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## Obtaining plants from crosses of seedless grapevine varieties by means of *in vitro* embryo culture

by

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### Die Gewinnung von Pflanzen aus Kreuzungen kernloser Rebsorten mit Hilfe der *in-vitro*-Kultur von Embryonen

**Zusammenfassung:** Zwischen den Neuzüchtungen Seedless hybrid VI-4 und Kishmish Moldavski wurden reziproke Kreuzungen durchgeführt. Aus den entstandenen Beeren wurden 52 bzw. 66 d nach der Vollblüte Samenanlagen isoliert. Mit Hilfe der *in-vitro*-Technik wurden auf einem festen Nährmedium (NIRSCH und NIRSCH 1969) mit optimal angepaßter Phytohormonkonzentration aus beiden Kreuzungskombinationen und zu beiden Kulturterminen vitale Embryonen herangezogen.

Der frühere Kulturbeginn, etwa 50 d nach der Anthese, erwies sich als günstiger. Die Embryonen aus der Kombination Kishmish Moldavski × Seedless hybrid VI-4 zeigten eine erhöhte Variabilität. Dementsprechend wurden aus ihnen mehr normale Pflanzen gewonnen. Das Wachstum der Embryonen war nicht von Kallusbildung begleitet. Die jungen Sämlinge wurden in einem Erdgemisch unter Gewächshaus- und Freilandbedingungen weiterkultiviert.

Die Technik der Embryokultur aus kernlosen Traubenbeeren könnte eine interessante Alternative zu den klassischen Methoden der Rebenzüchtung darstellen.

**Key words:** table grape, raisin, seedlessness, crossing, berry, embryo, tissue culture, selection.

### Introduction

In recent years, there is a growing interest in seedless table and raisin grapes, which demands genetic investigations and breeding work for creating improved grapevine varieties.

In cultivated grapevine, *Vitis vinifera* ssp. *sativa* L., two types of seedlessness of the fruit have been observed (STOUT 1936; NEGRULY 1936): parthenocarpy (Corinth type) and stenospermocarpy (Sultanina type).

Recovering of the embryo in a seed from a stenospermocarpic variety by means of *in vitro* technique is a very interesting alternative to the traditional methods of breeding seedless grapes, allowing to accomplish crosses between seedless parent forms and to obtain progenies. This technique theoretically increases the proportion of the seedless progeny within a selective cycle.

Two distinct methods of realizing *in vitro* embryo culture in grapevines have been reported. The first one utilizes liquid medium (EMERSHAD and RAMMING 1984; GRAY *et al.* 1987; GOLDY and AMBORN 1987) and the other method includes cultivation directly on solid medium (CAIN *et al.* 1983; SPIEGEL-ROY *et al.* 1985).

The objective of this research is:

- to study the possibilities for realizing *in vitro* embryo culture in seedless grape varieties created in or imported to Bulgaria;
- to check the efficiency of the procedure reported by SPIEGEL-ROY *et al.* (1985) which allows direct growth on solid medium.

### Materials and methods

In June 1988, reciprocal crosses were carried out between the seedless varieties Seedless hybrid VI-4 and Kishmish Moldavski, growing in the ampelographic collection of the Institute of Viticulture and Enology, Pleven, Bulgaria. The experimental work was carried out in 1988 and 1989 in the Central Laboratory of Genetic Engineering of the Agricultural Academy.

Berries were collected at two dates — 52 and 66 d after anthesis — and surface sterilized in 70 % ethanol and 0.2 %  $\text{HgCl}_2$ . Ovules were aseptically separated and put in groups of 20 in petri dishes containing 15 ml of culture medium.

We used the medium of NITSCH and NITSCH (1969) +  $10^{-6}$  M  $\text{Ga}_3$  +  $10^{-5}$  M IAA + 0.3 % active charcoal. Bearing in mind the information by MILYAEVA *et al.* (1984) about hormonal regulation of the female reproductive sphere in seedless grapevine varieties, we added to the above medium  $10^{-6}$  M kinetin, too. The cultivation was performed at a temperature of  $26 \pm 2$  °C and a photoperiod of 16 h, using cool fluorescence light.

The experiment includes 4 variants with 8 replicates each, consisting of 20 ovules per replicate.

Ovules were cultured for 12—16 weeks. The germinating seed rudiments were periodically counted. Observations were made on callus proliferation as well. The number of viable embryos recorded in the table refers to December 20, 1988.

After formation of cotyledons and a well developed root, the young established plantlets were transferred into test tubes 20 mm in diameter, containing 10 ml half concentration of original MURASHIGE and SKOOG (1962) medium for complete growing. When bearing 2—3 leaves, the seedlings were planted in small plastic pots 50 mm in diameter and 70 mm high, filled with sterile perlite. Later on, after adaptation to the external conditions having continued about 5 weeks, they were planted in pots 100 mm in diameter and 200 mm high in a soil mixture. In May 1989, the seedlings were cultivated in a non-heated polyethylene greenhouse. 3 of the plants were micro-propagated in lots of 3—4 individuals, allowing their parallel planting in a greenhouse and under field conditions, which aims at forcing the fruiting.

In the results presented here the number of the plants obtained and planted in a greenhouse is given.

### Results and discussion

In our experiment, direct growth of the embryos was obtained on solid medium, which confirms the results reported by SPIEGEL-ROY *et al.* (1985). Callus development was not observed. From each ovule only one plant was obtained (Figs. 1 and 2), which indicates the zygotic origin of the plantlets. From the total of 640 ovules cultured, 52 viable embryos were obtained. They gave the origin of whole plants, from which 18 were successfully cultivated in soil conditions and 3 were micro-propagated (Table).

The differences between the percentages of the embryos which had developed from the two crosses are not essential. This shows that a high rate of viable embryos has been preserved in the seed rudiments. The deviations obtained as a result of the term of setting of ovules are greater. Obviously the earlier term about the 50th d after anthesis is more suitable. The successful transferring of the plantlets from the cross Seedless hybrid VI-4 × Kishmish Moldavski proved to be problematic owing to their considerably smaller size (Fig. 1). Hence the small number of successfully cultivated plants — 3 in a test tube, 1 in a greenhouse. This necessitates further optimization of the procedure in order to avoid transferring in the early stage of development.

Number of viable embryos produced by cultured ovules from reciprocal crosses between cvs Kishmish Moldavski and Seedless hybrid VI-4 and number of plants derived from viable embryos

Anzahl der vitalen Embryonen aus kultivierten Samenanlagen, die aus reziproken Kreuzungen der Sorten Kishmish Moldavski und Seedless hybrid VI-4 hervorgingen, sowie Anzahl der aus den vitalen Embryonen gewonnenen Pflanzen

Crossing combination	Beginning of cultivation	Cultivated ovules	Viable embryos		Cultivated plants from embryos	
	d after anthesis	Number	Number	%	Number	%
1. Kishmish Moldavski × Seedless hybrid VI-4	52	160	18	11.2	12	66.7
	66	160	7	4.4	5	71.4
2. Seedless hybrid VI-4 × Kishmish Moldavski	52	160	20	12.5	1	5.0
	66	160	7	4.4	0	—

On the contrary in the reciprocal cross Kishmish Moldavski × Seedless hybrid VI-4, the growth resembles to that of the normal grape seed. The established plantlets are with well developed cotyledons and a rootlet, which considerably facilitates their successful transferring onto 1/2 MURASHIGE and SKOOG medium. In separate experiments, some of the plants were successfully cultivated directly in perlite. Thus the intermediate recultivation in sterile conditions could be omitted.

Our results confirm the earlier reports by CAIN *et al.* (1983), EMERSHAD and RAMMING (1984), SPIEGEL-ROY *et al.* (1985), GOLDY and AMBORN (1987), GRAY *et al.* (1987) and show that it is possible to recover embryos and to obtain plants of newly-created stenospermocarpic varieties belonging to the Sultanina group, and to do this by means of direct growth on solid medium.



Fig. 1: Seedless Hybrid VI-4 × Kishmish Moldavski, ovules germinating. Ovules planted on NITSCH and NITSCH medium (1969) + IAA + Ga<sub>3</sub> + kinetin.

Fig. 2: Kishmish Moldavski × Seedless Hybrid VI-4, ovules germinating. Ovules planted on NITSCH and NITSCH medium (1969) + IAA + Ga<sub>3</sub> + kinetin.

Abb. 1: Keimende Samenanlagen der Kreuzung Seedless hybrid VI-4 × Kishmish Moldavski auf Nährboden nach NITSCH und NITSCH (1969) + IAA + GA<sub>3</sub> + Kinetin.

Abb. 2: Keimende Samenanlagen der Kreuzung Kishmish Moldavski × Seedless hybrid VI-4 auf Nährboden nach NITSCH und NITSCH (1969) + IAA + GA<sub>3</sub> + Kinetin.

Considering the comparatively high percentage of viable embryos obtained on NITSCH's medium, it can be presumed that this is, due to its complex character, very suitable for the successful *in vitro* embryo culture in grapevines. However, balanced levels of phytohormones in the nutrient medium are essential. For limiting the necrosis of cultivated ovules, the addition of active charcoal is of special importance.

The use of *in vitro* embryo culture could considerably increase the efficiency of breeding new seedless forms in grapevines. Further investigations on hybrid populations obtained from seedless parents could also give a better understanding as to inheritance of the character seedlessness in grapes.

### Summary

Reciprocal crosses were performed between the new breedings Seedless hybrid VI-4 and Kishmish Moldavski. From the berries obtained ovules were aseptically isolated at two dates: 52 and 66 d after anthesis. By means of an *in vitro* procedure on solid medium of NITSCH and NITSCH (1969) with an optimized level of phytohormones, viable embryos were recovered in both crosses, as well as in both dates of culturing.

The earlier date of ovule cultivation by the 50th d after anthesis proved to be more suitable. The embryos from the cross Kishmish Moldavski × Seedless hybrid VI-4 showed higher variability. Correspondingly, a higher number of normal plants was obtained from them. The growth of the embryos was not accompanied by callus development. The young seedling plants were cultivated in soil in a greenhouse and under field conditions.

The procedure of culturing ovules from seedless grapes may be an important alternative to the traditional methods of selection.

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