THE WAYS OF CONVERSION OF IMMATURE EMBRYOS INTO PLANTS FOR SEEDLESS STENOSPERMOCARPIC GRAPEVINE

CĂILE DE CONVERTIRE ÎN PLANTE A EMBRIONILOR IMATURI LA GENOTIPURILE APIRENE STENOSPERMOCARPICE DE VIȚĂ DE VIE

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Abstract. *The investigations for optimization the ways of preventing the embryos abortion and to ensure their development into plants for five genotypes of seedless stenospermocarpic grapevine has been done. Two ways of conversion of excised embryos into plants were chosen depending on genotype. It was established that for cultivar Apiren extratimpuriu the dissecting of ovules after 70-90 days of cultivation on recovery medium should be done, and the excised viable embryos should be transferred on the MS medium (1962) with addition of 0.45 mg/l of BA during 7 days for their conversion into plants, and then subcultivated on the same basal medium with 0.2 mg/l IBA. For I-15-15 genotype the time of cultivation on recovery medium consist of 150-180 days, and embryos excised from seeds should be transferred on MS medium (1962) with addition of 0.45 mg/l BA, with consequent transfer on the same basal medium without growth regulators. The plants cultivated in vitro at the stage of 4-5 leaves and 3-4 roots were transferred on soil substratum with subsequent adaptation to ex vitro conditions.*

Key words: grapevine, embryos abortion, genotype of seedless stenospermocarpic

Rezumat. *S-au efectuat lucrări în scopul optimizării căilor de prevenire a avortării embrionilor şi de asigurare a dezvoltării lor până la obținerea plantelor pentru cinci genotipuri apirene stenospermocarpice de viță de vie. În funcție de genotip au fost trasate două căi de conversie a embrionilor excizați în plante. S-a stabilit că, pentru soiul Apiren extratimpuriu desecarea ovulelor trebuie efectuată după 70-90 zile de cultivare pe medii "de recuperare", iar embrionii viabili excizați se transferă pentru conversie în plante pentru 7 zile pe mediul MS (1962) cu adaos de 0,45 mg/l BA, iar apoi se subcultivă pe acelaşi mediu bazal ce conține 0,2 mg/l IBA. Pentru genotipul I-15-15 durata de cultivare pe medii "de recuperare" este 150-180 zile, iar embrionii extraşi din semințe se transferă pe mediul MS (1962) cu adaos de 0,45 mg/l BA, cu transfer ulterior pe acelaşi mediu bazal fără reglatori de creştere. Plantele cultivate in vitro la stadiul de 4-5 frunzulițe şi 3-4 rădăcini au fost transferate pe substrat sol şi ulterior adaptate la condiții ex vitro.*

Cuvinte cheie: viță de vie, avortare embrioni, genotip apiren stenospermocarpic

INTRODUCTION

Obtaining the seedless descendents from breeding programs is a priority objective in countries with traditions in cultivation the grapevine (Agüero et al., 2000; Popescu, Teodorescu, 2004). Utilization of seedless genotypes in breeding programs implies the use of methods preventing the embryos abortion. The efficiency of obtaining plants in case of crossing even one genitor with stenospermocarpic trait depends of choosing the adequate techniques of immature embryos "rescue" for seedless cultivars. In case of seedless x seedless combinations the rate of rescued embryos come up to 2-15 % and depends on used methods (Spiegel-Roy et al., 1985; Bouquet, Davis, 1989). A favorable "recovery" medium could contribute in resolving the problems of embryos rescue and their converting into plants. The results obtained previously (Chiriac, Savin, Smerea, 2007) demonstrated that only several local stenospermocarpic genotypes could be used as maternal genitors: Apiren extratimpuriu, Apiren roz Basarabean and I-15-15. Later, the investigations of immature embryos rescue and particularly optimization of embryos recovery mediums depending on genotype were evaluated (Chiriac, Smerea, Savin, 2008). The reaction of immature embryos to the *in vitro* cultivation depends essentially on genotype and that is why it is necessary to state the ways of converting into plants the embryos recovered by "*in ovulo*" manipulations, taking into account the peculiarities of the varieties that were involved in the crosses (Goldy, Amborn, 1987; Cain, Emershad, Tarailo, 1983).

The aim of the present research was to reveal the impact of cultivation medium and genotype in optimization the ways of conversion the embryos into plants after 3-6 months of cultivation on "recovery" medium for 5 newly created seedless grapevine genotypes used as maternal genitors.

MATERIAL AND METHODS

The berries resulted from natural (free) pollination of the seedless genotypes Apiren extratimpuriu, Apiren roz, Basarabean and I-15-15 were harvested 35-40 days after anthesis. Collected berries were cold pretreated and then surface sterilized with 70 % ethylic alcohol followed by 5.2 % calcium hypochlorite and washed with sterilized distillated water. The ovules were aseptically extracted and inoculated on the same cultivation medium in Petri dishes with subcultivation every 30 days until seeds residuum were dissected to recover viable embryos. Three "recovery" medium were selected: Murashige Skoog (1962) supplemented with 2 mg/l of 3-indolil acetic acid (IAA), and 0.5 mg/l of 6-benzilaminopurina (BA) (MS); Nitsch, Nitsch (1969) supplemented with 1.5 mg/l of IAA, 1 mg/l of zeatin and 0.2 mg/l of giberilinic acid (GA_3) (NN1) and Nitsch, Nitsch (1969) with addition of 2.5 mg/l of IAA, 0.2 mg/l of GA_3 and 0.3mg/l of putrescine (NN2). Ovules were cultivated for 30 days in total darkness conditions at 25 ± 2^0C, and then transferred in culture room in the same temperature regime and period of 16 hours of light. The seeds were dissected beginning 10-15 days after first germinations. Viable embryos were placed on "recovery" mediums. The composition of mediums was different by incorporated hormones as well as concentration, meanwhile the basal medium was the same: half strength of macro- and microelements, and vitamins after Murashige Skoog (1962) with 15 g/l of sugar, 100 mg/l of mio-inositol, 3 mg/l of glicine and 6.8 g/l of agar.

The embryos that evolved creating roots and sprouts were considered converted into plants.

RESULTS AND DISCUSSIONS

Properly equilibrated composition of cultivation medium defines an adequate development of immature embryos until the maturation stage (Emershad, Ramming, 1984). In our previous publication we have presented the results of immature embryos rescue (Chiriac, Smerea, Savin, 2008). Increase of rate of viable saved embryos with 27.19 % and 19.37 % for I-15-15 and Apiren roz genotypes, respectively, has been attested in comparison with results presented in 2006-2007 (Chiriac, Savin, Smerea, 2007). The less significant increase of survived embryos rate was attested for the genotype Apiren Alb - 7.82 %. The results presented in Table 1 reveal that cultivation of zygotic embryos on "recovery" medium differing by mineral components as well as by growth regulators define an increase of viable embryos rate after dissection of seeds residuum, as well as the rate of embryos converted into plants.

Table 1

Influence of "recovery" mediums of immature embryos on the rate of ovules with viable embryos after dissection of seeds residuum and embryos converted into plants for various genotypes

Maternal genitor	"Recovery" medium	Viable embryos after dissection, %	Embryos converted into plants	
			%	Limits
Apiren roz	MS	19,70	22,14	16,67 ÷ 27,63
	NN 1	38,72	16,23	10,70 ÷ 21,71
	NN 2	33,99	17,76	12,28 ÷ 23,24
Apiren alb	MS	12,22	0,00	-
	NN 1	7,75	2,22	0 ÷ 7,71
	NN 2	17,82	9,72	4,24 ÷ 15,21
Apiren extratimpuriu	MS	30,00	25,76	20,27 ÷ 31,24
	NN 1	34,59	39,46	33,97 ÷ 44,94
	NN 2	29,23	42,13	36,65 ÷ 47,62
Perlon	MS	47,70	28,23	22,74 ÷ 33,71
	NN 1	48,89	68,05	61,57 ÷ 73,54
	NN 2	58,63	33,90	28,48 ÷ 39,44
I-15-15	MS	55,37	35,58	30,10 ÷ 41,06
	NN 1	43,00	31,42	25,94 ÷ 36,91
	NN 2	59,41	36,59	31,11 ÷ 42,08

It was established, that depending on genotype and medium used for cultivation of embryos until their excision, the rate of converted into plants embryos reached the maxim values for I-15-15 (35.59 %) and Apiren extratimpuriu (42.13 %) genotypes in case of NN2 medium in comparison with 13.33 and 21.11 %, respectively, for the same genotypes in 2006-2007 in case of cultivation on Nitsch, Nitsch (1969) medium supplemented with 2.5 g/l of active

carbon, 3 mg/l of IAA and 5 mg/l of GA_3 (Chiriac, Savin, Smerea, 2007). In the present investigation more efficient "recovery" medium for Apiren roz genotype which determine an increased embryos conversion into plants (22.14 %), was attested MS medium with addition of 2 mg/l of IAA and 0.5 mg/l of BA. Using of this medium contributed to increases with 14.37 % of converted embryos rate compared with results obtained previously in 2006-2007. An interesting moment represents the fact that for two genotypes - Perlon and Apiren alb, the rate of embryos converted into plants reached the maximal values in case of saving zygotic embryos on NN2 medium with addition of putrescine. Similar results have been obtained by other researchers (Ponce, Guiunazu, Tizio, 2002). Comparison of the results obtained in 2007-2008 with those obtained 2006-2007 regarding the reaction to *in vitro* culture of Apiren alb genotype reveal that this cultivar is not suitable to be used as a maternal genitor, although the rate of converted embryos into plants has been increased from 0.0 to 9.72 %.

The obtained results suggest the necessity to fix up the components of the "recovery" mediums for immature embryos of each crossing combination. ANOVA test was used to estimate the impact of recovery mediums (MS, NN1 and NN2) and genotype on the rate of converted into plants embryos. Dispersion analysis reveal that this index depends on genotype (68.47 %), interaction of genotype and medium factors (21.75 %) and cultivation medium (4.85 %). The contribution of variation sources was significant at 99.9 % level. Above mentioned facts confirm the maximal impact of genotype on the development of embryos *in vitro* that was established for viable embryos (81.03 %) at the stage of embryos excision as well (Chiriac, Smerea, Savin, 2008).

Table 2

Variation of embryos rate converted into plants (ANOVA test)

Source of variation	Square sum	DF	Source contribution, %	Dispersion, S^2	F factor	P
A genotype	9012,58	4	68,47	22,53	104,19	0,0000
B medium	638,564	2	4,85	319,282	14,76	0,0000
AB	2863,22	8	21,75	357,902	16,55	0,0000
Residual	648,789	30	4,93	21,626		
Total	13163,2	44				

Usually, excised embryos are transferred for conversion on MS medium (1962) without growth regulators (Valdez, Ulanovski, 1997; Aguero et al., 2000; Valdez, 2005). In our previous researches we related that considerable plants number were resulted through cultivation of viable embryos on MS medium with addition of cytokinin, and then transferred to the same medium without growth regulators (Chiriac, Savin, Smerea, 2007). The present investigations regarding the capacity of embryos to develop apical meristem demonstrated, that rate of embryos converted into plants has increased in case of cultivation embryos on "conversion " medium supplemented with 0.45 mg/l of BA during 7 days and then transferred on MS medium with addition of 0.2 mg/l of IBA (Table 3).

Taking into account the "recovery" medium of cultivation embryos until dissection as well, the rate of embryos converted into plants has increased with 15.38-16.95 % in case of cultivar Apiren extratimpuriu and with 1.70-4.89 % for cultivar Apiren roz. The increase could be explained by the fact that rate of embryos without capacity to develop apical meristem on hormone-free medium could generate normal plants in case of addition IBA. As result, it was established a distinct dependence of IBA supplemented medium by genotype and this way of conversion could be used for cultivar Apiren extratimpuriu only. This fact suggests that the components of "conversion" medium should be established individually for each genotype, as well as in case of "recovery" medium.

Table 3

Modes of conversion of embryos into plants

Maternal genitor	"Recovery" medium	Tested embryos, Nr	Embryos converted into plants, %		Growing on MS medium with 0.2 mg/l IBA, %
			MS hormone free	MS with 0.2 mg/l of IBA	
Apiren extratimpuriu	MS	32	28,12	43,75	15,63
	NN 1	118	36,44	53,39	16,95
	NN 2	52	44,23	59,61	15,38
Apiren Roz	MS	143	20,28	25,17	4,89
	NN 1	118	15,25	16,95	1,70
	NN 2	72	16,67	20,83	4,16

In addition, it is important the moment of dissection begin as well, or surviving of embryos *in vitro* and ulterior plants formation depends on stage of the embryos have been placed on "conversion" medium (Liu, Sykes, Clingeleffer, 2003). The results obtained in 2007-2008 suggest that the optimal period for dissection of seeds residuum begins within 10-15 days after appearance of first germinations.

For the cultivar Apiren extratimpuriu this term is situated within 90-100 days of cultivation on "recovery" medium and for genotype I-15-15 within 150-180 days. The rest of cultivars are situated between these terms.

The plants cultivated *in vitro* were transferred on soil substrate on the stage of 4-5 leaves and 3-4 roots and then adapted for *ex vitro* conditions.

CONCLUSIONS

The techniques used for *in vitro* recovery of immature embryos of stenospermocarpic grapevine are determined significantly by genotype particularities, which comprise 68.47 % of variation for embryos converted into plants. Taking into account obtained results two ways of conversion embryos into plants were established:

The most efficient term for cultivation of ovules on recovery medium was 90-100 day for Apiren extratimpuriu. The excised embryos are cultivated on MS

medium with addition of 0.45 mg/l BA during 7 days and then transferred on the same basal medium supplemented with 0.2 mg/l IBA.

For the genotype I-15-15 is recommended cultivation on "recovery" medium during 150-180 days. Embryos excised from the seeds is transferred on MS (1962) medium with addition of 0.45 mg/l BA, and then on the same medium without growth regulators.

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REFERENCES

1. **Agüero C., Vigliocco A., Abdala G., Tizio R., 2000** - *Effect of gibberellic acid and uniconazol on embryo abortion in the stenospermocarpic grape cultivars* Emperatriz and Perlon. Plant Growth Regulation. 30, 9-16.
2. **Bouquet A., Davis H.P., 1989** - *Culture in vitro de ovule et de vigne (V.Vinifera L.) appliguee a la selection de varietes de raisin de table sanspepins*. Agronomie. 9, 6, 565-574.
3. **Cain D., Emershad R., Tarailo R., 1983** - *In ovulo embryo culture and seedlling development of seeded and seedliiess grapes (Vitis vinifera L.)*. Vitis. 22, 9-14.
4. **Chiriac Gh., Savin Gh., Smerea S., 2007** - *Embryo rescue in seedless genotypes.* Lucrări Şt. Univ. de Şt. Agricole şi Med. Veter. Iaşi, România, Seria Horticultură V.I. (50), 99-104.
5. **Chiriac Gh., Smerea S., Savin Gh., 2008** - *Cultivarea in vitro a embrionilor imaturi la vița de vie apirenă.* Conferința Naț. cu participare Internațională „Probleme actuale ale geneticii, fiziologiei şi ameliorării plantelor" Chişinău. 46-51.
6. **Emershad R.L., Ramming D., 1984** - *In ovulo embryo culture of Vitis vinifera L. cv "Thompson Seedless"*. Am. J. Bot. 71. 873-877.
7. **Goldy R., Amborn U.,** 1987 - *In vitro culturability of ovules from 10 seedless grape clones*. Hortscience. 22. 952.
8. **Liu S.M., Sykes S.R., Clingeleffer P.R., 2003** - *Improved in ovulo embryo culture for stenospermocarpic grapes (Vitis vinifera L.).* Australian Journal of Agricultural Research 54, 9: 869-876.
9. **Ponce M.T., Guinazu M.E., Tizio R., 2002** - *Improved in vitro embryo development of stenocarpic grape by putrescine*. Biocell. 26 (2), 263-266.
10. **Popescu C.F., Teodorescu A., 2004** - *Biotehnologii. Recuperarea in vitro la vița-de-vie cu aplicații în ameliorarea sortimentului*. Editura CERES. Bucureşti, 139 p.
11. **Spiegel-Roy P., Sahar N., Baron J., Lavi U., 1985** - *In vitro culture and plant formation from grape cultivars with abortive ovules and seedes*. J. Amer. Soc. Hort. Sci. 110. 109-112.
12. **Valdez J.G., 2005** - *Immature embryo rescue of grapevine (Vitis vinifera L.) after an extended periodof seed trace culture.* Vitis. 44 (1). 17-23.
13. **Valdez J.G., Ulanovsky S.M., 1997** - *In vitro germination of stenospermic seeds from reciprocal croses (Vitis vinifera) applying different techniques*. Vitis. 36. 105-107.