

The Use of In Ovulo Embryo Culture for Cross-Breeding Studies of Empty – Seeded Table Grape Cultivars

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Abstract

This experiment was carried out to improve the efficiency of cross-breeding programs of two types of a Turkish empty-seeded table grape cultivar “Cavus” (Beyaz Cavus and Bozcaada Cavusu), and Cardinal, which is an introduced early ripening table grape cultivar, by use of in ovulo embryo culture. Sultani and Alphonse Lavallée were used as pollinizer varieties.

Fruit-set ratios of above-mentioned cross combinations ranged from 66.7% (Beyaz Cavus x A. Lavallée) to 42.0% (Bozcaada Cavusu x A. Lavallée). Ovules excised at 42 and 60 days after pollination (dap) were cultured on solid Nitsch, E20A and ½ MS supplied with growth regulators. Rates of the empty-seededness were found as 95.2% in Beyaz Cavus, 92.4% in Cardinal, and 84.3% in Bozcaada Cavusu as the averages of pollinizers.

Highest embryo germination ratios were determined from the ovules excised at 60 dap and cultured on Nitsch (33.3%) for Beyaz Cavus x A. Lavallée; on Nitsch (68.8%) for Beyaz Cavus x Sultani; on E20A (41.6%) for Bozcaada Cavusu x A. Lavallée; on E20A (55.6%) for Bozcaada Cavusu x Sultani; excised at 42 dap and cultured on E20A (33.3%) for Cardinal x A. Lavallée; on ½ MS (48.4%) for Cardinal x Sultani. E20A, ½ MS and Nitsch were found to be as the most convenient media considering embryo germination for Bozcaada Cavusu, Cardinal and Beyaz Cavus, respectively.

All embryos germinated were developed in whole plant in the ovules cultured on Nitsch and ½ MS 60 dap for Beyaz Cavus x A. Lavallée; on Nitsch and ½ MS 42 dap, and on E20A 60 dap for Bozcaada Cavusu x Sultani; on Nitsch 60 dap for Cardinal x Sultani. These values were followed by on ½ MS 60 dap (75%) for Cardinal x A. Lavallée and E20A 42 dap (66.7%) for Cardinal x A. Lavallée.

INTRODUCTION

Very low in vivo germination potential of the seeds of early ripening seeded table grape varieties, mostly having immature zygotic embryos, limits their usefulness in cross-breeding studies when they are used as female parent (Ramming 1990, Marasali and Celik 1994, Karli and Celik 1996). Recent studies indicated that in ovulo embryo culture can be used successfully to rescue the abortive zygotic embryos coming from cross-breeding studies by use of seedless or empty - seeded genotypes as female parent to obtain seedless and seeded table grape varieties (Cain et al. 1983, Spiegel-Roy et al. 1986, Bouquet and Davis 1989, Singh and Brar 1992, Gribaudo et al. 1993, Pommer et al. 1995, Carimi et al. 1997, Tangolar et al. 1999), crosses between *Vitis vinifera* x *Vitis rotundifolia* (Ramming et al. 2000), polyploid hybrids (Park et al. 1999), and somatic embryogenesis from zygotic or somatic tissues (Tsolova and Atanasov 1994, Kiikert et al. 1997). Present study was aimed to rescue the immature zygotic embryos of crosses between empty-seeded x stenospermocarpic and seeded table grape varieties by use of in ovulo embryo culture.

MATERIALS AND METHODS

Experiments were carried out between 1997 and 2001 at the Department of Horticulture, Faculty of Agriculture, University of Ankara.

Plant Materials

Two types of Cavus (Beyaz Cavus with spherical berries and Bozcaada Cavusu with elliptic berries) which is mid-early, mid-large berried, white Turkish table grape variety with functionally female flowers and empty seeds, and Cardinal which is an early, round and large berried red table grape variety with perfect flowers and empty seeds, were pollinated with Sultani which is a white, mid-season, elliptic and small berried stenospermocarpic Turkish grape variety with perfect flowers. It is the most important raisin and table grape variety in the world, also known as Sultana, Sultanina or Kishmish. Alphonse Lavallée which is a black, midseason, oblate-round and large berried seeded table grape variety with perfect flowers, also known as Ribier.

METHODS

1. Emasculation and Pollination. Emasculated and isolated clusters (8 on each vine with 100 pistils) of mother varieties were pollinated by above mentioned pollinizer varieties during anthesis for three consecutive days twice in a day.

2. In Vitro Culture of Fertilized Ovules. Fertilized ovules were excised at 42 and 60 days after pollination (dap) and in vitro cultured in solid media of Nitsch (NN69) supplemented with $1 \mu\text{M}$ GA₃ and $10 \mu\text{M}$ IAA, E20A supplemented with 0.01 ppm IAA and 1/2 MS (Murashige and Skoog 1962) supplemented with 10^{-5} M IAA and 10^{-6} M GA₃ for 12 weeks in a growth chamber at 16 h daylength and $26 \pm 2^\circ\text{C}$ temperature.

Embryos isolated from in vitro cultured ovules were also cultured in the same nutrient media, and germinated embryos with a few exact leaves were then transferred into 1/2 MS medium supplemented with 1.0 mg/L BAP as a final stage of in ovulo embryo culture.

3. Acclimatization of the Plantlets. In vitro cultured plantlets were transferred to the substrates of perlite and sterilized peat-moss mixture (1:1) in the plastic pots covered with polyethylene sheet to control the rapid water loss and grown at 16 h daylength and $26 \pm 2^\circ\text{C}$ temperature.

4. Parameters

1. Fruit set (%)
2. Empty-seededness (%)
3. Embryo germination (%)
4. Final take of intact plants (%)

RESULTS AND DISCUSSION

Fruit Set (%) and Empty- Seededness (%)

Fruit set and empty-seededness ratios of the female varieties ranged in the same order as 66.3% - 95.2% for Beyaz Cavus, 59.2% - 92.4% for Cardinal and 47.4 %-84.3 % for Bozcaada Cavusu (Table 1).

Embryo Germination (%)

As shown in Table 2, embryo isolation could not be achieved from the ovules excised at 42 dap on all three media, and 60 dap on E20A in Beyaz Cavus x A.Lavallée crosses; 42 dap on Nitsch and 1/2 MS in the same variety crossed with Sultani. Best results for embryo formation and germination were obtained from the ovules excised at 60 dap on Nitsch (68.8 %), E20A (46.7%) and 1/2 MS (38.9 %) in Beyaz Cavus x Sultani. In Bozcaada Cavusu, although embryo isolation was resulted in all combinations, only the embryos isolated from the ovules excised at 60 dap on E20A pollinated by Sultani (55.6 %) and A.Lavallée (41.6 %), on 1/2 MS (50.0 %) and Nitsch (33.3 %) pollinated by Sultani

germinated satisfactorily.

Embryo isolation was mostly succeeded excepting the ovules excised at 60 dap and cultured on Nitsch medium in Cardinal. Satisfactory results were also obtained from the embryos of the crosses of Sultani (48.4 % on ½ MS) and A.Lavallée (33.3 % on E20A) in the ovules excised at 42 dap, and Sultani (33.3 % on Nitsch) in the ovules excised at 60 dap.

Present data indicated that the effects of excision stage of the ovules on embryo germination changed with the genotypes and nutrient media.

Although the embryo germination performances of the ovules of Beyaz Cavus, excised at the later stage (60 dap) were notably higher especially in the crosses with Sultani on all three nutrient media, stated as in Aguero et al. (1995), but early stages (42 dap) can be regarded as more successful in Bozcaada Cavusu and particularly in Cardinal, more distinctive in the crosses with Sultani, as explained in Horuichu et al. (1991), excepting the late excised ovules cultured on E20A. Among nutrient media, Nitsch for Beyaz Cavus, E20A for Bozcaada Cavusu, ½ MS or E20A for Cardinal can be recommended in ovulo embryo culture studies.

Final Take of Intact Plants (%)

Experimental data indicated that germinated embryos were mostly grown to intact plants or rescued on ½ MS medium. All germinated embryos were rescued in the ovules excised at 60 dap and cultured on Nitsch or ½ MS for Beyaz Cavus x A.Lavallée, on E20 A for Bozcaada Cavusu x Sultani, and on Nitsch for Cardinal x Sultani; in the ovules excised at 42 dap and cultured on Nitsch or ½ MS for Bozcaada Cavusu x Sultani. Success in embryo germination of other cross combinations to form intact plants were also highly acceptable with a few exceptions, and it can be said that ½ MS medium supplemented with 1 mg /L BAP is a convenient medium for culturing the germinated grapevine zygotic embryos.

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Tables

Table 1. Fruit set and empty - seededness ratios (%)^{a)}

Cross combinations		Fruit set (%)	Empty seededness (%)
Female	Pollinazor		
Beyaz	A.Lavallée	67.6 a	98.7 a
Cavus	Sultani	65.0 a	91.6 b
Mean		66.3	95.2
Bozcaada	A.Lavallée	42.1 a	82.7 a
Cavusu	Sultani	52.6 a	85.8 a
Mean		47.4	84.3
Cardinal	A.Lavallée	66.5 a	97.1 a
	Sultani	51.8 a	87.7 a
Mean		59.2	92.4
Gen. Mean		57.6	90.6

a) LSD (P=0.05)

Table 2. Embryo germination ratio (%)

Medium	Beyaz Cavus				Bozcaada Cavusu				Cardinal			
	A.Lavallée		Sultani		A.Lavallée		Sultani		A.Lavallée		Sultani	
	42 ^{a)}	60 ^{a)}	42	60	42	60	42	60	42	60	42	60
Nitsch	NEF	33.3	NEF	68.8	NEG	NEG	33.3	NEG	NEG	NEF	NEG	33.3
E20A	NEF	NEF	22.2	46.7	NEG	41.6	NEG	55.6	33.3	NEG	7.1	NEG
½ MS	NEF	16.7	NEF	38.9	22.2	NEG	50.0	NEG	NEG	18.2	48.4	10.0
Mean	NEF	25.0	22.2	51.5	7.4	13.9	27.8	18.5	11.1	9.1	18.5	14.4

a) Stage of ovule excision as the days after pollination (dap)

NEF: No embryo formation in ovulo cultures

NEG: No embryo germination in embriyo cultures

Table 3. Final take of intact plants (%)^{a)}

Medium ^{b)}	Beyaz Cavus				Bozcaada Cavusu				Cardinal			
	A.Lavallée		Sultani		A.Lavallée		Sultani		A.Lavallée		Sultani	
	42	60	42	60	42	60	42	60	42	60	42	60
Nitsch	NEF	100.0	NEF	63.6	NEG	NEG	100.0	NEG	NEG	NEF	NEG	100.0
E20A	NEF	NEF	33.3	42.9	NEG	25.0	NEG	100.0	66.7	NEG	NIP	NEG
½ MS	NEF	100.0	NEF	42.9	50.0	NEG	100.0	NEG	NEG	75.0	53.3	NIP
Mean	NEF	100.0	33.3	49.8	50.0	25.0	100.0	100.0	66.7	75.0	26.7	50.0

a) Success in germinated embryos

b) All germinated embryos were cultured on ½ MS

NIP: No intact plant