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Research Note

Induction of polyembryony and secondary embryogenesis in culture for embryo rescue of stenospermocarpic genotypes of *Vitis vinifera* L.

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K e y w o r d s : polyembryony, seedless grapes, stenospermocarpy.

Introduction: In stenospermocarpic genotypes of *Vitis vinifera* L. it is possible to rescue the embryo and produce seedling plants by a procedure of *in vitro* embryo culture (CAIN *et al.* 1983; EMERSHAD and RAMMING 1984; SPIEGEL-ROY *et al.* 1985; GRAY *et al.* 1987; TSOLOVA 1990).

Multiple embryos resulting from this procedure and spontaneous polyembryony in culture have been occasionally reported (EMERSHAD and RAMMING 1984; DURHAM *et al.* 1989). Studies on spontaneous polyembryony in some seeded grapevine varieties and interspecific hybrids (*V. vinifera* x *V. riparia*) were undertaken by BOUQUET (1978, 1980, 1982). He has reported a low percentage (0.35 %) in *V. vinifera* and a higher rate (up to 7.7 %) polyembryony in interspecific hybrids.

Polyembryony is a classical means for naturally occurring haploids in angiosperms (PODUBNAIA-ARNOLDY 1976; BOUQUET 1980). Studies on the possibilities to induce polyembryony in culture of fertilized ovules of stenospermocarpic vine genotypes have not been made for the time being.

The present research was undertaken with the following aims: 1. to study the possibilities for induction of relatively high percentage of polyembryony in culture during *in vitro* embryo rescue of seedless grapevine varieties; 2. production of multiple embryos and maintaining in culture of secondary, tertiary, etc. embryogenesis.

Materials and methods: *Plant material:* Five self-pollinated newly selected seedless grapevine cultivars (Kishmish Moldavski, Kishmish Luchistii, Smirnoff, Seedless hybrid VI-4, Russalka 3) and 3 traditional cultivars (Sultanina, Kishmish Rozovii and Sultanina gigas) and 2 crosses (Seedless hybrid VI-4 x Corynth white and Smirnoff x Corynth white) growing in the ampelographic collection of the Institute of Viticulture and Enology, Pleven, Bulgaria were tested. Berries were collected 60 d after anthesis and stored in the dark at 4 °C in the presence of SO_2 for a period of 5 weeks. Sultanina gigas is propagated in Bulgaria under the name of Seedless white big. It has been identified only by ampelographic characteristics as Sultanina gigas (tetraploid form of Sultanina) by NEGRULY and KATEROV in the 1960ies (KATEROV and DONTCHEV, unpublished data).

Embryo rescue and polyembryony induction: Ovules were aseptically separated and put in groups of 20 in petri dishes containing 20 ml of culture medium. The medium is NITSCH and NITSCH (1969) (NN69) supplemented with 10^{-6} M Ga_3 (gibberellic acid), 10^{-5} M IAA (indolyl acetic acid), 10^{-6} M kinetin, 0.3 % active charcoal, 20 g/l saccharose and 0.8 g/l agar, adjusted to pH 5.7 before it was autoclaved. Different levels of vitamins and hormones in the induction medium were tested for Kishmish Moldavski, Russalka 3 and Sultanina gigas cultivars.

The ovules were cultured on induction medium for 12-14 weeks, then the undeveloped ovules were transferred on fresh induction medium. The germinating ovules were periodically counted, the first counting being executed after 8 weeks in culture, the last one after 23 weeks. The results presented in Tables 1 and 2 are the summarized data for each cultivar from the three countings after 8, 16 and 23 weeks in culture. The cultivation was performed at temperature of 26±2 °C and a photoperiod of 16/8 h.

Induction of secondary embryogenesis: The embryos, 10-15 mm in length, were transferred on NN69 growth regulator-free medium for development and induction of secondary embryogenesis in the zone between the hypocotyl and the root.

Results and discussion: The spontaneous polyembryony in grape might be due to several mechanisms (BOUQUET 1978, 1980, 1982; EMERSHAD and RAMMING 1984; DURHAM *et al.* 1989; EMERSHAD *et al.* 1989): 1) fertilizing and development of more than one cell in the embryo sac; 2) adventive embryogenesis from the zygote and the proembryo; 3) embryogenic development of gametic cells in addition to the zygote in the embryo sac.

Possibilities for induction of polyembryony in the stenospermocarpic grapevine genotypes used in our research were available due to the fact that there were proembryonal structures in the initial explant. According to BOUQUET (1980) the genetic determination and some external conditions like climate, physiological state of the grapevine plant, improved nourishment etc. were found to influence greatly the naturally occurring polyembryony.

In our experiment we achieved a high percentage (from 20.0 to 85.7, depending on the variety) of developing embryos producing polyembryonic clusters. The cluster percentage is lower from 1 to 11 if the datum is referred to the total number of the cultured ovules (Tab. 1; Figure, a).

The cultivars Kishmish Moldavski and Russalka 3, positively reacting to low temperature, were subjected to the influence of 2, 4, 6, 8 and 10 times increased concentration of vitamins and hormones and the cultivar Sultanina gigas to 3,5 and 9 times. It might be concluded that 4-5 times increasing of the vitamin and hormone level in the induction medium might increase the percentage of polyembryogenic clusters. The higher values suppressed the percentage of the rescued embryos in these variants.

In most of the studied genotypes we were able to induce secondary embryogenesis in the transition zone between the hypocotyl and the root (Figure, c) of embryos 12-15 mm in length (Tab. 1). The percentage of embryoids

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Table 1

Genotype influence upon the induction of polyembryony and secondary embryogenesis in stenospermocarpic grapevines (100 ovules cultured for each genotype; culture medium NN69 + 10^{-6} M GA_3+10^{-5} M IAA + 10^{-6} M kinetin).

Cultivar	RE	SP	PC	PC(%)	SE
Kishmish Moldavski	11	7	4	36.4	+
Kishmish Luchistii	10	8	2	20.0	-
Smirnoff	20	19	1	5.0	-
Seedless Hybrid VI-4	7	1	6	85.7	+
Sultanina	15	4	11	73.3	+
Kishmish Red	10	6	4	40.0	-
Russalka 3	20	14	6	30.0	+
Seedless Hybrid VI-4 x Corynth white	14	8	6	42.8	+
Smirnoff x Corynth white	22	11	11	50.0	+

RE: number of rescued embryos; SP: number of single plants; PC: number of polyembryogenic clusters; PC(%): clusters (%) of rescued embryos; SE: secondary embryogenesis.

having secondary embryogenesis in 1 cluster for the cvs Kishmish Moldavski, Russalka 3 and Sultanina gigas is presented in Tab. 2. Secondary embryogenesis in vine has been reported by several authors (KRUL and WORLEY 1977; EMERSHAD *et al.* 1989).

We registered the plantlet production of 1 polyembryogenic cluster and the resulting plantlets with secondary embryogenesis until 18 March 1993, i.e. for 23 weeks in culture for the cultivar Russalka 3. The total number of regenerated plants produced in this period is 107. These data illustrate quite cursorily the possibilities provided by the parallel application of polyembryony and secondary embryogenesis as methods for progeny rescue in seedless grapevine cultivars.

Table 2

Genotype influence upon the average number of plantlets/cluster and the percentage of plant with secondary embryogenesis. The results refer to induction medium for which the percentage of produced polyembryogenic clusters for each cultivar is the highest: NN for Kishmish Moldavski, NN4 for Russalka 3, NN3 for Sultanina gigas.

Cultivar	Polyembr. clusters (%)	Aver.number of plantlets in 1 cluster	Plantlets with sec. embryogenesis in 1 cluster (%)
Kishmish Moldavski	7.0	11.5	13.8
Russalka 3	5.0	32.3	30.9
Sultanina gigas	8.0	21.0	33.3

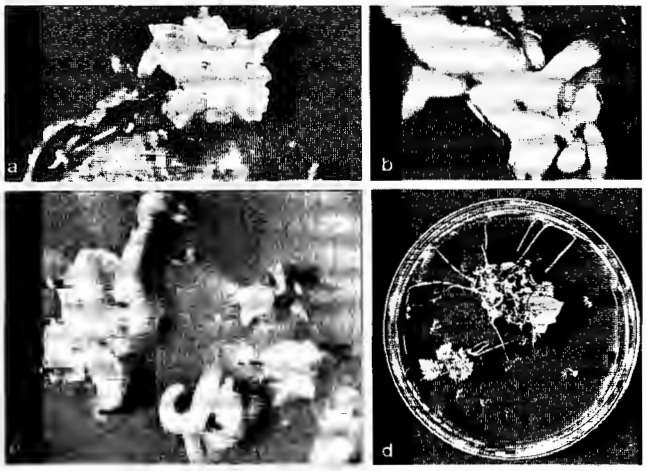

Figure: Induction of polyembryony and secondary embryogenesis in culture for embryo rescue of stenospermocarpic genotypes of *Vitis vinifera*. a. Polyembryonic cluster (cv. Russalka 3) (1 cm ≙ 1.7 mm); b. adventive embryogenesis (cv. Kishmish Moldavski) (1 cm ≙1.7 mm); c. Secondary embryogenesis (cv. Sultanina gigas) (1 cm ≙1.6 mm); d. Cluster of regenerated plants (cv. Russalka 3).

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