THE INCREASING OF THE *IN VITRO* MULTIPLICATION RATE FOR GRAPEVINE GENOTYPES BY APPLICATION OF LOW-INTENSITY MILLIMETER WAVES

SPORIREA COEFICIENTULUI DE MULTIPLICARE *IN VITRO* LA VIȚA DE VIE PRIN UTILIZAREA UNDELOR MILIMETRICE DE INTENSITATE JOASĂ

***SMEREA Svetlana*[1], *ANDRONIC Larisa*[1]**
e-mail: smerea_svetlana@yahoo.com

Abstract. *The aim of this work involves the application of low-intensity millimeter waves on the meristematic apex for increasing of the in vitro multiplication rate for six grapevine genotypes. After the first 25-35 days of inoculation it was attested the proliferation and development of regenerants, which were subsequently transferred to nutritive media for micropropagation. The rate of explant with shoots varied between 8.39 % and 46.04 % for evaluated genotypes. The millimeter waves conducted to the increasing of this parameter. The major growth was established for Apiren extratimpuriu (by 2.24 times) and the smaller for Prezentabil (by 1.1 times). The number of shoots per explant had the minimal and maximal values certified between 1.0 ÷ 5.98 for control and 2.75 ÷ 27.83 for experimental traits. The growth capacity of this index ranged from 1.48 for Gen Moldova to 4.91 for 1-5-71 genotype.*
Key words: *multiplication rate, in vitro culture, low-intensity millimeter waves, grapevine*

Rezumat. *Scopul cercetărilor constă în sporirea coeficientului de multiplicare in vitro prin iradierea meristemelor cu unde milimetrice de intensitate joasă. În studiu au fost incluse 6 genotipuri de viță de vie. La primele 25-35 zile de la inoculare s-a atestat proliferarea și dezvoltarea regeneranților, care ulterior au fost transferați pe medii nutritive pentru micropropagare. La genotipurile cercetate, cota explantelor cu lăstărași a variat la martor între 8,39 % și 46,04 %, iar iradierea apexurilor a condus la sporirea acesteia. Cea mai majoră creștere (de 2,24 ori), a fost atestată la Apiren extratimpuriu, iar cea mai lejeră (de 1,1 ori) a fost înregistrată la Prezentabil. Numărul de lăstărași per explant a atins valori min/max de 1 și 5,98 pentru varianta martor și respectiv 2,75 și 27,83 pentru varianta experimentală. Potența creșterii acestui indice a fost cuprinsă între 1,48 pentru genotipul Gen Moldova și 4,91 la genotipul 1-5-71.*
Cuvinte cheie*: rata de multiplicare, cultura in vitro, unde milimetrice de intensitate joasă, vița de vie*

INTRODUCTION

The microclonal multiplication is applied for rapid propagation of the virus-free material (Grout and Brian, 1999) or for the production, conservation and

[1] Institute of Genetics, Plant Phyisiology and Protection, Academy of Sciences, Chişinău, Republic of Moldova

improvement of plant resources (Bairu *et al.,* 2011, Ngezahayo and Liu, 2014), enabling establishment in a short period of time for obtaining of sufficient seedlings. Technical date is justified by efficiency and economic benefit of this procedure.

According to the certification requirements of grapevine planting material the microclonal multiplication methods gets applicability in practice. It is known more techniques for *in vitro* vegetative multiplication or micropropagation. The classic procedure (Chee *et al.,* 1984) involves selection of the shoot, *in vitro* inoculation of apex, shoot production and obtaining of regenerants, shoot multiplication from the subcultured shoots, rooting, *ex vitro* adaptation and transfer of the plantlets in soil substrate. The authors reported the significant increasing of multiplication rate after the first 3-4 passages. The potential for vegetative micropropagation was found after the passage 6, which corresponds to the duration of approximately 330 days of *in vitro* cultivation, which is followed by major spending. Also, prolonged subcultivation in *in vitro* conditions may conduct to the inducing variations somaclonal.

Millimeter waves therapy was found to be highly effective in biology and medicine being demonstrated positive impact on cell proliferation (Betskii *et al.,* 1998).

The aim of the present research is to study the impact of low-intensity millimeter wave irradiation on micropropagation of grapevine meristems with *in vitro* positive response in order to increase the multiplication rate.

MATERIAL AND METHOD

As biological material for *in vitro* micropropagation were selected 6 grapevine genotypes from the collection of Research and Practical Institute for Horticulture and Food Technologies, Chisinau: 1-15-15, 1-5-71, Apiren roz, Apiren extratimpuriu, Gen Moldova and Prezentabil.

Selected shoots for *in vitro* culture (6-10 cm long) were striped of leaves and rinsed in water with three drops of Tween-20 (0.1%) and under running tap water for 15 min, after that the terminal shoot tips (5-10 mm) containing the apical meristem were excised by a sharp blade.

The explants were sterilized for about 2-3 seconds with 70% ethylic alcohol and 5.2 % calcium hypochlorite (dilution with distillate water 1:1), following by rinsing in three baths of sterile water, of 5 minutes each, to remove the total chlorine.

All sterilization operations, apex excising and their inoculation on culture media were conducted under aseptic conditions. The explants were inoculated in tubes containing 2 ml of Murashige & Skoog nutrient medium (Murashige and Skoog, 1962) supplemented with 6-benzilaminopurine (BAP) (5 μM), 1-naphthylacetic acid (NAA) (0.5 μM), 2% sucrose and 0,7% agar, which proved optimal for all analyzed genotypes. The pH of the nutrient medium was adjusted to 5.7 before autoclaving. To induce regeneration, the tubes were incubated under controlled conditions with a 16 h-light photoperiodicity temperature of 25 ± 2^0C and light - 2000lx. At 2-3 weeks after the inoculation, the explants which established a positive response were irradiated for 20-25 minutes with the low-intensity millimeter wave (λ = 5.6 mm, 53.8 GHz), using the device "IAVI-1" (flux density 1mW/cm^2÷10 mW/cm^2). After treatment the apices

were restored in growth chamber under the same controlled conditions. Untreated explants were used as control.

The obtained regenerants in the first 25-35 days after inoculation were transferred in Magenta glass jars (100/150 ml) containing 10-12 ml the media for multiplication. Every 4-5 weeks the shoots with 3 - 4 nodes were cutting and inoculated on fresh nutrient media. This procedure has being made up to 6-7 cycles of subculturing. From each cycle of subculturing the plantlets with 3-4 internodes were transferred to medium for risogenesis (Murashige & Skoog, 1962) supplemented with NAA (0.4 µM).

At 6-7 days after passage on this substrate, the regenerants presenting a rooting system with well developed were transferred to 500ml plastic pots with soil mix: peat (1:3). The pots were covered with polyethylene film and transferred to the culture room at 25±2^{o}C with a 16 h-light photoperiodicity. After the occurrence of 2-3 new leaves the plantlets were transferred to soil substrate according to the standard techniques.

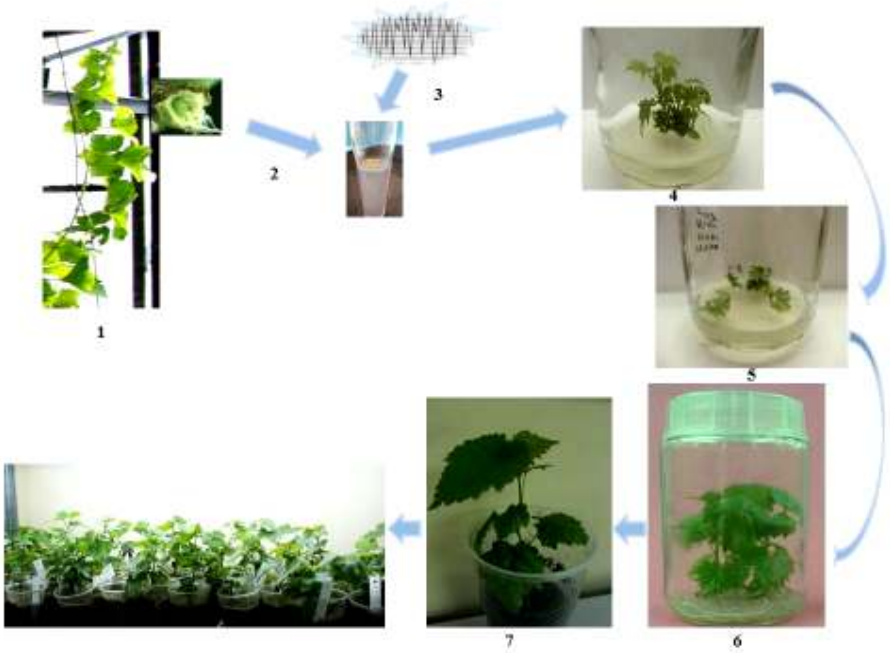

Fig.1 - Scheme of microclonal multiplication of the grapevine apex
(1) the selection of the shoot, (2) apex inoculation, (3) irradiation with low-intensity millimeter wave, (4) obtaining regenerants (5) subcultivation and in vitro multiplication (6) rooting and (7) *ex vitro* adaptation of plantlets to soil substrate.

RESULTS AND DISCUSSIONS

According to the obtained results, the number of survival explants was slight influenced by the applying scheme of multiplication based on the using of millimeter waves (Table 1). For analyzed genotypes, the percentage of explants

that formed shoots had ben ranged from 8.39 for Apiren extratimpuriu to 46.04 for 1-1-15. According to the literature data (Chee *et al.,* 1984) the response of the explants to *in vitro* culture conditions and the number of formed shoots priority are dependent on the genotype. Similar reaction was established and by our results.

The irradiation of apices led to a significant increase on the rate of explants with shoots. The major growth was attested for cultivar Apiren extratimpuriu being noticed an increase of 2.24 times, while the lowest influence was registered for cv. Prezentabil (up 10%).

Table 1

Effect of low-intensity millimeter waves on regeneration of grapevine meristems

Genotype	Treatment	Inoculated apex (number)	Survival explant (number)	Explants with shoots (%)	Plantlets per explant (number)
1-15-15	control	21	19	46.04	5.98
	mmW	18	17	75.00	9.88
1-5-71	control	16	14	38.89	5.67
	mmW	12	11	52.78	27.83
Gen Moldova	control	40	37	23.99	2.64
	mmW	31	29	55.83	3.92
Apiren Roz	control	13	12	16.67	1.00
	mmW	13	12	25.00	2.75
Apiren extratimpuriu	control	25	24	8.39	2.00
	mmW	19	18	18.83	9.75
Prezentabil	control	9	8	25.00	1.00
	mmW	14	14	27.50	3.50

Also for all six investigated genotypes was found a significant increase of the number of shoots derived from an explant. The potency of growth of this index was between 1.48 and 4.91 for the varieties Gen Moldova and 1-5-71 respectively. Based on ANOVA test was found that the number of shoots per explant is determined primarily by genotype. The application of low-intensity millimeter waves had an effect 11.4% (Tab. 2).

Table 2

Analysis of variance of shoots frequency per explant for different grapevine cultivars

Source of variance	Sum of Squares	Degree of freedom	Dispersia	Test F	Contribution of the source of variance (%)
Genotip (A)	930,348	5	186.07	3.90**	30.87
Millimeter waves (B)	343,904	1	343.904	7.20*	11.41
Interaction AB	501,2	5	100.24	2.10	16.63
Rezidual	1242,03	26	47.7704		
Total	3014,04	37			

*; ** - significant difference from the control at P ≤ 0.05; 0.01

Significant differences between control and irradiated apices were found for the number of shoots per explant according to the subcultivation cycle (Tab.3). If for control the main multiplication potency is observed after the third cycle of *in vitro* subcultured, while the irradiation conducted to an increase of the number of shoots after the first subculturing. The efficiency of multiplication had the major impact yet 2-3st subculturing cycles.

The accelerating of multiplication was also accompanied by a major augmentation of microclonale rate, and as a result by increasing number of obtained shoots in a short period of time.

The reduction of duration of *in vitro* subcultivation conducted to significant diminution of cost related to the nutrient medium components, growth conditions, number of working days and prevent the risk of inducing somaclonal variations and/or culture vitrification, which appeared in cultures corresponding to the multiplication stage (Bairu *et al.,* 2006).

Table 3

Proliferation of shoots with 3 - 4 nodes in dependence of *in vitro* subculture cycle

Subcultivation cycle	Number of shoots per explant					
	Gen Moldova		1-5-71		1-15-15	
	control	mmW	control	mmW	control	mmW
First multiplication	-	1	-	-	0.67	1
1st subculture	0	4	0	12.33	0	8
2st subculture	0	4.33	0	19.67	0	14.75
3st subculture	1.33	4.33	1	12.33	1.33	10.5
4st subculture	2.33	-	4.33	15	4.25	4
5st subculture	3	-	5.5	-	2.5	-
6st subculture	7	-	2	-	2	-
7st subculture	3	-	-	-	1	-

According to the obtained results the effect of waves is distinguished by accelerating of multiplication, and also by increasing of the microclonal reproduction rate. Thus, if in the control treatment the value ranged between 2.38 and 3.63 for Gen Moldova and 1-5-71, then as a result of irradiation with millimeter waves this index increase to 4.2 and 14.8 respectively from the same genotypes (Tab. 4).

Table 4

Theoretical potential of micropropagation via *in vitro* multiplication for some grapevine genotypes

Subcultivation cycle	Days of culture	Number of shoots with 3 and more nodes					
		Gen Moldova		1-5-71		1-15-15	
		control	mmW	control	mmW	control	mmW
Cultivated explants		13	15	8	10	9	12
Rate of multiplication		2.38	4.2	3.63	14.8	2.77	10.75
Multiplicated explants*		18	28	45	15	31	90
1st subculture*	60	32.9	78.4	204.3	222	95.5	806.4

2nd subculture*	90	60.3	219.5	927.5	3285.6	294.1	7225.3
3st subculture*	120	110.3	614.7	4210.9	48626.9	905.8	64739.1
4st subculture*	150	201.9	1721	19117.7	719677.8	2789.8	580062.2
5st subculture *	180	369.4	4818.9	86794.4	10651231.8	8592.4	5197357.1
6st subculture *	210	676.1	13492.9	394046.7	1576382 30.6	26464.6	46568319.7

*** -** theoretical potency of multiplication reported to ten subcultured regenerants

Based on the data related to this study it is presented that the microclonal multiplication potency in case of application of low-intensity millimeter waves could conduct during 6 subcultivations to an increasing of tens or to thousands of times (Gen Moldova - 19.9 times, 1-5-71 - by 400 and for 1-15-15 - more than 1750 times).

CONCLUSIONS

Apex irradiation by low-intensity millimeter waves has a positive effect on microclonal propagation leading to an increase in the rate of 1.53 to 3.26 for different analyzed genotypes of *Vitis vinifera*.

As practical perspective the results are more efficient in order to speed multiplication during 3-4 subcultivation, which provide a sufficient number of materials for foundation of grapevine nurseries or other plant species of interest.

REFERENCES

1. **Bairu M.W., Aremu A.O., Staden J.V.**, **2011** - *Somaclonal variation in plants: causes and detection methods*. Plant Growth Regul. vol. 63: 147–173.
2. **Betskii O.V., Kislov,V.V., Devyatkov,N.D.**, **1998** - *Low-intensity MM waves in medicine and biology.* Biomeditsinskaya Radioelektronika, No. 4:1 3–29, (in Russian).
3. **Chee R., Pool R. M., Bucher D., 1984 -** *A method for large scale in vitro propagation of Vitis.* New York's food and life sciences bulletin. Nr. 109: 1-9.
4. **Grout W., Brian W., 1999** - *Meristem-tip culture for propagation and virus elimination*. In Methods in Molecular Biology, R. D. Hall, Ed., p. 115–125, Plant Cell Culture Protocol, Humana Press, Totowa, NJ, USA.
5. **Murashige T, Skoog F., 1962 -** *A revised medium for rapid growth and bio assays with tobacco tissue cultures.* Physiol Plant., V.15(3): 473-497.
6. **Ngezahayo F., Liu B., 2014 -** *Axillary Bud Proliferation Approach for Plant Biodiversity Conservation and Restoration*. Hindawi Publishing Corporation, International Journal of Biodiversity, Vol. 2014: 1- 9.
7. **Sharma U., Mohan J.S., 2006 -** *Reduction of vitrification in in vitro raised shoots of Chlorophytum borivilianum Sant. & Fernand., a rare potent medicinal herb*. Indian J.Exp.Biol., Vol. 44:.499-505.