IMMATURE EMBRYO RESCUE OBTAINED BY INTRASPECIFIC HYBRIDIZATION PERFORMED ON THE GRAPEVINE

RECUPERAREA EMBRIONILOR IMATURI OBTINUȚI PRIN HIBRIDĂRILE INTRASPECIFICE EFECTUATE LA VIȚA DE VIE

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Abstract. *In contrast to conventional breeding methods, biotechnological approaches in recovery in ovules embryos rescue opening new perspectives in grapevine varieties. Works were performed the optimization of recovery paths for immature embryos from 4 genotypes hybrid vines in the collection of Research Station for Development Viticulture and Oenology Bujoru. Culture medium and genotype that participated in hybridization has strongly influenced the viability of eggs inoculated. Use of liquid culture medium under the bridges of filter paper showed a higher rate of egg viability. Supplementation of medium with 100 mg ascorbic acid and citric acid 100 mg resulted in greater potential results favorable. Un viable eggs was obtained from hybrid combinations Madeleine Angevine x Afuz Ali si Apiren alb x Kişmiş alb.*
Key words: grapevine, hybridization, seedless varieties, in vitro culture, embryons

Rezumat. *Spre deosebire de metodele conventionale de reproducere, aplicarea biotehnologiei in recuperarea embrionilor in ovulo a deschis noi perspective in obtinerea soiurilor apirene de viță de vie. S-au efectuat lucrări privind optimizarea căilor de recuperare a embrionilor imaturi de la 4 genotipuri hibride de viță de vie din colecția Statiunii de Cercetare Dezvoltare pentru Viticultura si Vinificatie Bujoru. Mediul de cultură şi genotipurile care au participat la hibridare, au influențat semnificativ viabilitatea ovulelor inoculate. Utilizarea mediilor de cultură lichide prevăzute cu punți din hârtie de filtru a evidențiat o rată a viabilității ovulelor mai mare. Suplimentarea mediilor de cultură cu acid ascorbic 100 mg şi acid citric 100 mg a condus la rezultate favorabile.Un potențial mai mare de ovule viabile s-a obținut la combinatiile hibride Madeleine Angevine x Afuz Ali si Apiren alb x Kişmiş alb*
Cuvinte cheie: vița de vie, hibridare, soiuri apirene, cultura in vitro, embrioni

INTRODUCTION

The study seedless varieties existing in Romania has shown that they, in addition to quality, they have some shortcomings under the aspect adapting them to specific vineyards preserve ecosystems. This situation requires the need to obtain new seedless varieties which to adapt to environmental conditions

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characteristic vineyard climate in Romania, to ensure production quality, used either that grapes of table varieties or as a raw material for industrialization.

In ovulo embryo rescue thus provides a solution not only for obtaining intraspecific and interspecific crosses, but also allows using seedless vines as female parents (Cain et al. 1983; Emershad and Ramming 1984). Results of the previous works pointed out that not only the composition of the medium culture but also the genotype affects the success of the culture (Bouquet and Davis 1989).

Problem in creating seedless varieties genitor is the choice that must be to obtain the form large grain, more productive, with periods of maturation, to transmit to progeny seedless, to a lesser degree as high as possible (Cachiță, 1987). Recovery and in vitro culture of the zygotic embryos at a stage early stage of development, is the only way to get the hybrids from crosses in which one or both parent varieties are seedless (Bharathy et al., 2005). Embryo culture propagation is used for obtaining and selection.

MATERIAL AND METHODS

In the method conventional have been carried out four hybrid combinations, using the genitor seedless varieties and varieties of flowers normal hermaphrodite .The objective pursued in experiences carried out has been developing culture media favorable seed to maintain the viability and excised-sample test results by default save immature embryos. For this purpose have been tested several types of nutrient media. In vitro growth processes are directed in particular to the hormone use in the environment. Nutrient media are distinguished by the content of macro and trace elements, but also on the report auxine / cytokinine thus, in dependence of the intended purpose they have been used following media:

1. Immature embryos to save environments have been used:

• M1 - macroelemente Nitsch & Nitsch and micronutrients after MS (Murashige-Skoog , 1962) supplemented, 6 g/l agar, 30 g/l sucrose, 100 mg ascorbic acid and 100 mg citric acid: M2 - micro, macroelemente after MS (Murashige-Skoog , 1962), and liquid. The medium was supplemented with: 10 mg/l THAT (indolilacetic acid) 0,05 mg-biotin, 100 mg ascorbic acid and 100 mg citric acid; M3 - micro, macroelemente after MS (Murashige-Skoog , 1962). The medium was supplemented with: 10 mg/l (indolilacetic acid), 0,05 mg-biotin.

2. For maintaining viable seed has used the medium MS (Murashige-Skoog, 1962). supplemented with: 20 mg/l that is why (indolilacetic acid), 3 mg/l BAP, 0, 05 mg-biotin, 6 g/l agar, 30 g/l sucrose, 100 mg ascorbic acid and 100 mg citric acid Environments described differs from both after the factor a hormonal and after pH value, which varies within the range 5.8 -7.0. Explants inoculation has been carried out under aseptic conditions into the speaker or in laminar air flow sterile.

Material has been taken from the SCDVV Bujoru collection ampelographic where they carried out the work of hybridization. Grapes were harvested at 69 and 86 days after pollination. After seed inoculation on the medium culture, the vessels have been kept under controlled conditions of the medium 25-27 °C, ensuring a photoperiod 16 hours light and 8 hours dark. Choosing the optimal temperature, the optimization of other factors externally induced, which contributes to the completion framework for the conduct of the multiplication process, growth and differentiation, has been taking account of the circumstances to which it is adapted to ecological vines in natural circumstances of cultivation. The bird seed in early stage on nutrient media

fresh is mandatory to maintain the viability. This procedure has been carried out at regular intervals to 5 weeks. Duration of a subculture has been 30 days.

RESULTS AND DISCUSSIONS

As a result intraspecific hybridization has been obtained in all four grape hybrid combinations. Kernels were harvested at 2 different stages namely: 69 days to 86 days after pollination. Most have had an average weight of between 0, 35 -0, 47 g to those harvested at 69 days after pollination (table 1). The difference in weight was not significant. The smallest weight is obtained to the combination Seedless white x Kismis white. The ratio of grain/ova signifies the number of ova recovered from grain usable. The report heavily/ovum has been greater than to the combination Gelu x Kismis black 1.44 , while the combination Seedless white x Kismis white has had the lowest report 1.27 user's manual (table 1). Variation in seed grain can be amended by begets pattern and/or endogens hormones levels. Another aspect would be that the number of seeds/heavily is a character intrinsic genotype.

Table 1

The genitors influence on grain characteristics

Combination hybrid	Days after pollination	No. of the berries used	Weight of berries (g)	No. of seed excised	Report berries/seed	Seed size mm	Average weight of the seed mg
Madeleine Angevine x Afuz Ali	69	300	0,37	420	1,40	0,5	11,20
	86	445	2,47	623	1,40	1,0	14,63
Azur x Kismis black	69	150	0.44	208	1,39	0.4	9,61
	86	149	2,30	193	1,39	0,9	10,76
Seedless white x Kismis white	69	120	0,35	152	1,27	0,3	8,14
	86	129	1,55	154	1,27	0,8	9,75
Gelu x Kismis black	69	200	0,47	288	1,44	0,5	9,81
	86	203	2,38	284	1,40	1,0	11,27

The variation in weight of the seed has been observed in all 4 combinations. The greatest weight was recorded to the combination Madeleine Angevine x Afuz Ali with 11.20 mg. The variation in weight of the seeds is given by genotypic parental characteristics, compatibility and the nutritional factories involved in the process of hybridization.

Berries harvest at 86 days after pollination brought us a plus as regards weight of grain which varied from 1.55 to Seedless white x Kismis white, and 2.47 to Madeleine Angevine x Afuz Ali. It should be noted that the ratio of

grain/ova has not changed in relation to stage of 69 days after pollination. Low ratio of ova despite a greater amount of weight of grain can be attributed to the effect of consagvinisation strenuousness, loss and exhaustion of the nutrient of the tissues in the developing berries. The variation in weight of the ovum has been observed in all four combinations. The greatest weight was recorded to the combination Madeleine Angevine x Afuz Ali with 14,63 g, and the lowest it has been registered with Seedless white x Kismis white respectively white 9.75 g.

Recorded results in experiments to save the embryos reflect the important role that clarity composition of the culture medium it owns in the assembly factors which makes the continued viability of seeds, conclusive proof in this respect be the effect different combinations of the same hormones in environments with basic composition sensitive different salts.

In figures 1-2 are highlighted the results of the summary on the influence of the culture medium of the percentage of viable seed after pollinisation able to germinal seeds. Seed viability rate has been associated with the type of culture medium and with the size/age inoculated seed. Significant results have been reported in case inoculation in culture medium M2- liquid medium. The supplement of the culture medium, 10 mg/l indolilacetic acid, 0, 05 mg-biotin, 100 mg ascorbic acid and 100 mg citric acid led to favorable results. The lowest rate of viability has been recorded in the case of the culture medium M3 to all hybrid combinations. In the case of M3, that there is no citric acid and the role-ascorbic antioxidant is reflected on viability low during the period of the seed inoculation. By comparing seed growing in their two stages, it has been demonstrated that the seed excised-sample test results at 86 days after pollination survived in a percentage of more than. Differences in nutrition, growth regulators and the age seeds lead to differences in survivability and germination of embryos.

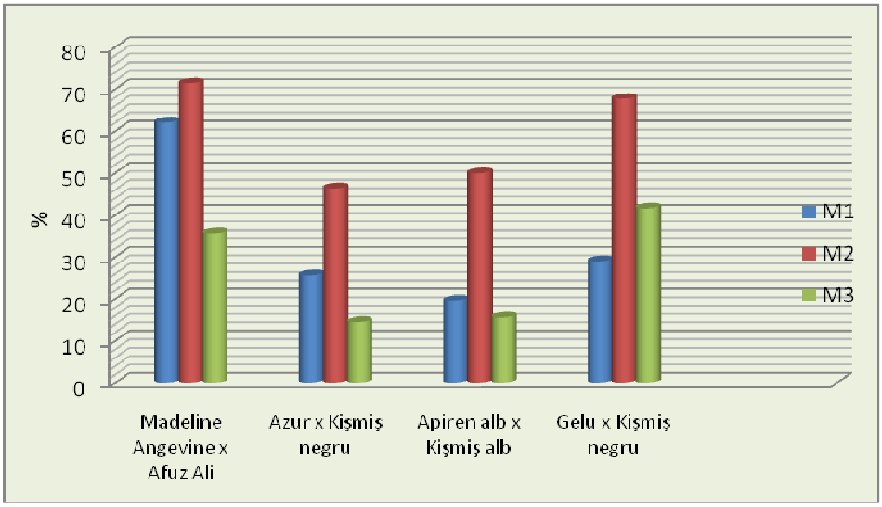

Fig. 1 - Influence of the culture medium of viability seeds harvested at 69 days after pollination

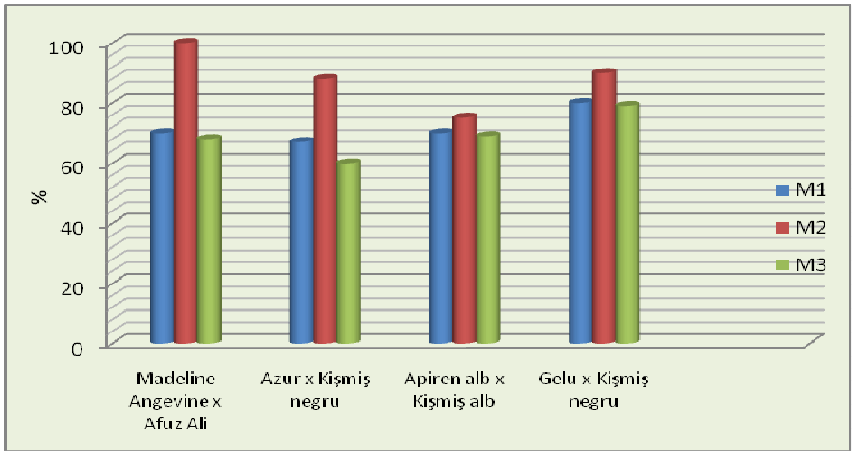

Fig. 2 - Influence of the culture medium of viabilty seeds harvested at 86 days after pollination

Increase in embryos has been obtained directly on solid medium. It was not noticed callus development. In each seed has been obtained from single seedlings; what zygotic indicating the origin of these. Of a total of 2322 immature seed grown have been obtained 189 embryos viable (8 %), of which only 38 have generated vitro plants (table 2). The rate of viability of the embryos and the generation vitro plants was slightly greater than the 1.6 - 3, 7 % of seeds harvested at 86 days after pollination. Significant differences between the number embryos that have developed of the four crossovers, it shows that an important factor is genitors who participate in crossing. In the case combination Kismis white x Seedless white, the transfer vitro plants are problematic due to their considerable size small. As to the other three hybrid combinations that have a parent with seeds, vitro plants have had a good development of cotyledons.

Table 2

Number of viable embryos obtained by growing seeds

Combination hybrid	Days after pollination	No. of seeds cultivated	Viable embryos		Vitro plants regenerate from embryos	
			No.	%	No.	%
Madeleine Angevine x Afuz Ali	69	420	32	7,6	7	21,9
	86	623	58	9.3	15	25,9
Azur x Kismis black	69	208	18	8,7	3	16,7
	86	193	15	7,8	2	13,3
Seedless white x Kismis white	69	152	8	5,3	0	0
	86	154	13	8,4	1	7,7
Gelu x Kismis black	69	288	20	6,9	4	20
	86	284	25	8,8	6	24

CONCLUSIONS

1. To avoid necrosis seed it is very important addition in the environment for cultivation of 100 mg/l, abscises acid and citric acid, substances with role antioxidant;

2. Use of the culture medium liquid provided with miss bridges of filter paper to highlight the rate of seed viability greater.

3. The culture medium and genotypes which participate in hybridize, the period of harvest of grain were influenced by embryo germination inoculate significantly.

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