame of one and the same genotypes depending on the health status. It is suggested that virus infection represent an important source of variance in regeneration. The analysis of variance revealed the highest effect of genotypes on the regenerative capacity of the grapevine meristems (75,8 %).

Keywords: grapevine; *in vitro* culture; meristem; regenerative capacity; virus eradication

Introduction

Meristem culture in combination with heat-treatment or chimiotherapy is a effective procedure used in rapid production of high quality, disease-free and uniform planting material. *In vitro* techniques can be used for mass clonal propagation of diverse horticultural species, including *Vitis vinifera*. Some reports concerning mass propagation of Vitis species by shoot apices [3; 8; 11; 14; 15] or axillary buds [9; 16] demonstrate the feasibility of producing vines via somatic embryogenesis [13; 18]. *In vitro* cultivation of plants is one of the rapid methods to produce healthy plants with significant economy [2; 12].

Viticulture is one of the most important branches of the agriculture complex of the Republic of Moldova, providing important revenues sources in national budget. In order to restore the viticulture sector of economy, a "National Program for restoration and development of viticulture and winemaking in the Republic of Moldova for the period 2002-2020" was elaborated and accepted. According to this Program the area of vineyards in the republic must be established at level of 100 thousand hectares in 2020. Because at the beginning of this period all existent vineyards are more than 20 years old, during this period all old vineyards will be stubbed and the young vineyards will be founded. Considerable damage for this sector is caused by diseases, especially viruses.

The International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG), recognizes over 70 infectious agents affecting grapevine (viruses, viroids and mycoplasmas) [7]. Many of them cause disorders that reduce the plant vigour and longevity or

the quality and quantity of the yield. Infected propagating material is largely responsible for the spread of these diseases among and within viticulture regions.

The only way to prevent illness of plants is eradication of pathogen germs; obtain virus-free clones and their multiplication in condition excluding the following infection. A great efficiency is achieved using thermotherapy and meristem tip culture [1; 19].

The objective of the present work was the study of regenerative capacity of meristem in autochthonous grapevine cultivars.

Materials and methods

Tips of 11 grapevine genotypes from the collection of Research and Practical Institute for Horticulture and Food Technologies, Chisinau, served as biological material.

Virological expertise of analysed materials was conducted through enzyme-linked immunosorbent assay (ELISA) [4] and immonosorbent electron microscopy (ISEM) [6] tests. Immunological analysis was carried out using diagnostic kits from SEDIAG (France) against Grapevine fleck virus (GFkV), Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine leafroll-associated virus 1 (GLRaV-1) and Grapevine leafroll-associated virus 3 (GLRaV-3).

Selected shoots for *in vitro* culture were striped of leaves and rinsed in water with three drops of Tween-20 (0.1%) and under running tap water for 10 min, after that the terminal shoot tips (5-10 mm long) containing the apical meristem were excised and sterilized with 70% ethylic alcohol followed by 5.2 % calcium hypochlorite (dilution with distillate water 1:1). At last, in culture cabinet (laminar airflow hood) were excised the tips (1-2 mm) from sterilized materials and incubated in culture medium.

The explants were inoculated on three variants of Murashige&Skoog (MS) [16] medium: A) MS supplemented with 4,44µM 6-benzilaminopurine (BA); B) MS supplemented with 4,44µM BA and 1,1µM 1-naphthylacetic acid (NAA); C) MS with 4,44µM BA and 2,85µM 3-indolil acetic acid (IAA). Each medium variant was added with 2% sucrose and 0,8% agar. The explants were incubated at 25 ± 2^0C under illuminated conditions (16h photoperiod). Explants were cultured in test tubes (100x15) containing 5 ml of culture medium. In dependence of growing intensity the material was subcultured to fresh medium every 3-4 weeks under similar conditions.

After the regeneration was initiated the materials were transferred in Magenta glass jars (100/150 ml) containing 20 ml of the same medium for 3-4 cycles of multiplication. The minishoots were transferred for rhizogenesis on medium with different concentration of NAA (5,37-26,85 µM).

The statistical processing of data was carried out using the software package Statgraphics Plus for Windows (version 2.1; Microsoft Corp., Redmond, WA, USA) and Microsoft Excel. The contribution of variation sources was computed following the ANOVA test results [5].

Results and Discussion

For *in vitro* propagation in grapevines of great interest for viticulture the sanitary status was evaluated in the following genotypes: Apiren alb, Apiren roz Basarabean, Apiren roz, Apiren extratimpuriu, Apiren Negru de Grozesti, Gen Moldova, Prezentabil, Romulus, 1-5-58, 1-5-15 and 1-5-71. Immunological analyses confirm the virus particle presence in 70-80% of analyzed cases. All 6 tested viruses were identified serologically: Grapevine fanleaf virus, Grapevine virus A, Grapevine virus B, Grapevine fleck virus, Grapevine leafroll-associated virus 1 and 3 (table 1).

Table 1. Results of serological tests (ELISA and ISEM)

Nr	Genotype	Plant index	GFLV	GLRaV -1	GLRaV -3	GVA	GVB	GFkV
1	Apiren alb	CC-III-11(21)						+
		A 35(54)					+	
		A 40(51)		+				
		A9(33)		+	+			
		A37(52)	+			+	+	
2	Apiren roz Basarabean	CC-III-20(20)		+				
		A6(20)			+			
		A29(28)				+		
3	Apiren roz	CC-III 18 (40)	+		+			
		A 37(46)					+	
		A38 (43)			+			
		CC-III 18 (31)		+				
4	Gen Moldova	CC-IV-24(18)	+	+	+			
5	Romulus	CC-I-7(3)	+					
		CC-I-7(24)	+					+
		CC-I-7(25)	+				+	
		CC-I-7(26)	+				+	
6	1-5-15	CC-II-29(37)	+					
7	1-5-58	A37(34)			+	+		
8	1-5-71	CC-III 27(2)						
9	Apiren extratimpuriu	A30(26)		+				
		CC-III-3(21)		+				
10	Apiren Negru de Grozesti	A3(46)				+	+	
		A3(50)					+	
		A10(7)					+	
11	Prezentabil	CC-II-11(4)			+	+		+
		CC-II-11(8)			+			

As shown in table 1, which summarized the results of serological tests, the GFLV, GLRaV-1, GLRaV-3 and GVB were the most widespread. In many cases the GVA was present in mixed infections with GLRaV-3 (for example in Prezentabil, index CC-II-11(4)) or with GVB (in Apiren alb, index A 37(52); Apiren Negru de Grozesti, index A 3(46)).
GFLV was present in grapevines showing the fanleaf degeneration . Fanleaf disease is a major viticulture problem, causing reduced yields due to poor berry set. Symptoms of leaf disease may include downward rolling of leaves, leaf reddening, poor fruit development and delayed fruit maturation [21].

According the task of the virus eradication the research was developed in direction of virus elimination by meristem culture.

Application of dispersional analyses revealed a positive response of meristems on all three used medium culture ($P \leq 0{,}001$). The generalized results indicate that the regenerative rate of meristem varies for different genotypes. The highest rate of meristem regeneration was obtained for Apiren extratimpuriu (87,5%), Romulus (83,41%) and 1-5-71 (81,79%). A more reduced rate of regenerative meristems was established for Apiren Negru de Grozesti (41,99%), 1-15-15 (41,51%) and Apiren Roz (39,66%) (table 2). These values of regeneration represent the summarized data for 4-5 plants of each genotype. In frame of the same genotypes the rate of regeneration significantly varied. Such deviation may influence the

phytosanitary status for each plant used as explant donor. The lowest values of regeneration have been noted at genotypes with a higher degree of infection.

Table 2. The percents of the regenerative meristems for different grapevine genotypes, %

Nr	Genotype	Average and standard error	Min÷max
1	Apiren alb	52,25 ± 4,35	20,00 ÷ 80,00
2	Apiren roz	39,66 ± 3,34	25,00 ÷ 57,14
3	Apiren roz Basarabean	58,20 ± 4,21	40,00 ÷ 75,00
4	Apiren extratimpuriu	87,50 ± 4,43	75,00 ÷ 100,0
5	Apiren Negru de Grozesti	41,99 ± 2,20	33,33 ÷ 50,00
6	Gen Moldova	79,29 ± 1,63	75,00 ÷ 85,71
7	Prezentabil	46,43 ± 1,59	42,86 ÷ 50,00
8	Romulus	83,41 ± 2,12	75,00 ÷ 88,89
9	1-5-58	73,02 ± 2,52	61,54 ÷ 79,31
10	1-5-71	81,79 ± 2,15	75,00 ÷ 90,00
11	1-15-15	41,51 ± 2,22	33,33 ÷ 50,00

Specialized literature contains extensive evidence of virus influence on regenerative capacity of meristems in *in vitro* culture for different species [9], including grapevine [20]. B.Walter [22] also showed that the presence of virus infection produces a negative effects on micropropagation.

After 4-6 weeks of *in vitro* meristems subcultivation regenerants were acquired. In the most cases the direct meristem regeneration was achieved. In some cases, the induction of adventitious shoots was registered. It must be noted that during the two subcultivations a genotypic specify dependent on medium culture was observed, resulting in different number of bud primordia and adventitious shoots (fig. 1).

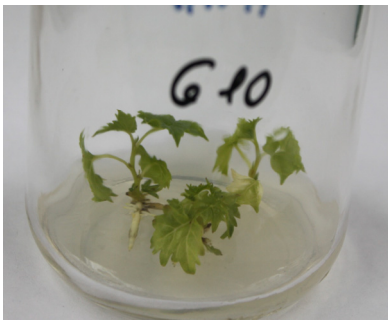
a)  b)  c) 

Fig. 1. *In vitro* culture of meristems from virus infected grapevine. Regenerants derived from Apiren extratimpuriu (a); Prezentabil (b); 1-5-71(c).

The experimental results show a regenerative rate dependent primordialy on genotypes (fig.2.). Such, for Apiren roz Basarabean the extreme values variation in proportion of 31,19; 38,73; 49,05 % for A, B and C culture mediums or 87,5; 85 and 90,0% for Gen Moldova respectively.

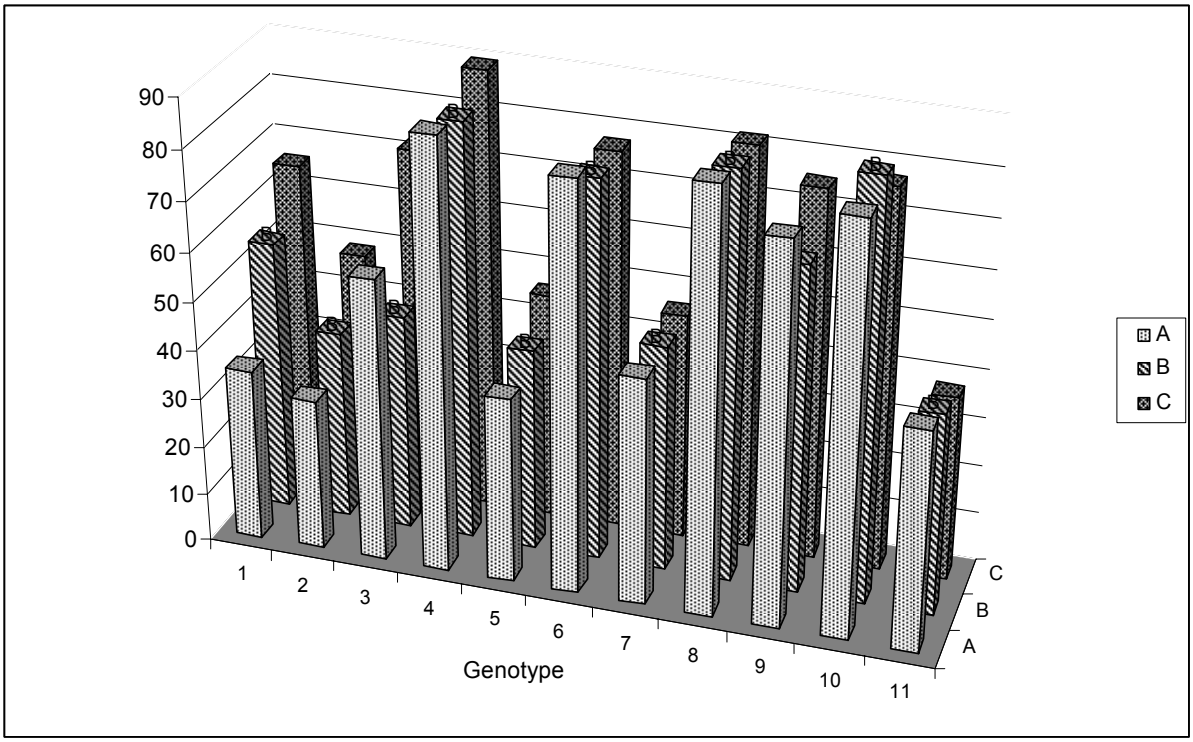


Fig. 2. Genotypic specifity of meristem regeneration in dependence on medium culture: 1 - Apiren alb; 2 - Apiren roz Basarabean; 3 - Apiren roz; 4 - Gen Moldova; 5 – Romulus; 6 - 1-5-15; 7 - 1-5-58; 8 - 1-5-71; 9 - Apiren extratimpuriu; 10 - Apiren Negru de Grozesti; 11 – Prezentabil.

For such genotypes as Gen Moldova, 1-5-15, 1-5-58; 1-5-71, Apiren Negru de Grozesti and Prezentabil differences induced by medium culture have not been observed. Hormonal balance had a significant influence on regenerative capacity of meristems derived from Apiren alb, Apiren roz Basarabean, Apiren roz and Romulus.
Application of ANOVA test revealed a significant influence of genotype on the regenerative capacity of meristems with a confidential level at 99,9% (table 3). In the aggregate, the effect dependent on the plant genotype consists in 75,8%. In spite of that, the solitary effect of medium culture was insignificant, but the interaction of both factors genotype-culture medium have a contribution of variance ranged at 10,9% (at a level a confidence 99%).

Table 3. Analysis of variance of meristematic regenerative frequency in different grapevine cultivars

Source of variance	Contribution of the source of variance (%)	Degree of freedom	Sum of Squares	Mean Square	F-Ratio	P-Value
A: Genotype	75,80	10	23676,60	2367,66	36,74	0,0000
B: Culture medium	1,26	2	392,81	196,409	3,05	0,0567
Interaction AB	10,90	20	3405,64	170,282	2,64	0,0030
Total		80	31234,30			

After 10-12 days of shoots transfer on medium for rhizogenesis, the roots initiation has been registered. The best results were obtained for MS supplemented with 26,85 μM NAA (fig.3). A high rate of rhizogenesis was obtained also for adventitious shoots, that lead to the increase of the number of derived plantlets. In the rooting procedures less variation between genotypes was registered.

a)  b) 

Fig. 3. Roots induction of shoots of Apiren alb (a) and Apiren extratimpuriu (b) on MS supplemented with 26,85 µM NAA.

Conclusion

The registered deviations in the regenerative capacity of 11 grapevine genotypes on three culture media were dependent primordially on genotype.

In spite of the fact that healthy status significantly influenced the rate of meristem regeneration capacity, the selected conditions of sterilization and the medium composition may conduct to a real scheme leading to including of analyzed genotypes in programs of virus eradication and propagation of plantlets.

In vitro meristem culture method showed the possibility to obtain plantlets from virus infected of autochthonous grapevine genotypes widely used in Republic of Moldova in breeding programs for selection of new varieties with high productivity and quality, advanced resistance to unfavorable conditions of environment, adaptability to local climatic conditions.

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