UDC 634.8:57.017.642:575.222.72

Biological sciences

INITIATION OF PROEMBRYOGENIC CELL SUSPENSIONS IN NINE INTERSPECIFIC GRAPEVINE HYBRIDS

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Petioles, taken from *in vitro* grown plants of nine interspecific grapevine hybrids: rootstocks *Berlandieri* x *Riparia* 'Kober 5BB' and *Riparia* x *Rupestris* '101-14' and cultivars (cvs): 'Bianca', 'Zigfridrebe', 'Podarok Magaracha', 'Pervenets Magaracha', 'Tsitronnyi Magaracha', 'Intervitis Magaracha' and hybrid form 'Magarach 100-74-1-5' were cultured on solid NN medium supplemented with different levels of 2,4-D and BA at various concentrations. In order to initiate cell suspensions, proembryogenic calluses were subcultured to liquid NN medium supplemented with 1.0 mg/L 2,4–D and 0.2 mg/L BA. Subculturing these suspensions to liquid NN medium supplemented with 2 mg/L NAA and 0.1 mg/L BA led to the development of embryo aggregates while subculturing to liquid NN medium supplemented with 0.5 mg/L BA resulted in development of single globular and heart-stage embryos. Proembryogenic cell suspensions consisting of prevailing single cells can be used in gene transformation and cell selection with the aim of decreasing the probable formation of chimeric plants.

УДК 634.8:57.017.642:575.222.72

03.00.00 Биологические науки

ИНИЦИАЦИЯ ПРОЭМБРИОГЕННЫХ КЛЕТОЧНЫХ СУСПЕНЗИЙ У ДЕВЯТИ МЕЖВИДОВЫХ ГИБРИДОВ ВИНОГРАДА

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Черешки листьев, взятых из растений ин витро, девяти межвидовых гибридов винограда: подвоев Берландиери х Рипариа 'Кобер 5ББ', Рипариа х Рупестрис '101-14' и сортов (cvs): 'Бианка', 'Зигфридребе', 'Подарок Магарача', 'Первенец Магарача', 'Цитронный Магарача', 'Интервитис Магарача' и гибридной формы 'Магарач 100-74-1-5' культивировали на твердой среде NN, содержащей различные концентрации 2,4-D и BA в различных сочетаниях. Для того, чтобы инициировать рост клеточных суспензий, проэмбриогенные каллусы пересаживали в жидкую среду NN, дополненную 1.0 мг/л 2,4-Д и 0,2 мг/л BA. Субкультивирование этих суспензий в жидкую среду NN, дополненную 2 мг/л NAA и 0,1 мг/л BA, привело к развитию агрегатов эмбриоидов, а пересадка в жидкую среду с 0,5 мг/л BA вызвала развитие единичных глобулярных и сердцевидных эмбриоидов. Проэмбриогенные клеточные суспензии, состоящие из преимущественно отдельных клеток, могут быть использованы в трансформации генов и селекции

на клеточном уровне с целью уменьшения вероятности появления химерных растений

Keywords: VITIS, GROW REGULATOR, IN VITRO, LIQUID MEDIUM, SOMATIC EMBRYO

Ключевые слова: ВИНОГРАД, РЕГУЛЯТОРЫ РОСТА, В ПРОБИРКЕ, ЖИДКАЯ СРЕДА, СОМАТИЧЕСКИЕ ЭМБРИОИДЫ

INTRODUCTION

Grapevine cell suspensions have been used to select cells which are resistant to low temperature [1], high levels of sodium chloride [2] and active lime contained in the media [3]. Genotypes with fungal resistance and enhanced secretion of chitinase have been selected at the cell level in media containing *Elsinoe ampelina* culture filtrate [4, 5]. Transgenic grapevine plants produced by biolistic transformation of embryogenic cell suspensions [6] were characterized by different integration and expression of marker genes and appearance of chimeric plants [7]. As shown by the *gus*-test, a ratio between chimeric and non-chimeric transgenic plants was approximately 1:1 [8]. In order to avoid the formation of chimeric plants which can appear as a result of gene transformation and selection at the cell level, suspension cultures must include prevailing single proembyogenic cells, and not cell clusters or early-stage globular embryos. Somatic embryogenesis has been reported in liquid media from grapevine cell suspensions initiated from calluses which had been derived from nucellus [9] anthers [2, 10], from leaf discs [11] and leaf petioles [12, 13].

This paper reports the results pertaining to the adjustment of optimum growth regulators in liquid media for initiation of grapevine proembryogenic cell suspensions and development of single globular and heart-stage somatic embryos in nine interspecific hybrids of the genus *Vitis.*

MATERIALS and METHODS

Plant Materials

In our study we used grapevine rootstocks *Berlandieri* x *Riparia* 'Kober 5BB' and *Riparia* x *Rupestris* '101-14', interspecific hybrids cvs. 'Bianca' and

'Zigfridrebe', cultivars released by the Institute for Vine and Wine 'Magarach' via crossing between *Vitis vinifera* L. cultivars and Franco-American hybrids: cvs. 'Podarok Magaracha', 'Pervenets Magaracha' and 'Tsitronnyi Magaracha' and between *Vitis vinifera* and *Vitis rotundifolia* Michx.: cv. 'Intervitis Magaracha' and hybrid 'Magarach 100-74-1-5'.

Media Preparing and Culture Conditions

Media were autoclaved at 103 kPa for 25 min. The pH of all media was adjusted to 5.6 with NaOH before autoclaving. Each 100 ml Erlenmeyer flask contained a 20 ml aliquot of solid or liquid medium and was sealed with aluminium foil. Approximately 200 mg fresh weight of callus was placed in liquid medium of each flask to initiate cell suspension. Suspension cultures were incubated on a shaker at 60 rpm. Subculturing was done every 21 days to respective liquid medium. Suspensions were subcultured to fresh liquid media using an inoculum ratio of approximately 1:5 (v:v). Calluses, cell and embryo suspensions were incubated at 27°C in the dark. Plantlets and plants were cultured in a growth chamber under a photon flux density at the culture surface of 55 $\mu mol/m^2 \cdot s$ provided by cool white fluorescent tubes during a 16-h photoperiod at 27°C.

Initiation of Proembryogenic Cell Suspensions and Development of Globular and Heart-stage Embryos of cv. 'Podarok Magaracha' in Liquid Media

Leaf petioles collected from *in vitro* grown plants of cv. 'Podarok Magaracha' were established on solid full-strength NN medium [14] supplemented with 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.5 mg/L N6-benzyladenine (BA). After 90 days in culture proembryogenic calluses were subcultured to liquid NN medium supplemented with 1.0 or 2.0 mg/L 2,4-D + 0.2 or 2.0 mg/L BA in different combinations to initiate cell suspensions.

After 21 days in culture cell suspensions were subcultured to different versions of liquid NN medium: without growth regulators, supplemented with

0.2 or 2.0 mg/L 1-naphthaleneacetic acid (NAA) + 0.1 or 1.0 mg/L BA in different combinations and with 0.5 mg/L BA only.

Formation of Proembryogenic Calluses, Initiation of Cell Suspensions and Development of Globular and Heart-stage Embryos in Nine Grapevine Genotypes

Leaf petioles collected from *in vitro* grown plants of various grapevine genotypes were placed on solid NN medium supplemented with different levels of 2,4-D and BA adjusted to each cultivar for development of proembryogenic calluses [11]: 2.0 mg/L 2,4-D + 2.0 mg/L BA for rootstock 'Kober 5BB' and cv. 'Bianca'; 2.0 mg/L 2,4-D + 1.0 mg/L BA for rootstock '101-14', cvs. 'Zigfridrebe' and 'Intervitis Magaracha' and hybrid 'Magarach 100-74-1-5'; 2.0 mg/L 2,4-D + 0.2 mg/L BA for cv. 'Tsitronnyi Magaracha'; 0.5 mg/L 2,4-D + 2.0 mg/L BA for cv. 'Pervenets Magaracha' and 0.5 mg/L 2,4-D + 1.5 mg/L BA for cv. 'Podarok Magaracha'.

After 91 days in culture proembryogenic calluses were subcultured to liquid NN medium supplemented with 1.0 mg/L 2,4-D + 0.2 mg/L BA to initiate cell suspensions (Fig. 1). Obtained suspension cultures were subcultured to two versions of liquid NN medium: supplemented with 2.0 mg/L NAA + 0.1 mg/L BA and with 0.5 mg/L BA only. Embryo suspensions developed in each version of medium were again subcultured to the above versions of liquid NN medium: supplemented with 2.0 mg/L NAA + 0.1 mg/L BA and with 0.5 mg/L BA only. Subsequently these cell suspensions were subcultured to one version of liquid NN medium supplemented with 0.5 mg/L BA: (Fig. 1). Production of heart- to late-stage embryos and plantlets with green cotyledons and hypocotyls in liquid media and shoot growth from these plantlets on solid medium were carried out by methods of Zlenko et al. [15, 16].

Assessment of Results and Statistical Analysis

Formation of globular and heart-stage embryos per flask was assessed using a binocular microscope MBS-6 providing a 16-fold multiplication. Globular embryos (0.1 - 0.4 mm) and heart-stage embryos (0.3 – 0.8 mm) were counted by the method of Zlenko et al. [13].

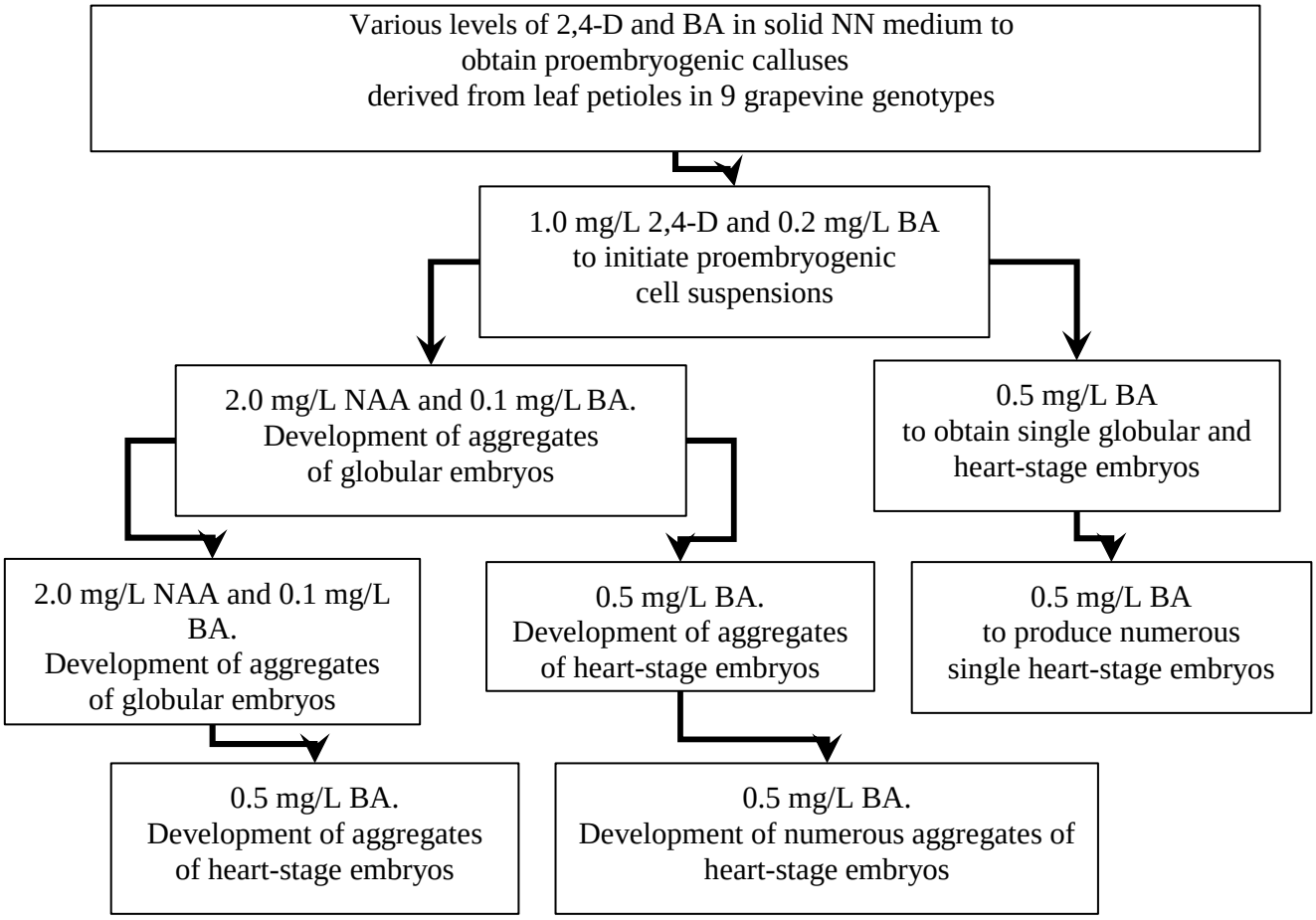

Fig. 1. Design of subculturing proembryogenic calluses and proembryogenic cell and embryo suspensions in 9 interspecific grapevine hybrids. Full strength NN medium was used as basal medium at all stages of subculturing.

Each version of liquid media for incubation of cell and embryo suspensions was in 15 replications. The variation coefficient (V) in all cases was not more than 31% ($P < 0.05$). Statistical assays were done by the Student's *t*-test ($\pm$ SE, $P < 0.05$).

RESULTS and DISCUSSION

Leaf petioles collected from *in vitro* grown plants of cv. 'Podarok Magaracha' were cultured on solid NN medium supplemented with 0.5 mg/L 2,4-D + 1.5 mg/L BA. Proembryogenic callus was subcultured to liquid NN medium supplemented with 1.0 or 2.0 mg/L 2,4-D + 0.2 or 2.0 mg/L BA in different combinations to initiate cell suspensions (Table). These suspensions were inoculated into different versions of liquid NN medium: without growth regulators, with 0.2 or 2 mg/L NAA + 0.1 or 1 mg/L BA in different combinations as well as with 0.5 mg/L BA only. A cell suspension which had been initiated in medium (1) with 1 mg/L 2,4-D + 0.2 mg/L BA and subcultured to liquid hormone-free medium (a) formed larger globular embryos (0.05-1.0 mm**),** than that which had been initiated in medium (2) with 2,4-D at a higher concentration (2 mg/L) + 0.2 mg/L BA (< 0.05 mm embryos) (Table). Somatic embryos were formed after subculturing proembryogenic calluses from the media with 2,4-D and BA to the hormone-free media [2, 10, 17, 18].

Aggregates of globular embryos were formed after initiating proembryogenic cell suspensions in liquid media with 1 or 2 mg/L 2,4-D + 0.2 mg/L BA and subsequent subculturing these suspensions to liquid medium with 2 mg/L NAA + 0.1 mg/L BA (Table). Somatic embryos of hybrid 'Seyve Villard 52-76' were formed after subculturing proembryogenic callus from solid medium [18] (MS) with 2,4-D and BA to solid MS medium with the above levels of NAA and BA [19]. Somatic embryogenesis in grapevine was achieved on different media with NAA [21], 1-naphthalene oxyacetic acid (NOA) + BA [11, 22] and NOA + IAA + BA [23]. Secondary grapevine embryos were developed from primary somatic embryos in liquid medium with NOA + BA [10] as well as on solid media without growth regulators [8] and with NOA + IAA + BA [24].

The best results pertaining to cv. 'Podarok Magaracha' were achieved after initiating cell suspensions in liquid NN medium supplemented with 1 or 2 mg/L

2,4-D + 0.2 mg/L BA and subculturing these suspensions to liquid medium supplemented with 0.5 mg/L BA (e) which gave rise to single globular and heart-stage embryos. In this case a lower level of 2,4-D (1 mg/L compared to 2 mg/L) in medium for initiating cell suspensions led to a higher number of globular embryos (3911 ± 1212 and 1874 ± 618, respectively) and heart-stage embryos (3285 ± 1018 and 345 ± 80, respectively) in liquid media with 0.5 mg/L BA (Table). Somatic embryos were formed in liquid NN medium supplemented with BA [9, 12]. Other designs of suspension subcultures did not enable the development of somatic embryos in cv. 'Podarok Magaracha' (Table).

Table. - Effects of growth regulators on the initiation of proembryogenic cell suspensions and development of globular and heart-stage embryos of cv. 'Podarok Magaracha' in liquid NN medium.

Levels of growth regulators in versions of liquid NN medium (mg/L)		Results of subculture II		Notes
Subculture I. Initiation of cell suspensions from proembryogenic calluses[1] →	Subculture II. Development of cell and embryo suspensions	Number and size of single embryos in 20 ml of liquid medium after 21 d in culture		
		Number	Size	
1) 2,4-D (1.0 mg/L) + BA (0.2 mg/L) →	a) Without growth regulators	Numerous globular embryos (>4000)	0.05-0.1 mm	Friable callus containing single globular embryos
2) 2,4-D (2.0 mg/L) + BA (0.2 mg/L) →	a) Without growth regulators	Numerous globular embryos (>4000)	<0.05 mm	
1) 2,4-D (1.0 mg/L) + BA (0.2 mg/L); 2) 2,4-D (2.0 mg/L) + BA (0.2 mg/L) →	b) NAA (0.2 mg/L) + BA (0.1 mg/L)	0	—	Loose, cotton like callus
1) 2,4-D (1.0 mg/L) + BA (0.2 mg/L) →	c) NAA (2.0 mg/L) + BA (0.1 mg/L)	0	—	Aggregates of 3-5 globular embryos. Each embryo is 0.2-0.5 mm
2) 2,4-D (2.0 mg/L) + BA (0.2 mg/L) →	c) NAA (2.0 mg/L) + BA (0.1 mg/L)	0	—	Aggregates of 10-15 globular embryos. Each embryo is 0.1-0.2 mm
1) 2,4-D (1.0 mg/L)	d) NAA (0.2 mg/L)	0	—	Loose, cotton like

+ BA (0.2 mg/L); 2) 2,4-D (2.0 mg/L) + BA (0.2 mg/L) →	+ BA (1.0 mg/L)			callus
1) 2,4-D (1.0 mg/L) + BA (0.2 mg/L) →	e) BA (0.5 mg/L)	3911±1212 globular embryos and 3285±1018 heart-stage embryos	0.1-0.4 mm globular embryos, 0.3-1.0 mm heart-stage embryos	Single globular and heart-stage embryos
2) 2,4-D (2.0 mg/L) + BA (0.2 mg/L) →	e) BA (0.5 mg/L)	1874±618 globular embryos and 345±80 heart-stage embryos	0.1-0.4 mm globular embryos, 0.3-0.8 mm heart-stage embryos	Single globular and heart-stage embryos
3) 2,4-D (1.0 mg/L) + BA (2.0 mg/L) →	a) Without growth regulators; b) NAA (0.2 mg/L) + BA (0.1 mg/L); c) NAA (2.0 mg/L) + BA (0.1 mg/L)	0	—	Loose, cotton like callus
4) 2,4-D (2.0 mg/L) + BA (2.0 mg/L) →	a) Without growth regulators; b) NAA (0.2 mg/L) + BA (0.1 mg/L)	0	—	Loose, cotton like callus
3) 2,4-D (1.0 mg/L) + BA (2.0 mg/L); 4) 2,4-D (2.0 mg/L) + BA (2.0 mg/L) →	d) NAA (0.2 mg/L) + BA (1.0 mg/L); e) BA (0.5 mg/L)	0	—	Crumbs of hard callus among the mass of soft callus

[1] Proembryogenic callus obtained on solid NN medium supplemented with 0.5 mg/L 2,4-D and 1.5 mg/L BA was subcultured to different versions of liquid media.

Figure 1 shows the initiation of proembryogenic cell suspensions in interspecific hybrids of the genus *Vitis* and different subculture designs for development of globular and heart-stage embryos. Subculturing cell suspensions which had been initiated in liquid NN medium supplemented with 1 mg/L 2,4-D + 0.2 mg/L BA to liquid NN medium supplemented with 2 mg/L NAA + 0.1 mg/L BA led to development of aggregates of globular embryos. Subsequent once more subculturing these embryo-aggregate suspensions to NN medium supplemented with 2 mg/L NAA + 0.1 mg/L BA resulted in the development of aggregates of globular embryos while subculturing embryo-aggregate suspensions to medium supplemented with 0.5 mg/L BA led to development of aggregates of globular and heart-stage embryos. Subculturing cell suspensions to

liquid medium with 0.5 mg/L BA and conducting one or two repeated cultures with this growth regulator (0.5 mg/L BA) resulted in the development of single globular and heart-stage embryos. Genotypic differences between 9 interspecific hybrids of grapevine as to the development of somatic embryos included different levels of 2,4-D and BA in solid NN medium for obtaining proembryogenic calluses from leaf petioles (see 'Materials and Methods') and the need of one or two subsequent cultures of cell and embryo suspensions in liquid NN medium supplemented with 0.5 mg/L BA for the development of numerous heart-stage embryos. Cultivars 'Podarok Magaracha', ‘Intervitis Magaracha', 'Tsitronnyi Magaracha' and 'Pervenets Magaracha' and hybrid 'Magarach 100-74-1-5' required only one culture of cell and embryo suspensions in liquid NN medium supplemented with 0.5 mg/L BA to develop globular and heart-stage embryos while rootstocks 'Kober 5BB' and '101-14' and cultivars 'Zigfridrebe' and 'Bianca' required two cultures in this medium with 0.5 mg/L BA.

The use of methods by Zlenko et al. [15, 16] for regenerating plants from grapevine somatic embryos has shown that even torpedo-stage embryos both cv. 'Podarok Magaracha' (Fig. 2) and other 8 grapevine genotypes were connected

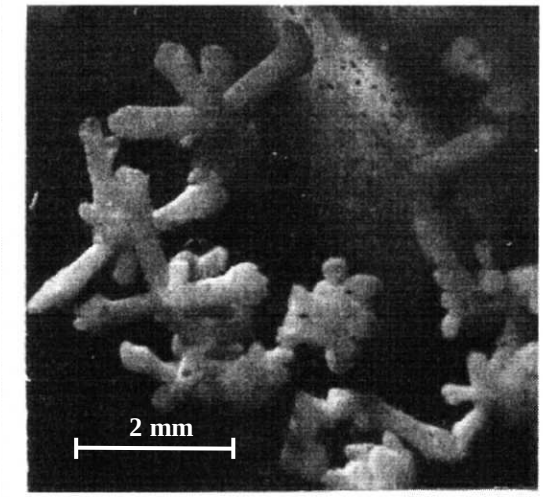

Fig. 2. Effects of different growth regulators in liquid NN medium used for induction of somatic embryogenesis in cv. 'Podarok Magaracha' on the character of the following development of heart- and torpedo-stage embryos in liquid HTE medium supplemented with 0.1 mg/L IAA + 30 mg/L sodium hummate: Embryo aggregates after the following subculture

design: 1.0 mg/L 2,4-D + 0.2 mg/L BA → 2.0 mg/L NAA + 0.1 mg/L BA → 0.5 mg/L BA → 0.1 mg/L IAA + 30 mg/L sodium hummate.

into aggregates after subculturing cell and embryo suspensions in liquid media (NN medium + 1 mg/L 2,4-D + 0.2 mg /L BA, cell suspensions → NN medium + 2 mg/L NAA + 0.1 mg/L BA, globular embryo aggregates → NN medium + 0.5 mg/L BA, heart-stage embryo aggregates → HTE medium + 0.1 mg/L IAA + 30 mg/L sodium hummate, torpedo-stage embryo aggregates). Aggregates disintegrated in the process of the development of plantlets with green cotyledons and hypocotyls in liquid HTE medium supplemented with 0.5 mg/L GA_3. In order to produce shoots, plantlets were placed onto solid M2MS medium with 0.5 mg/L BA.

Development of single globular and heart-stage embryos in liquid NN medium with 0.5 mg/L BA led to subsequent development of single heart- and torpedo-stage embryos in liquid HTE medium with 0.1 mg/L IAA + 30 mg/L sodium hummate using the following subculture design: NN medium + 1 mg/L 2,4-D + 0.2 mg/L BA, cell suspensions →NN medium + 0.5 mg/L BA, single globular and heart-stage embryos → HTE medium + 0.1 mg/L IAA + 30 mg/L sodium hummate, single heart- and torpedo-stage embryos (Fig. 3).

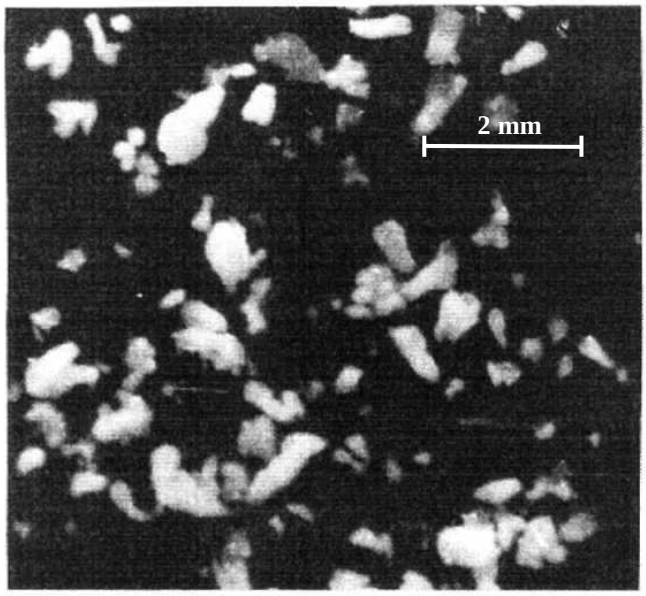

Fig. 3. Effects of different growth regulators in liquid NN medium used for induction of somatic embryogenesis in cv. 'Podarok Magaracha' on the character of the following

development of heart- and torpedo-stage embryos in liquid HTE medium supplemented with 0.1 mg/L IAA + 30 mg/L sodium hummate: Prevailing single embryos after the following subculture design: 1.0 mg/L 2,4-D + 0.2 mg/L BA →0.5 mg/L BA →0.1 mg/L IAA + 30 mg/L sodium hummate.

CONCLUSIONS

Based on the results for development of single globular and heart-stage embryos from proembryogenic cell suspensions it may be concluded that:

1. Although the development of proembryogenic calluses from explants of leaf petioles in different grapevine genotypes required different levels of 2,4-D and BA in solid NN medium (see 'Materials and Methods'), subculturing calluses to liquid NN medium supplemented with 1 mg/L 2,4-D + 0.2 mg/L BA led to the maintenance of the cell proembryogenic pontential in these suspension cultures.

2. Initiation of proembryogenic cell suspensions in cv. 'Podarok Magaracha' in liquid NN medium supplemented with 0.2 mg /L BA and a lower level of 2,4-D (1 mg/L compared to 2 mg/L), gave rise to the increased number of globular and heart-stage embryos after subculturing to NN medium supplemented with 0.5 mg/L BA.

3. In cv. 'Podarok Magaracha' and other 8 interspecific hybrids initiation of proembryogenic cell suspensions in liquid NN medium supplemented with 1 mg/L 2,4-D + 0.2 mg /L BA and their subsequent subculturing to liquid NN medium supplemented with 2 mg/L NAA + 0.1 mg/L BA led to the development of aggregates of globular embryos while subculturing proembryogenic cell suspensions to NN medium with 0.5 mg/L BA led to development of single globular and heart-stage embryos. To obtain numerous heart-stage embryos in some cultivars, two repeated cultures of cell and embryo suspensions in liquid media with 0.5 mg/L BA were needed.

Transformation of genes into single proembryogenic cells of suspension cultures and selection at the cell level with subsequent single embryo development enable to decrease the probable regeneration of chimeric plants.

LITERATURE CITED

1. *Zhang M., Rajashekar C.B.* Selection of cold tolerant cells of grapes in suspension culture // Plant Science (Shannon). - 1994. - V. 97. - P. 69-74.

2. *Lebrun L., Rajasekaran K., Mullins M.G.* Selection *in vitro* for NaCI-tolerance in *Vitis rupestris* Scheele // Ann. Bot. - 1985. - V. 56. - P. 733-739.

3. *Zlenko V.A., Kotikov I.V., Troshin L.P.* A mathematical design of experiment to optimize concentrations of substances in nutrient medium for the grape callus development with a subsequent genotype selection on the cellular level (in Russian) // Biotechnology (Moscow). - 2005. - V. 6. - P. 63-73.

4. *Volynkin V.A., Likhovskoi V.V., Oleinikov N.P.,Pavlova I.A., Levchenko S.V.,Zlenko V.A., Vasylyk I.A., Sheloudko Y.V., Gerasimenko I.M.* Classical and innovative approaches in the field of cell biology and genetic engineering for improvement of characteristics of grape varieties // Magarach. Viticulture and winery. – 2014. – V.4. – P.4-6.

5. *Jayasankar S., Li Z., Gray D.J. In vitro* selection of *Vitis vinifera* 'Chardonnay' with *Elsinoe ampelina* culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase // Planta. - 2000. - V. 211. - P. 200-208.

6. *Kikkert J.R., Herbert-Soule D., Wallace P.G., Striem M.J., Reisch B.I.* Transgenic plantlets of 'Chancellor' grapevine *(Vitis* sp.) from biolistic transformation of embryogunie cell suspensions // Plant Cell Rep. - 1996. - V. 15. - P. 311-316.

7. *Striem M.J., Reisch B.I., Kikkert J.R.* Differences in GUS expression among grapevine transformants // Acta Hort. - 2000. - V. 526. - P. 437-443.

8. *Oláh R., Scegedi E., Ruthner S., Korbuly J.* Thidiazuron - induced regeneration and genetic transformation of grapevine rootstock varieties // Vitis. - 2003. - V. 42. - P. 133-136.

9. *Mullins M.G., Srinivasan C.* Somatic embryos and plantlets from an ancient clone of the grapevine (cv. Cabernet Sauvignon) by apomixis *in vitro* // J. Exp. Bot. - 1976. - V. 27. - P. 1022-1030.

10. *Rajasekaran K., Mullins M.G.* Embryos and plantlets from cultured anthers of hybrid grapevines // J. Exp. Bot. - 1979. - V. 30. - P. 399-407.

11. *Bornhoff B., Harst M.* Establishment of embryo suspension cultures of grapevines *(Vitis* L.) // Vitis. - 2000. - V. 39. - P. 27-29.

12. *Zlenko V.A., Troshin L.P.* Somatic embryogenesis of grapevine from cell suspensions (in Russian) // Cytology and Genetics (Kiev). - 1993. - V. 27. - P. 53-63.

13. *Zlenko V.A., Kotikov I.V., Troshin L.P.* Effects of IAA and BA on development of globular, heart- and torpedo-stage embryos from cell suspensions of three grape genotypes // Scientia Hort. - 2005. - V. 104. - P. 237-247.

14. *Nitsch J.P., Nitsch C.* Haploid plants from pollen grains // Science. - 1969. - V. 163. - P. 85-87.

15. *Zlenko V.A., Kotikov I.V., Troshin L.P.* Efficient GA_3-assisted plant regeneration from cell suspensions of three grape genotypes via somatic embryogenesis // Plant Cell Tiss. Org. Cult. - 2002. - V. 70. - P. 295-299.

16. *Zlenko V.A., Kotikov I.V., Troshin L.P.* Plant regeneration from somatic embryos of interspecific hybrids of grapevine formed in liquid medium // J. Hort. Science Biotechnol. - 2005. - V. 80. - P. 461-465.

17. *Jayasankar S., Gray D.J., Litz R.E.* High-efficiency somatic embryogenesis and plant regeneration from suspension cultures of grapevine // Plant Cell Rep. - 1999. - V. 18. - P. 533-537.

18. *Lopez-Perez A.J., Carreño J., Martinez-Cutillas A., Dabauza M.* High embryogenic ability and plant regeneration of table grapevine cultivars *(Vitis vinifera* L.) induced by activated charcoal // Vitis. - 2005. - V. 44. - P. 79-85.

19. *Murashige T., Skoog F.* A revised medium for rapid growth and bioassays with tobacco tissue cultures // Physiologia Plantarum. - 1962. - V. 15. - P. 473-497.

20. *Krul W.R., Worley J.F.* Formation of adventitious embryos in callus cultures of 'Seyvarl 'a French hybrid grape // J. Amer. Soc. Hort. Sci. - 1977. - V. 102. - P. 360-363.

21. *Salunkhe C.K., Rao P.S., Mhatre M.* Plantlet regeneration via somatic embryogenesis in anther callus of *Vitis latifolia* L. // Plant Cell Pep. - 1999. - V. 18. - P. 670-673.

22. *Stamp J.A., Meredith C.P.* Somatic embryogenesis from leaves and anthers of grapevine // Scientia Hort. - 1988. - V. 35. - P. 235-250.

23. *Martinelli L., Gribaudo I., Bertoldi D., Candioli E., Poletti V.* High efficiency somatic embryogenesis and plant germination in grapevine cultivars Chardonnay and Brachetto // Vitis. - 2001. - V. 40. - P. 111-115.

24. *Croce P., Vannini C., Bracale M.* An easy and convenient method for maintenance of embryogenic cultures of *Vitis vinifera //* Vitis. - 2005. - V. 44. – P. 197-198.