BARD

FINAL REPORT

PROJECT No. US-338-80

Development and ^{adoption} Adaptation of New Techniques to
Increase the Efficiency of Grape Breeding with
Emphasis on Seedlessness and Early Ripening

D.W. Ramming, P. Spiegel-Roy

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Development and adoption of new techniques to increase
efficiency of grape breeding with emphasis on seedlessness and early-ripening.

Names of Investigators:

Principal Investigator: David W. Ramming

Cooperating Investigator(s): P. Spiegel-Roy

Name and Address of Affiliated Institutions:

Principal Institution: USDA-ARS, Fruit Genetics and Breeding Research,

2021 South Peach Avenue, Fresno, CA 93727

Cooperating Institution(s): ARO Volcanic Center, P.O. Box 6, Bet-Degan,

Israel

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Signatures:

Principal Investigator

David Ramming

Institution's Authorizing Official

W. G. Chase

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Cooperation

Reprints

Abstract

ARO - Inheritance of the seedless trait in Vitis vinifera was investigated. Stenospermocarpy seems to depend on two complementary recessive genes. About 2/3 of the hybrids bearing stenospermocarpic fruit have noticeable seed traces, while about 1/3 bear seedless fruit.

A method of in-vitro culture (in agar) and plant formation from cultivars with abortive ovules and seeds has been developed. Nearly all plants developed from seedless X seedless crosses and analyzed so far bear seedless fruit.

A method was developed to culture immature grape embryos of early-ripening cultivars. Two chemical methods for assessment of seed traces have been developed. Gibberellin sprays converted fruit of 4 hybrids out of 16 from the class with seed traces to seedless ones. Methods allowing for immediate germination after harvest have been developed.

Field treatment with a cytokinin and an antigibberellin was more effective than treatments in the greenhouse for increasing the percentage of hybrids flowering in response to treatment. An effective protocol for shortening the period from crossing to flowering enabled us to obtain up to 40% flowering two years after crossing, and up to 78% flowering hybrids three years after making the cross.

USDA - The percent progeny with the seedless trait (traces <20 mg FW) averaged 30-35% when seeded females were hybridized with seedless males. Thompson Seedless and Black Monukka were exceptions with 18 and 15% seedless. This still raises doubt if seedlessness is simply inherited.

A method to culture ovules from seedless genotypes and produce plants from the embryo has been developed. Over 700 seedless X seedless hybrid seedlings have been produced and planted in the field using this method.

A method for culturing seed from early-ripening genotypes that normally do not germinate was developed. Over 2,000 hybrid seedlings from early-ripening genotypes crossed with seedless have been produced and planted in the field using this method.

Greenhouse methods to produce flowering and fruiting of seedlings in 6 months on a large scale were not successful.

Objectives of the research.

- 1) To expedite and increase the efficiency of breeding for seedlessness by refining and implementing partially developed techniques of pre-selecting at an early stage in the greenhouse.
- 2) To develop in-vitro techniques for culturing ovules of normally abortive seeds to allow the use of 'seedless' phenotypes as female parents.
- 3) To adapt embryo culture techniques presently used in Prunus to permit culture of immature grape embryos from early-ripening genotypes.
- 4) To develop an adequate classification method to characterize stages of seed abortion using anatomical and quantitative techniques.
- 5) To measure genotypic and environmental variance of the seedless character.
- 6) To study the inheritance of seedlessness using crosses with (a) pistillate females, (b) hermaphroditic females, and (c) progenies with a seedless female parent crossed to seedless and seeded types.

I. Increasing breeding efficiency by developing preselection methods.

a) Greenhouse experiments.

ARO - Greenhouse facilities, enabling growth of hybrids, with drip irrigation, nutrient fertilization, supplementary illumination and modification of environmental conditions (humidity) have been set up. Progenies have been treated with PBA (6-benzylamino)-9-(2-tetrahydro-pyranyl)-9H purine), and with a combination of PBA and CCC (Cycocel). Seedlings of the cultivar French Colombard have responded by flowering about 8 months from seed germination. However, in order to achieve flowering in a large percentage of hybrids, modifications by the use of a more available cytokinin (such as benzyladenine), repeated sprays and lower plant density will be required.

USDA - Two spray treatments based on work by Mullins consisting of 1) 500 uM PBA spray the first day followed by 250 uM PBA sprays applied daily for 10 days, 2) 500 uM PBA + 3000 uM CCC spray the first day followed by 250 uM PBA sprays applied daily for 10 days, were applied in 1982. Some of the tendrils that emerged showed small undeveloped flowers at the tip, but they failed to bloom and fruit.

Applications of PBA to the roots of 6 week old Muscat Hamburg seedlings in 1985 did not induce flowering.

b) Field experiments.

ARO - Induction of early flowering by use of cytokinins and gibberellin antagonists.

In an experiment PBA (125 ppm) + CCC (400 ppm) and BA (150 ppm) + CCC (400) ppm (on different vines) were sprayed (15 May 1984) onto recently planted seedlings of a Muscat Hamburg X Perlette progeny. Twenty plants of each group were treated, with 100 plants of the same progeny used as a check. Results are given in Table 1.

Table 1. The effect of cytokinin + antigibberellin sprays on hastening flowering of field planted vines of Muscat Hamburg X Perlette progeny (hybrids planted 10 April 1984, sprayed 15 May 1984). Flowering checked during 1984 (ARO).

Treatment	Number of vines	Number of vines flowering during 1984	Percentage of vines with flowering
Check	100	5	5
Sprayed with 124 ppm PBA + 400 ppm CCC	20	10	50
Sprayed with with 150 ppm BA + 40 ppm CCC	20	4	20

In addition, open pollinated seedlings of Muscat Hamburg have been planted in the field in June 1985 and will be treated in 1986 with a combination of a cytokinin (PBA or BA) and an antigibberellin, paclobutrazol (PP 333) in order to hasten flowering.

c) Time table from seed to flowering.

ARO - Our present time table includes a three months cold stratification of wet seed. All lots are germinated with alternating temperatures (20-30°C) from December 1. Transplanting into larger pots in a heated greenhouse is done by the middle of January and planting in the field during May and June. Percentage of fruiting hybrids obtained, on the average, has been 40% the next year (flowering 2 years after performing the cross) with 78% flowering 3 years after performing the cross and 2 years after planting in the vineyard. Some further shortening of the cycle especially with early-ripening seed parents could perhaps be achieved by shortening or even dispensing with the chilling period (see IV of this report), planting in the field in winter (8-9 months after making the cross) and possibly by field application of certain chemicals (see I. b).

II. In-vitro culture and plant formation from cultivars with abortive ovules and seeds.

a) Seedless in-vitro ovule culture improvement experiments.

ARO - The development of a method for in-vitro culture and plant formation from grape cultivars with abortive ovules and seeds has been summarized in our attached publication in J. Amer. Soc. Hort. Sci. 110(1): 109-112, 1985. Analysis of progeny from hybrids that have fruited so far is given in Part VII of the report. Total number of seedling hybrids and selfs established in the vineyard up to fall 1985, from seedless genotypes as female parents is given in Table 2.

USDA - A method for in-vitro culture and plant formation from grape cultivars with abortive seeds has been reported in the attached publication, Amer. J. Bot. 71(6): 873-877, 1984. This showed plants could be produced from embryos in seedless grapes, but the % plants developed was low and not efficient for a breeding program. Analysis of progeny from the first plants of seedless genotypes (Thompson Seedless open pollinated and P60-58 open pollinated) is given in Part VII of the report.

Media improvement and determination for the best time to collect material for culture need to be defined better so additional experiments were performed.

To study the effect of date of culture, cuttings of Vitis vinifera cv. Flame Seedless (a stenospermocarpic seedless variety) were grown in the greenhouse according to Mullins and Rajasekaran (1981). Fertilized ovules from these plants were used. Ovules were taken 2, 4 and 6 weeks post anthesis, cultured on liquid modified Whites medium + 6% sucrose + 0.3% charcoal + 10 mM cysteine on a gyratory shaker (80 oscillations/min.). 125 ml flasks containing 50 ml media and filter paper support designed by Stewart

Table 2. Number of plants established in vineyard from seedless X seedless crosses. Count made October 1985 (ARO).

<u>Female parent</u>	<u>Male parent</u>	<u>Number plants</u>
Perlette	Perlette	13
L 12	L 12	3
Perlette	Flame Seedless	14
Flame Seedless	Perlette	28
L 12	Perlette	12
L 12	Flame Seedless	12
L 12	Sultanina	5
Perlette	O.P.	23
Flame Seedless	O.P.	64
Sultanina	O.P.	40
L 12	O.P.	93

and Hsu (1978) were used. One half of the cultures were evaluated 2 and 3 months after bloom for the number of ovules containing an embryo and the size of the embryos. The remaining 1/2 were stratified for 45 days in the original medium after being cultured for 2 or 3 months and then evaluated. One flask containing 20 ovules was used per treatment. When embryos were found they were transferred to 20 x 150 mm test tubes on filter paper wicks with the above medium. The 2 and 3 month culture period, with or without stratification, showed no difference for the number of ovules containing embryos. However, combined totals for the 2, 4 and 6 week collection dates totaled 4/60, 12/80 and 5/40 ovules with embryos/ovules cultured respectively. It appears 4 and 6 weeks post anthesis is better than 2 weeks post anthesis. Ovules were also taken 2, 4, 6, 8 and 10 weeks post anthesis from the field and cultured 2, 3 and 4 months (2 flasks per treatment with 20 ovules/flask).

In Thompson Seedless starting at 4 weeks past bloom greater numbers of embryos were found in-vitro equal to or greater than .5 mm in length (Table 3). In P60-58 many embryos were found in-vitro equal to or greater than .5 mm starting 6 weeks past bloom (Table 4). At 6 weeks post bloom P60-58 has larger abortive ovules than Thompson Seedless in-vivo and after culture P60-58 had a higher percentage of ovules with embryos. The larger ovule of P60-58 when grow in-vivo may offer more protection for the embryo and allow embryos to remain viable at later dates and grow in culture. It appears that the longer an embryo is in culture, the larger they become. However, what seems to be more important is ovule maturity at the time of culture. It appears that our culture medium and system supports minimal embryo growth in ovules cultured 2 weeks past bloom. Beginning at 4 weeks past bloom in Thompson Seedless, we see a change and are able to obtain a greater number of

Table 3. The number and size of embryos produced after 2, 3 and 4 months in vitro ovule culture from Thompson Seedless collected 2, 4, 6, 8 and 10 weeks post anthesis. (USDA).

Treatment	% Ovules with embryos ²	Avg. embryo length (mm)	No. embryos <.5 mm	No. embryos >.5 mm
<u>2 wks. Post Bloom</u>				
2 mos. culture	2.5	.3	1	0
3 " "	7.5	.2	3	0
4 " "	22.5	3.0	0	9
<u>4 wks. Post Bloom</u>				
2 mos. culture	42.5	.5	8	9
3 " "	17.5	1.2	2	5
4 " "	85.0	6.7	0	34
<u>6 wks. Post Bloom</u>				
2 mos. culture	27.5	.9	2	9
3 " "	25.0	2.4	0	10
4 " "	27.5	3.5	0	11
<u>8 wks. Post Bloom</u>				
2 mos. culture	30.0	.9	2	10
3 " "	27.5	1.8	0	11
4 " "	20.0	7.8	0	4
<u>10 wks. Post Bloom</u>				
2 mos. culture	22.5	1.0	1	8
3 " "	5.0	1.8	0	2
4 " "	12.5	11.9	0	5

² N = 40

Table 4. The number and size of embryos produced after 2, 3 and 4 months in vitro ovule culture from P60-58 collected 2, 4, 6, 8 and 10 weeks post anthesis. (USDA).

Treatment	% Ovules with embryos ^z	Avg. embryo length (mm)	No. embryos < .5 mm	No. embryos > .5 mm
<u>2 wks. Post Bloom</u>				
2 mos. culture	0	0	0	0
3 " "	0	0	0	0
4 " "	17.5	4.4	1	4
<u>4 wks. Post Bloom</u>				
2 mos. culture	2.5	1.0	0	1
3 " "	12.5	.4	3	2
4 " "	7.5	.6	1	2
<u>6 wks. Post Bloom</u>				
2 mos. culture	32.5	.7	7	6
3 " "	35.0	1.2	4	8
4 " "	22.5	1.4	0	9
<u>8 wks. Post Bloom</u>				
2 mos. culture	27.5	.5	5	6
3 " "	27.5	1.2	0	11
4 " "	15.0	1.3	0	6
<u>10 wks. Post Bloom</u>				
2 mos. culture	32.5	1.3	3	10
3 " "	22.5	3.4	2	7
4 " "	30.0	3.6	0	12

^z N - 40

embryos and also larger embryos. The data seems to indicate many in-vivo embryos are viable and continue to develop well into their 4th week past bloom on the vine. These same embryos when subcultured in our medium and culture system continue to enlarge. The optimum culture date for Thompson Seedless was 4 weeks past bloom, but P60-58 did equally well 6, 8 and 10 weeks past bloom. Time in culture was only significant for the 2 week past bloom culture date when 3 months in culture produced 22.5 and 17.5% ovules for Thompson Seedless and P60-58 respectively.

A number of experiments to refine the nutrient medium to achieve greater embryo development and a higher % of plants from seedless genotypes were performed. In 1982, fertilized ovules from Thompson Seedless and P60-58 grown in the greenhouse were cultured 4 weeks post anthesis on liquid medium. The following addenda were added individually: IBA (Indole butyric acid) (4, 8, 12 ppm), Ergostin (.25, .5, .75%), arginine (1.0, 10.0, 15.0 mM), cysteine (1.0, 10.0, 15.0 mM), and tartaric acid (0.5, .1, .2%). Ovules were cultured for 3 months and evaluated for number of ovules containing embryos and for size of embryo. The IBA, Ergostim and arginine treatments were not significantly different in number or size of embryos from the basal medium. The best treatment was 0.1% tartaric acid (significant at 1% level from basal medium) with 7 embryos/20 ovules, but 0.05% + 0.2% tartaric acid were not significantly different from the basal medium. Cysteine at 1.0 and 15.0 mM were significantly different (at 5% level) from the basal medium. Cysteine at 10.0 mM was not significantly different from the basal medium or from 1.0 and 15.0 mM level. Cysteine appears beneficial and will be used routinely as an additive to the medium.

The development of shoots and plants from embryos after the 3 month ovule culture has been difficult, resulting in low % plants produced from

embryos. In 1983 embryos from seedless genotypes were subcultured after the initial 3 month ovule culture period on media containing 1 ppm zeatin, 1 ppm benzyl adenine or 360 mg/l ammonium nitrate. Less than 6% embryos produced shoots in the cytokinin treatments, while 33% of the embryos treated with ammonium nitrate had shoots, compared to 6% in the control (n=15). This preliminary test seems to indicate the importance of the nitrogen level for shoot development.

b) Survey of seedless genotypes in culture.

USDA - In 1983 a number of ovules from seedless genotypes were cultured to determine their potential for embryo enlargement and plant development. Embryos developed in all but two genotypes. The percent ovules with embryos after culture ranged from 0 to 74% (Table 5). This survey provided valuable information in 1984 and 1985 for the selection of seedless genotypes to consider and use as female parents in the breeding program.

c) Seedless X seedless crosses cultured and plants produced.

ARO - Number of hybrid plants between seedless genotypes as well as selfs established so far from in-vitro culture in 1985, and transplanted either in pots in the greenhouse or placed in the incubator (in a 10.5 cm x 8 cm container) are given in Table 6. A further number of less developed hybrids in-vitro (mainly from late-ripening seedless cultivars as female parents) are still in the first stage, before transplanting into a larger container. Some reciprocal crosses between seeded and seedless cultivars have been made in 1985 (with seedless parent as female parent). The list of the few plants established is given in Table 7.

USDA - Crosses using seedless genotypes as the female have been made in 1983, 1984 and 1985. In 1983 Thompson Seedless and P60-58 seedless genotypes were hybridized with seedless, seeded (see Part VII) and V. labrusca (Table

8). A reflex seedless genotype, P79-101, was hybridized with a homozygous perfect flowered genotype, C32-68. Twenty-three plants have been planted in the field and will fruit in 1986. In 1984, 14 seedless X seedless crosses were made (Table 9) and 15 crosses with seeded males were made for an inheritance study (Table 10). As a result 604 plants from the seedless X seedless crosses were planted in the field. The remaining 120 were plants for the inheritance study and were planted in the field (see Part VII). In 1985, 19 seedless X seedless crosses were made and 7971 ovules cultured, resulting in 1991 embryos, which were subcultured (Table 11). The crosses for the seedless inheritance study were repeated and 9113 ovules cultured from which 1788 embryos developed and were subcultured (Table 12). It will be very interesting to observe the % seedlessness in the progeny. This information will be helpful in determining the inheritance of the seedless trait.

Table 5. The number of ovules cultured, embryos developed, size of embryo and plants planted in the field from seedless genotypes cultured in-vitro in 1983 (USDA).

Genotypes	Seed trace wt. (mg FW)	No. ovules cultured	No. embryos developed (%)	Average embryo size	No. ovules with multiple embryos	No. plants planted in the field
A73-150	11.55	55	26 (48)	1.49	7	
A74-61	15.2	64	16 (25)	1.30	8	
A8-2	1.64	23	0			
B1-68	2.24	37	14 (38)	0.61	0	
B18-185	14.44	68	26 (39)	2.22	0	1
B29-82	9.66	30	10 (34)	2.3	0	
B31-164 Arvin	-	62	29 (47)	1.42	0	
B31-164 CSUF	20.78	58	30 (52)	1.49	6	
B44-2	9.15	62	16 (26)	2.31	0	
B46-112 Self	11.17	74	51 (69)	9.45	1	2
B5-15	4.70	60	23 (39)	1.38	0	
B73-167	-	61	24 (40)	2.65	4	
B74-198	-	60	44 (74)	1.40	10	
C18-36	21.10	52	1 (2)	.10	0	
C20-149	15.52	40	3 (8)	0.67	0	
C22-121	4.59	34	13 (39)	7.65	0	
C29-171	42.31	53	22 (42)	1.54	0	9
C33-199	4.79	23	0 -			

Table 5. (Continued) The number of ovules cultured, embryos developed, size of embryo and plants planted in the field for seedless genotypes cultured in-vitro in 1983 (USDA).

Genotypes	Seed trace wt. (mg FW)	No. ovules cultured	No. embryos developed (%)	Average embryo size	No. ovules with multiple embryos	No. plants planted in the field
C35-33	10.51	60	24 (40)	1.90	0	
C58-22	-	74	10 (14)	4.5	0	
C67-7	1.73	67	33 (50)	1.91	1	1
C76-79	7.07	68	9 (14)	0.78	1	
C84-116	-	46	13 (29)	0.83	0	
C85-82	-	64	37 (58)	2.09	7	
C88-89	8.8	55	13 (24)	2.04	2	
C89-173		66	26 (40)	1.88	3	
Fiesta		57	23 (41)	0.78	2	
Flame Seedless	2.24	63	21 (34)	3.12	1	
P60-58	7.80	61	31 (51)	3.47	1	
Thompson Seedless	1.42	56	12 (22)	10.60	1	

Table 6. Number of germinating plants from seedless X seedless crosses performed in 1985 (ARO).

<u>Female parent</u>	<u>Male parent</u>	<u>Number germinating hybrids</u>
L 12	Flame Seedless	127
L 12	Perlette	100
L 12	Centennial	96
L 12	Ruby Seedless	66
L 12	Sultanina	21
Perlette	L 12	170
Perlette	Flame Seedless	57
Flame Seedless	L 12	30
Flame Seedless	Perlette	8
Sultanina	Flame Seedless	16
L 12	Black Kishmish	8

Table 7. Number of germinating plants from seedless (♀) X seeded (♂) crosses performed in 1985 (ARO).

<u>Female parent</u>	<u>Male parent</u>	<u>Number germinating hybrids</u>
L 12	Shaharit	16
L 12	Dan Ben Hana	4

Table 8. The crosses made, ovule cultured, embryos developed and plant planted in the field for seedless culture 1983. (USDA).

Parentage (♀ x ♂)	Type	No. ovules cultured	No. embryos developed (%)	Average embryo length (mm)	No. plants to the field
T.S. x Petite Sirah	Seedless X seeded	25	0 (0)	-	0
T.S. x B4-120	Seedless X seeded	459	45 (9.8)	6.47	1
T.S. x B5-15	Seedless X Seedless	186	11 (5.9)	1.2	0
T.S. x B4-86	Seedless X Seedless	420	39 (9.3)	1.70	8
T.S. x Venus	Seedless X Seedless Marker	33	0 (0)	-	0
T.S. x Concord	Seedless X Seeded Marker	130	40 (30.8)	1.03	4
P60-58 x Petite Sirah	Seedless X Seeded	81	25 (30.9)	1.09	0
P60-58 x B4-120	Seedless X Seeded	210	34 (16.2)	0.75	12
P60-58 x B5-15	Seedless X Seedless	215	59 (27.4)	0.78	1
P60-58 x B4-86	Seedless X seedless	147	45 (30.6)	0.77	0
P60-58 x Venus	Seedless x Seedless Marker	89	24 (27.0)	0.96	0
P60-58 x Concord	Seedless x Seeded Marker	77	7 (9.1)	0.55	0
P79-101 x C32-68	Seedless X Seedless	2,003	317 (15.8)	-	23
Total		4,075	647 (21.3)		49

Table 9. The crosses made, number ovules cultured, embryos developed and plants planted in the field for seedless culture in 1984. (USDA).

Parentage ♀	♂	No. emasculations	No. ovules cultured	No. embryos developed (%)	No. plants to the field
C67-7	B1-68	1930	508	242 (48)	0
C67-7	C76-104	2329	7	2 (29)	0
B46-112	C18-36	1525	409	256 (63)	29
B46-112	C76-104	2952	235	111 (47)	0
B31-164	C78-68	2954	59	7 (12)	15
B31-164	C76-104	1371	7	0 (0.0)	0
B31-164	C20-164	1575	27	4 (15)	5
C85-82	B1-68	2166	122	42 (34)	5
C85-82	C20-149	2044	510	216 (42)	56
A71-185	C32-68	-	514	209 (41)	125
P79-101	Flame Seedless	-	522	265 (51)	129
P79-101	C33-199	-	506	151 (30)	50
P79-101	C35-33	-	674	408 (61)	147
P79-101	B31-164	-	257	135 (53)	43
Total			4357	2048 (48)	604

Table 10. The crosses made, number ovules cultured, embryos developed and plants planted in the field for seedless culture in 1984 for inheritance study. (USDA)

Parentage	♀	♂	No. emasculations	No. ovules cultured	No. embryos developed (%)	No. plants to the field
Fl. Seedless		B18-185	2097	662	297 (45)	15
Fl. Seedless		A81-110 Seeded	2800	927	367 (40)	39
Fl. Seedless		C15-162 Seeded	1916	308	113 (37)	17
Fl. Seedless		T.S.	2618	300	87 (29)	18
Fl. Seedless		C78-53 Seeded	2424	242	34 (14)	10
B18-185		Fl. Seedless	2228	93	26 (28)	0
B18-185		A81-110 Seeded	1950	38	4 (11)	0
B18-185		C15-162 Seeded	2264	16	5 (31)	0
B18-185		T.S.	2101	80	21 (26)	8
B18-185		C78-53 Seeded	2027	202	50 (25)	3
T.S.		Fl. Seedless	2973	200	26 (13)	0
T.S.		A81-110 Seeded	2564	407	20 (05)	4
T.S.		C15-162	2186	189	42 (22)	3
T.S.		C78-53 Seeded	2400	238	29 (12)	3
T.S.		B18-185	2400	600	34 (6)	0
Total				4,302	1,155 (27)	120

Table 11. The crosses made, number ovules cultured, and embryos developed for seedless culture in 1985. (USDA).

Parentage		No. emasculations	No. ovules cultured	No. embryos developed (%)	No. ovules containing multiple embryos
♀	♂				
C67-7 x B1-68		1746	432	123 (29)	3
C67-7 x C49-38		2144	420	139 (33)	17
C67-7 x A73-150		1783	357	114 (33)	15
B46-112 x C49-165		1593	433	193 (45)	27
B46-112 x B1-68		1407	940	392 (42)	77
B46-112 x C76-104		1350	400	131 (33)	19
C23-92 x A74-61		1620	81	9 (11)	4
C23-92 x C49-38		1780	210	21 (10)	2
C23-92 x C80-155		1471	185	17 (09)	1
C78-68 x C20-120		1460	334	82 (25)	17
C78-68 x C87-147		1724	624	193 (16)	34
C78-68 x C20-149		1487	350	8 (24)	12
C78-68 x C102-26		1776	270	52 (19)	5
A73-150 x C18-36		1180	518	110 (21)	5
A73-150 x C36-145		1134	255	77 (30)	30
A73-150 x B4-175		1215	330	79 (24)	6
A73-150 x C67-7		1020	72	11 (15)	0
B73-215 x B46-112		-	1148	132 (11)	5
B73-215 x C17-120		-	612	108 (18)	24
Total			7971	1991	

Table 12. The crosses made, ovules cultured, embryos developed and plants planted in the field for seedless culture in 1985. (Inheritance study). (USDA).

Parentage	No. emasculations	No. ovules cultured	No. embryos developed (%)	No. ovules containing multiple embryos
♀ ♂				
T.S. x C85-82	2617	698	34 (5)	2
T.S. x F.S.	2018	698	24 (3)	6
T.S. x A81-110 Seeded	1900	494	9 (2)	1
T.S. x C15-162 Seeded	2200	510	4 (3)	2
T.S. x C78-53 Seeded	1908	36	2 (6)	
T.S. x Self	-	1019	13 (1)	6
F.S. x T.S.	2035	546	166 (30)	18
F.S. x C85-82	2276	868	181 (21)	24
F.S. x A81-110 Seeded	1739	448	72 (19)	5
F.S. x C78-53 Seeded	1930	584	104 (18)	4
F.S. x C15-162 Seeded	2076	223	29 (13)	
F.S. x Self	-	609	87 (14)	6
C85-82 x T.S.	1591	264	104 (39)	11
C85-82 x F.S.	1982	808	376 (46)	79
C85-82 x A81-110 Seeded	1793	383	123 (32)	36
C85-82 x C78-53 Seeded	1880	311	187 (62)	67
C85-82 x C15-162 Seeded	1463	434	248 (57)	24
C85-82 x Self	-	180	25 (14)	2
Total		9113	1788	

III. In-vitro culture of seed from early ripening genotypes.

ARO - Abstract. A method was developed to culture immature grape embryos of early-ripening Vitis vinifera genotypes. Sivan and Cardinal seed germinated on Orchid agar 28 and 23 percent, respectively, compared to 8 and 9 percent in-vivo. Differences between male pollenizers were significant. No significant differences in favor of adding daminozide or GA₃ to the medium were found.

In-vitro methods have been used in order to culture immature embryos of many species (Turkey 1933, 1934, Maheshwari, 1958; Ramming, 1983; Collins, 1985). Grape seed from early-ripening genotypes has a low percentage of germination. Germination in-vivo of such genotypes is also highly inconsistent and cannot be depended upon to yield large progenies. Balthazard (1977) has shown that the threshold range for successful germination is when the embryo reaches a minimum length of 1 mm. Inadequate embryo size does not enable or at least, severely restricts use of early-ripening cultivars as female parents in a breeding program. This is even more of a problem in breeding stenospermocarpic cultivars, in which the latter are generally used as male parents. Ramming and Emershad (1984) have recently reported on the successful culture of early-ripening genotypes in the grape. Seed germination has been increased from 7 to 16 percent, on the average. Our work reported here was performed by culturing still earlier ripening genotypes.

Materials and methods.

Seed of Sivan and Cardinal have been removed close to maturity. Berries were sterilized in 1% sodium hypochlorite for 10 minutes, then rinsed 3 x with sterile water. Embryos were placed in 5 cm diameter Petri dishes containing Orchid agar without the addition of growth substances. No attempt was made

to separate floaters from sinkers. Four replications of 10 embryos per dish were used. Medium was adjusted to pH 5. Petri dishes were placed in an incubator at 26°C, 16 h daily illumination. Seed from the two cultivars was obtained by pollinating emasculated flowers with pollen of two seedless genotypes, Flame Seedless and a selection, L 12. A further variable included incorporation of either GA₃ at 10⁻⁶M, or daminozide (500 ppm) in the medium. Seeds were chilled in the Petri dish for 3 weeks (August 9-26, 1984) at 4°C before being placed in an incubator at 26°C.

For in-vivo germination rate, seeds of Sivan and Cardinal were placed in a volcanic tuff: perlite mixture (1:1 v/v) in a heated greenhouse (30°C day, 20°C night) and germination percentage noted. In the in-vivo germination test, only sinkers (usually seed with endosperm) were employed. This procedure very probably enables one to obtain higher germination rates in vivo than the rate that would have been obtained by using both sinkers and floaters, as the latter are mostly devoid of endosperm.

In-vitro germinating embryos that developed into seedlings were transplanted first in an incubator and later brought into the greenhouse by methods used in our laboratory for transplanting plantlets from in-vitro culture of abortive ovules from stenospermocarpic cultivars (Spiegel-Roy et. al. 1985).

Results show an appreciable increase in germination by the in-vitro method using Orchid agar medium compared to the low percentage of germination obtained in-vivo with both cultivars, Sivan and Cardinal. Use of daminozide in the medium seemed to result in a higher rate of germination compared to control (Orchid agar without daminozide) in 3 out of 4 cases. However, the effect of treatment according to the factorial analysis, has been found insignificant (Table 13).

Table 13. Percent germinating embryos from culturing undeveloped seed of early-ripening table grape cultivars Sivan and Cardinal. (ARO).

Sivan: pollinated 5/2/84, seed cultured 7/25/84.

Cardinal: pollinated 4/29/84, seed cultured 7/25/84.

Orchid agar medium.

<u>Genotype</u>	<u>Male parent^x</u>	<u>Treatment^y</u>	<u>Germinating embryos (%)^z</u>
Sivan	1 ^x	a	42
"	1	b	46
"	1	c	26
"	2	a	28
"	2	b	27
"	2	c	32
Cardinal	1	a	38
"	1	b	20
"	1	c	28
"	2	a	21
"	2	b	16
"	2	c	17

^x1 = Flame Seedless

^x2 = L 12 Seedless

^ya = daminozide 4×10^{-3} M in medium.

^yb = GS₃ 10^{-6} in medium.

^z = Percentage germination in vivo: Sivan 8.0 ± 2.5
Cardinal 9.0 ± 3.1

Factorial analysis. Interaction NS.

	<u>DF</u>	<u>F</u>	<u>PR F</u>
Genotype	1	4.70	0.0374
Male parent	1	5.08	0.0310
Treatment	2	1.04	0.3655

USDA - Abstract. Three early-ripening seeded grape genotypes, Early Muscat, C64-97 and C66-151, were hybridized with seedless genotypes in 1983. Their germination was increased from 0, 7 and 14% in the soil to 8, 18 and 22% respectively by culturing in-vitro. From the seed that did not germinate, 633, 67 and 850 embryos were excised and subcultured, resulting in 164, 15 and 137 plants that were planted in the greenhouse. This culture procedure gave a total germination of 22, 25 and 30% respectively. Seed without endosperm from Early Muscat, C64-97 and C66-151 were also cultured and contained 25, 12 and 34% embryos respectively. These seed are normally discarded because they do not germinate in soil. When the embryos were subcultured they produced 7, 0 and 10 plants (13, 0 and 7%) respectively. This shows in-vitro culture increases germination of early-ripening genotypes and is useful for breeding programs. (This result is from abstract published in HortScience).

A major problem in the development of early-ripening grapes is the poor germination of early-ripening seeded genotypes used as females. This same phenomenon occurs in cherries as described by Tukey (1933). He suggests and uses embryo culture to overcome the problem. Ovule culture has been used successfully in cotton interspecific crosses (Steward and Hsu, 1978) to recover plants from immature seed that do not germinate in soil. Techniques similar to this would be useful in facilitating the production of progeny from early X early-ripening grape crosses and the development of better early-ripening grape cultivars.

Materials and methods:

Three early-ripening genotypes (Early Muscat, C64-97 and C66-151) were hybridized with seedless genotypes. The fruit was harvested at maturity, surface sterilized with 70% ETOH for 1 minute and 10% chlorox for 5 minutes

and the seed extracted aseptically. Seeds were separated into sinkers and floaters, which indicates the presence or absence of endosperm. The seed with endosperm was cultured in Cain's medium which consists of White's 1954 macroelements plus CaNO_3 (600 mg/l), KNO_3 (160 mg/l) and Norstrog's 1973 microelements and vitamins, plus glycine (3 mg/l), Casein Hydrolysate (50 mg/l), Myo-inositol (50 mg/l), 2% sucrose, 0.3% activated charcoal and 0.6% agar. Individual seed were cultured in 20 x 150 mm test tubes containing 10 ml medium. The seeds were then stratified for 45 days and allowed to germinate at room temperature. As the strong seed germinated they were planted in the greenhouse. Seed (sinkers) germination in 15 x 125 mm Petri dishes with moist filter paper was used to estimate seed germination in-vivo. Seed not germinated or contaminated after 45 days were cut open and the embryo subcultured on Cain's medium without agar. Twenty embryos were cultured in 125 ml Erlenmeyer flasks on filter paper supports with 50 ml medium. As the embryos produced shoots they were planted in the soil.

We have found that seed without endosperm (floaters) can also contain embryos. These were also cultured at fruit maturity on CM without agar on filter paper in 125 Erlenmeyer ml flasks for 3 months at room temperature, after which they were cut open and the embryos were subcultured on the same medium.

Results:

The in-vitro culture of Early Muscat, C64-97 and C66-151 greatly enhanced the germination and production of plants from these early-ripening genotypes. The percent germination for the sinkers in the test tubes was 8.1, 17.9 and 21.8%, while only 0, 7 and 14% respectively germinated in the Petri dishes on moist filter paper (Table 14). The seed that did not germinate contained 72.9, 69.8 and 68.9% embryos respectively (Table 15). From these embryos 164, 15 and 137 plants grew and were transplanted to the

Table 14. The number seed cultured and germinated in test tubes and plants developed compared to standard germination procedure. (USDA).

Genotype	# Seed	# Germinated (%) in test tube	# Planted in field	% Germinated in Petri dish
Early Muscat	1,264	102 (8.1%)	41	0%
C64-97	212	38 (17.9%)	34	7%
C66-151	1,737	379 (21.8%)	308	14%

Table 15. The number seed not germinated containing embryos and the number plants produced when they were subcultured. (USDA).

Genotype	# Seed not germinated	# Embryos subcultured	# Plants to greenhouse	# Planted in field
Early Muscat	922 (72.9%)	633 (71.9%)	164 (24.7%)	138
C64-97	148 (69.8%)	67 (45.3%)	15 (22.4%)	7
C66-151	1,197 (68.9%)	850 (71.0%)	137 (16.1%)	107

Table 16. The number of seed without endosperm (floaters) containing embryos after culture and the number of plants produced from embryos subcultured. (USDA).

Genotype	# Floaters cultured	# Embryos (%) subcultured	# Plants to greenhouse
Early Muscat	221	56 (25.3%)	7
C64-97	139	17 (12.2%)	0
C66-151	424	143 (33.7%)	10

greenhouse and those that survived acclimation were planted in the field for fruit evaluation. The total germination of the seed and embryos was 21.8, 25.0 and 29.7% respectively. This is significantly greater than seed germinated in-vivo. The medium used for embryo culture seems to induce greater root growth than shoot growth. More plants might be produced if better shoot development could be induced.

The 221, 139 and 424 floaters that were cultured contained 25.3, 12.2 and 33.7% embryos after three months culture (Table 16). When subcultured, 7, 0, and 10 plants were produced and transplanted to the greenhouse. This shows that even floaters that have embryos can be grown into plants. It will be interesting to determine if plants from these floaters average more seedless progeny. The cost benefit of culturing floaters to produce progeny compared to the cost of just making additional emasculations and pollinations also need to be determined.

References:

Stewart, J. and C. Hsu. 1978. Hybridization of diploid and tetraploid cottons through in-ovulo embryo culture. J. Hered. 69: 404-408.

Tukey, J. B. 1933. Artificial culture of sweet cherry embryos. J. Hered. 24: 7-12.

The optimum time to collect seed for culture needed to be better defined as well as determining if improvements could be made in the culture medium. Additional experiments were performed.

In 1982, three early-ripening genotypes (P59-150, B4-120 and A42-80) and Red Malaga, a mid-season variety with high % germination, were cultured 6 and 10 weeks post anthesis to determine the optimum time to collect seed for culture and to see if a pre-stratification culture period would be beneficial. The seeds were cultured in 20 x 150 mm test tubes containing

10 ml modified Whites medium + 0.3% charcoal + 10 mM cysteine x 0.4% Difco Bacto agar. The % sucrose and culture treatments were varied as follows:

- 1) Cultured @ 80°F for 2 months on 6% sucrose and 45 days @ 33°F on 2% sucrose and germinated on same.
- 2) Cultured @ 80°F for 2 months on 2% sucrose and 45 days @ 33°F on 2% sucrose and germinated on same.
- 3) Cultured for 45 days @ 33°F on 6% sucrose and germinated on 2% sucrose.
- 4) Cultured for 45 days @ 33°F on 2% sucrose and germinated on same.
- 5) Stratified in plastic bags and germinated on 2% sucrose.

In all treatments no germination occurred after 1 month. The high L-cysteine level was suspected as the cause of inhibition of germination. When germination did not occur, half the seeds were cut open and the number of embryos present and their lengths were recorded (Table 17). Culturing on 2% sucrose at 10 weeks past bloom and immediately chilling them at 33°F resulted in large embryos with the greatest percentage of seed possessing embryos. This treatment and time of culture with the deletion of L-cysteine from the medium is now being used to culture early-ripening seeded grapes in our breeding program.

In 1983, seed of early-ripening genotypes from fruit grown in the greenhouse were cultured at verasion and maturity. Seed cultured the first date (28 days after bloom = verasion) which were scarified had a greater percent of seeds with endosperm and embryos than not scarified. Red Malaga and B4-120 contained 30 and 20% seed with embryos and 80 and 37% with endosperm respectively and Cardinal contained 73% seed with endosperm. Embryo size was not increased. Non-scarified seed contained 3 and 7% embryos for Red Malaga and B4-120, respectively. Seed cultured the second

Table 17. The percent germination, number of seed with endosperm and embryos, and embryo length from scarified and non-scarified seed cultured July, 21, 1983 after 3½ months culture. (USDA).

	Sinkers		Floaters	
	- ^z	+	-	+
<u>Red Malaga</u>				
% with endosperm	95	100	50	100
% with embryo (avg. length)	90(1.8)	75(2.3)	50(1.7)	40(2.8)
% Germination	0	15	0	40
<u>A8-158</u>				
% with endosperm	95	100	35	80
% with embryo (avg. length)	50(2.0)	90(4.5)	25(1.4)	70(4.5)
% germination	0	0	0	0
<u>C55-37</u>				
% with endosperm	100	100	20	84
% with embryo (avg. length)	85(1.8)	78(5.1)	80(1.1)	74(4.9)
% germination	0	0	0	0
<u>Total</u>				
% with endosperm	97	100	35	88
% with embryo (avg. length)	75(1.8)	76(4.1)	52(1.4)	71(4.3)
% germination	0	5	0	17

^z - = no scarified, + = scarified

date (maturity) showed no difference in percent seed containing embryos or endosperm for scarified and non-scarified or for two different sucrose concentrations. However, embryos seemed to enlarge more in scarified seeds. Seed without endosperm were also cultured and embryos did enlarge.

In 1983 Seed (sinkers and floaters) were cultured from the field to see if the results were similar to that obtained from the greenhouse material. Three genotypes, including Red Malaga, were collected July 21 and cultured in liquid Cain's medium for $3\frac{1}{2}$ months before germination and embryo size was measured (Table 18). Embryos developed more when seeds were scarified (4.09 and 4.32 mm) than when not scarified (1.84 and 1.37 mm). Germination occurred only from scarified sinkers and floaters. This shows that scarifying seed has a beneficial influence on embryo development and seed germination if no stratification is given.

b) Early-ripening seeded X seedless crosses cultured and plants produced.

USDA - In 1982 four early-ripening genotypes were hybridized with seedless genotypes and 96, 263, 308 and 155 seed cultured in-vitro. Their germination in Petri dishes (similar to germination in soil) was 30, 26, 22 and 12%. As a result of in-vitro culture, 27, 57, 26 and 34% of the seed cultured grew into plants and were planted in the field in 1983.

In 1984, six early-ripening genotypes were hybridized with seedless grapes. Twenty-seven cross combinations were made, resulting in 3104 seed that were cultured (Table 19). Cain's medium solidified with 0.6% agar and 10 mM cysteine was used. The cysteine seemed to inhibit seed germination and was deleted from the medium in 1985 when NH_4NO_3 (360 mg/l) was added. The seed generally germinated better in-vitro than in-vivo. Embryos from ungerminated seeds were excised and subcultured on liquid Cain's medium.

Table 18. The percent seeds containing embryos and average embryo length of seed of early-ripening genotypes 30 days after seeds put at 20°C to germinate.
(USDA).

Treatment	% seed with embryos	Avg. embryo length (mm)
<u>Culture 6 weeks post bloom</u>		
Stratified in plastic bags Germinated on 2% sucrose	.25 a ^z	0.0 a
Stratified on 6% sucrose Germinated on 2% sucrose	1.75 ab	0.29 ab
Stratified on 2% sucrose Germinated on 2% sucrose	1.50 ab	0.58 abc
Culture on 6% sucrose 60 days Stratified and germinated on 2% sucrose	4.25 abc	0.74 abc
Culture on 2% sucrose 60 days Stratified and germinated on 2% sucrose	7.00 bc	0.99 bcd
<u>Culture 10 weeks post bloom</u>		
Stratified on 6% sucrose Germinated on 2% sucrose	5.50 abc	1.71 cd
Stratified on 2% sucrose Germinated on 2% sucrose	8.50 c	1.51 d

^z = followed by different letters are significantly different at the 5% level (Duncan multiple range test).

Table 19. The number and % plants obtained from early-ripening seeded grapes crossed with seedless genotypes in 1984 and germinated in-vitro and in-vivo. (USDA).

Genotype	No. Seed cultured	No. grew from seed	(%)	No. grew from embryos	No. planted in field	% germination in vitro	% germination in vivo (n=50)
C19-71	571	86	(33)	135	212	39	18
C64-97	163	12	(07)	26	35	23	0
B1-166	283	3	(01)	106	109	39	32
C66-151	364	9	(02)	65	74	20	0
C66-144	1250	126	(10)	301	419	34	32
C74-123	473	235	(50)	53	268	61	42
Total	3104	471	(15)	686	1117	37	21

More plants developed from the subcultured embryos than germinating seed in test tubes. This procedure increased the average germination of early-ripening seeded grapes from 21% to 37%. The most dramatic benefit is for genotypes that have less than 10% germination and are not usable unless cultured.

In 1985, 10 early-ripening seeded genotypes were hybridized with seedless genotypes. Thirty cross combinations were made resulting in 4730 seed that were cultured in test tubes (Table 20). To date, 1246 (26%) seed have germinated compared to 14% germination in-vivo. Embryos from ungerminated seeds were subcultured and are currently growing into plants.

Germinating the seed in test tubes is advantageous because it reduces loss due to the spread of contamination. It is also simple and allows germination of strong embryos without excising them, thereby reducing labor.

In 1985, 950 floaters from three early-ripening genotypes were cultured. After three months the seed were cut open and 769 embryos subcultured. As of January 1, 1986, 40 plants had developed and were planted in the greenhouse. Embryos from very valuable hybrid seed can be saved by this method.

Table 20. The number and % plants obtained from early-ripening seeded grapes crossed with seedless genotypes in 1985 and germinated in-vitro and in-vivo. (USDA).

Genotype	No. seed cultured	No. grew from seed	(%)	Additional plants will develop from subcultured embryos.	% germination in vivo (n=50)
B4-120	662	159	(24)		8
C64-33	991	141	(15)		4
C63-188	835	82	(10)		2
C49-200	338	124	(37)		40
C64-97	165	36	(22)		16
B1-166	106	27	(25)		12
A8-130	557	184	(33)		18
C19-71	628	285	(45)		20
C85-41	199	106	(53)		8
C66-144	329	102	(31)		16
Total	4,730	1,246	(26)		14

IV. Improvement in seed germination.

ARO - In addition to developing in-vitro techniques for early seedless and seeded female parents, experiments were conducted to improve and hasten germination of seed by use of Alzodef (cyanamide) and PPP 333 (paclobutrazol).

a) The effect of Alzodef (cyanamide) on seed germination.

Experiments with Alzodef dips have been conducted during 1984 and 1985. Germination of check (without Alzodef treatment; 90 days of wet cold stratification of seed) will be determined only during December 1985. Results given for 1985 are thus somewhat incomplete. However, definite conclusions can already be drawn. Results are given in Tables 21 and 22. Results given in Table 21 (for 1984) show, that while germination at the time of harvest for two of the earlier cultivars is practically nil, Alphonse and Dabouki seed collected late in the season germinated 20-23% without cold or Alzodef treatment. Alzodef treatment doubled percentage of germination of fresh Alphonse and Dabouki seed. Highest germination rates were achieved with 45 days chilling in Alphonse and with the 1% Alzodef dip applied with fresh seed and immediate sowing. Keeping seed in cold stratification with Alzodef dip performed immediately after harvest tended to decrease germination percentages, except with 0.5% Alzodef dip, and seeds kept for 45 days thereafter in cold stratification. In Queen of Vineyards the 16% germination attained with 2% Alzodef dip with fresh seeds and the 24% germination attained from seeds dipped immediately in 2% Alzodef, followed by stratification for 45 days, was of interest. Seed of Queen of Vineyards, an early ripening cultivar, does not germinate generally in-vivo at a rate exceeding 15-20% because of many immature embryos in the sample, according to our experience for several reasons. With Oz, maximum germination was obtained with 45 days chilling, followed by Alzodef dip.

Table 21. The effect of Alzodef (cyanamide) on germination percentage of chilled and unchilled *Vitis vinifera* seed in 1984. Fifty seeds per treatment, with Queen of Vineyards and Oz. 4 replicates of 16 seeds with Alphonse Lavallee and Dabouki. Only sinkers used; floating seed discarded. (ARO).

Treatment	Cultivar				
	Sowing days ^x	Days chilling	Queen Vineyards	Oz	Alphonse Lavallee
None	1	0	0	0	20 ± 3.5
0.5% Alzodef dip ^{xx} (1)	1	0	2	26	35 ± 5.4
1.0% " (1)	1	0	4	28	37 ± 2.9
2.0% " (1)	1	0	16	28	42 ± 6.2
None	2	45	12	18	52 ± 3.5
0.5 Alzodef dip (2)	2	45	8	34	42 ± 1.8
1% " (2)	2	45	2	36	28 ± 6.9
2% " (2)	2	45	8	34	34 ± 9.5
0.5% " (1)	2	45	6	28	52 ± 6.8
1% " (1)	2	45	8	10	34 ± 11.1
2% " (1)	2	45	24	26	23 ± 5.4
None	3	90	16	16	34 ± 6.3
0.5% Alzodef dip (1)	3	90	2	10	30 ± 4.5
1% " (1)	3	90	12	4	13 ± 5.5
2% " (1)	3	90	4	18	27 ± 6.2
None					23 ± 5.4
					34 ± 11.2
					44 ± 5.9
					20 ± 4.5
					39 ± 4.5
					20 ± 3.5
					34 ± 5.9
					20 ± 3.6
					25 ± 5.1
					19 ± 5.1
					8 ± 4.2
					31 ± 5.9
					20 ± 5.4
					16 ± 2.1
					11 ± 3.8

^x₁ Immediately after harvest.
 2 45 days later.
 3 90 days later.

^{xx} In brackets time of dip relative to harvest.
 (1) Immediately after harvest (2) - 45 days later.

Table 22. The effect of Alzodef (cyanamide) on germination of chilled and unchilled seed in 1985. Five replications of 20 seed per treatment for each genotype. (ARO).

Treatment	Queen of Vineyards	Oz	Cardinal
No treatment immediate sowing	0	0	0
1% Alzodef immediate sowing	10 $\pm$ 4.0	19	8
2% Alzodef immediate sowing	21 $\pm$ 3.7	22	8
No treatment 45 days chill	20 $\pm$ 5.0	18	13
45 days chill then 1% Alzodef	40 $\pm$ 9.4	27	14
45 days chill then 2% Alzodef	27 $\pm$ 6.5	18	11
1% Alzodef then 45 days chill	28 $\pm$ 5.8	18	8
2% Alzodef then 45 days chill	23 $\pm$ 3.8	17	2
No treatment, 90 days cold	38 $\pm$	Not yet determined	11 ^x

Freshly harvested seed, when treated with Alzodef, germinated 26-28% compared to no germination without treatment. The value of Alzodef dip in substituting for the cold treatment and enabling immediate germination has been thus proven. Results for 1985 have been concluded with Queen of Vineyards. Again, seed treated with Alzodef immediately after harvest enabled us to obtain 21% germination, against no germination with untreated seed and 20% germination with untreated seed stratified in the cold for 45 days. However, using Alzodef dip (1%) after 45 days of cold stratification, doubled the number of germinating seeds compared to the control. Results in 1984 and 1985 are very similar (36% with Alzodef 1% against 18% without Alzodef after 45 days of chilling, in 1984 and 40% with Alzodef against 20% in the check in 1985).

Consistency of results with Queen of Vineyards shows that Alzodef can be advantageously used either as a dip immediately after harvest, allowing this earlier germination (3 months earlier than past practice and 1 1/2 months earlier compared to a shortened chilling period of the seed). In addition, for optimal germination results, Alzodef can be used as a dip after 45 days of cold stratification of the seed, enabling doubling of germination percentage compared to cold stratification alone.

b) The effect of paclobutrazol (PP 333) on grape seed development.

Use of compounds with antigibberellin activity was found (Sherman, 1983, Spiegel-Roy, unpublished) to increase germination and survival of immature peach embryos in-vitro. We have compared in-vivo germination rates of early maturing Vitis vinifera cultivars from vines in soil treated with the antigibberellin compound paclobutrazol (PP 333).

In 1984 vines have been treated with 75 mg active material paclobutrazol 60 days before collecting the seed. In this preliminary experiment 4

replications of 25 seeds of Cardinal were germinated in a volcanic tuff peat mixture and compared to 4 replications of 25 seeds from berries from paclobutrazol treated vines. Germination of seed from paclobutrazol treated vines amounted to 24.0%, compared to 9.6% in the check.

In 1985 paclobutrazol was applied in the cultivars Shaharit, Sivan and Cardinal. Treatment with paclobutrazol was made on May 1, 1985 at the rate of 75 mg active material per vine. Six vines of Cardinal, 3 vines each of Shaharit and Sivan have been treated. Seeds have been collected at the usual time of ripening for each of these early ripening cultivars. Percentage of seed sinking to the bottom of a water filled container was noted, as well as the number of floaters. A comparison of seed from paclobutrazol treated vines with seed from untreated vines show a significantly larger percentage of sinkers in seed from paclobutrazol treated vines (Table 23). This should also be reflected in the increased percentage of seed germination from paclobutrazol treated vines (not yet completed).

Table 23. Relative percentage of seed with endosperm (sinkers) to seed without endosperm (floaters) in early-ripening grape cultivars with and without soil treatment with paclobutrazol (application made May 1, 1985). (ARO).

Cultivar	Treatment ^x	Number floating seeds	Number sinkers	Percentage sinkers
Shaharit	A	71	294	80.5
	B	395	99	20.0
Cardinal	A	93	341	78.5
	B	366	121	24.5
Sivan	A	73	232	30.5

A 75 mg paclobutrazol active material per vine.

B Control.

The percentage of germination is now being tested.

V. Classification of seedlessness by anatomical and quantitative techniques.

a) New methods for seed trace determination

ARO - In an effort to create a simple, convenient and reliable test for the determination of degree of seedlessness (presence or lack of seed traces, hard seed coats) two chemical methods have been devised. One (Vitis 22: 306-310, 1983) is based on the determination of phenolic compounds in the berry, the other (J. South. Afr. 1985) is based on luciferase activity. Reprints are attached.

b) Classification of seedlessness in grapes.

USDA - Botanically there are several types of seed development in grapes: 1) Those in which the ovule is not fertilized and does not develop. 2) Stenospermic - the egg cell is fertilized but at some stage the seed aborts. 3) Seeded - fully developed seeds with hard seed coat: a) without endosperm (generally occurs in early-maturing varieties) and b) those with endosperm and embryo. Fruit from 6 seedless (stenospermic) and 4 seeded genotypes were collected on 8/6/81 and evaluated for berry weight, the number, size and weight of seed, and the development of embryo and endosperm (Table 24). Thompson Seedless and Fiesta have very small seed rudiments without endosperm. C63-5 has very small seed rudiments containing endosperm and embryos. Flame Seedless, BlA-27 and C35-33 have medium size seed rudiments with some endosperm and without visible embryos and P59-150, A82-64, Cardinal and Black Rose have mature seeds. Based on the average weight of the seed > 4 mm or weight of largest seed found, we could differentiate between genotypes with fully developed seeds and those with aborted seed for the genotypes observed. Stenospermic seedless genotypes can be differentiated from seeded genotypes based on seed weight and appearance.

There was no relation between the size of seed and presence of endosperm or embryo between genotypes. From a consumer's viewpoint, different evaluation criteria is needed. The seedless types in this case would be very small (undetectable) seed rudiments without endosperm or visible embryo.

Table 24. Berry size and seed development of 6 seedless and 4 seeded genotypes picked on 8/6/81. (USDA).

Genotypes	Avg. Berry Wt. (g)	No. Seeds + Traces ^z Per Berry		Avg. Length (mm)		Avg. Width (mm)		Avg. Wt. (mg) x		No. 2y Seed 4mm Per Berry	Avg. Length (mm)		Avg. Width (mm)		Weight Largest Seed (mg) x	Ovules with Endo-sperm		No. Embryos ^w Fill + Avg. Lenth (mm)
		Avg. Length (mm)		Avg. Width (mm)		Avg. Wt. (mg) x		Avg. Length (mm)			Avg. Width (mm)			Ovules with Endo-sperm				
Thompson Seedless	1.52	2.7	2.9	1.1	1.26	0.5	4.4	1.5	3.00	6	-	-	-	-	-	-	-	-
Fiesta	2.10	2.9	3.0	1.3	2.21	0.8	4.1	2.1	5.13	6	-	-	-	-	-	-	-	-
C63-5	2.40	3.1	4.5	2.4	6.78	3.0	4.7	2.5	7.20	10	42.0	77	2 @ .55					
Flame Seedless	2.31	2.7	4.5	2.6	9.10	2.0	5.3	3.1	11.80	19	18.5	35	-					
B1A-27	3.62	1.6	4.6	2.2	11.50	1.2	5.8	2.5	14.92	22	25.0	63	-					
C35-33	2.85	3.1	3.9	1.7	5.13	2.0	4.8	2.2	7.40	23	6.4	100	-					
P59-150	4.34	1.7	4.8	3.4	34.30	1.0	6.5	4.9	56.50	74	47.0	94	2 @ 1.75					
A82-64	5.14	2.6	4.4	2.8	23.69	1.5	5.8	3.8	39.67	70	50.0	100	2 @ 1.00					
Cardinal	6.05	2.3	6.0	3.3	33.10	2.2	6.2	3.4	34.55	63	69.5	88	1 @ .50					
Black Rose	6.09	2.7	7.9	5.1	87.30	7.9	7.9	5.1	87.30	126	88.8	96	1 @ 1.00					

^zNumber of seed and seed traces from 10 berries.

^yClassified as seed or trace based on visual observation of size, separated at about 4 mm length.

^xExpressed in fresh weight.

^wHard to see embryo because samples were frozen prior to examination.

VI. The effect of gibberellin on seed traces.

ARO - As shown in Part VII, the proportion of hybrids with noticeable seed traces highly exceeded the percentage of hybrids with barely noticeable seed traces. Seed trace content may be nearly unnoticeable in Sultanina and Perlette, larger in Flame Seedless and considerably larger in Black Monukka. Seed trace content and development have been found to vary from season to season and influenced by rootstock with Fiesta (Christensen, Ramming and Andris, 1983).

In our breeding work the main goal is that of producing seedless table grapes. This is being done mainly by crossing seeded seed parents with pollen of seedless cultivars, though crosses between seedless cultivars have also been initiated. Analyzing progeny of crosses between seeded and seedless cultivars, we obtained, on the average, 3 seeded to 1 seedless, with about 2/3 of the seedless progeny yielding fruit with noticeable traces.

It seems, therefore, warranted to subject some selected progenies to treatment with gibberellic acid in order to clarify whether also hybrids with a certain amount of traces (and otherwise satisfactory quality) can be selected from a progeny, multiplied and tested. It was also hoped to find criteria that could point to a minimum of traces or other characters, worthy of further effort. In any case, an affirmative answer could increase the percentage of hybrids that pass the "seedless criteria".

Within the framework of our work, hybrids with noticeable seed traces have been subjected to treatment with gibberellic acid during full bloom and to a second spray days thereafter. Results obtained and analyzed for each hybrid are given in Table 25. Seed trace content in each of these, with and without gibberellin treatment have been also classified by taste panel into 3 categories. N = normal sized seed or seeds, B = with noticeable seed

Table 25. The effect of two gibberellin (GA₃) sprays (one during bloom at 75 ppm, and the other 12 days after full bloom at 30 ppm) on seed trace number, and dry weight, berry weight and berry classification^x of hybrids in 1984. (ARO).

No.	Progeny	Treatment ^x	Traces dry weight mg	Avg. number traces	Avg. berry weight,g	Classification ^{xx}
28	Cardinal Perlette	A	18	1.68	4.9	T
		B	19	1.81	3.3	T
30	M. Hamburg Sultanina	A	25	2.30	3.1	T
		B	33	3.05	2.0	T
32	Oz Sultanina	A	17	1.92	4.1	T
		B	26	2.50	3.9	T
34	Oz Sultanina	A	1	0.04	3.5	S
		B	11	1.06	1.5	T
55	Cardinal ^x Perlette	A	4	0.81	3.6	S
		B	9	1.32	3.3	T(small)
58	Cardinal ^x Perlette	A	5	1.36	2.7	T(small)
		B	5	1.61	1.4	T(small)
62	Queen of Vineyards ^x Flame Seedless	A	14	1.48	4.4	T
		B	19	2.34	4.0	T

^{xx} S = Seedless
 T = Noticeable traces
 N = Normal seeded

^xA2 Gibberellin sprays
 B Unsprayed check

traces(s), S = seedless. Sprays have been usually applied to 2 clonally propagated (grafted) vines of each hybrid, while 2 other cloned vines have been left unsprayed. These experiments have been performed in 1984 (on a limited number of genotypes) and in 1985 with a larger number of genotypes. Results are given in Tables 25 and 26.

Examination of the tables shows that the treatment was successful (in converting into totally 'seedless') in 6 cases out of 21. Four hybrids (no. 32, 34, 58 and 62) have been treated during both seasons. Of these, no. 34 consistently yielded seedless berries in both seasons. A sibling of 34 was treated successfully in 1984 and 1985 (nos. 59 and 62). Treatment in both seasons was not successful. Many treated berries of no. 58 were seedless in both seasons, but other berries had traces. The experiment will be concluded in 1986. This will allow repeated treatment (two seasons) of more hybrids. So far, no guidelines can be established as to feasibility of success in choosing hybrids that can be treated with reasonable chance of success. Dry weight (in mg) of traces in hybrids successfully treated with gibberellin was 9 and 11 mg in 1984, 21 mg in one hybrid, and 6 and 3 mg in other hybrids in 1985. In hybrids, where treatment was unsuccessful, trace dry weight in 1984 ranged between 5 and 33 mg, and in 1985 from 5 to 24 mg. As to the average number of traces, hybrids successfully treated had, in 1984 (untreated) 1.06 to 1.32 traces per berry, 1.48, 1.66, 1.12 and 2.13 traces in 1985. Some of the unsuccessfully treated hybrids had a lower number of traces per berry (no. 12 - 0.66, no. 31 - 0.68). Increase in average berry weight accompanied virtually all cases of gibberellin treatment. Gains were sometimes slight (10% in no. 63 and less in no. 32), sometimes very noticeable 4.3 g against 2.0 g) in no. 34 in 1984, a genotype in which berries were also converted to completely seedless ones by the gibberellin sprays.

Table 26. The effect of two gibberellin (GA³) sprays (one during bloom at 75 ppm, the other 12 days after full bloom at 30 ppm) on seed trace number and dry weight and classification in diverse progeny in 1985. (ARO).

No.	Progeny	Treatment	Traces dry weight mg	Avg. number traces	Avg. berry weight, g	Classification*
8	Cardinal	A	5	0.81	4.0	S
	Perlette	B	21	2.13	3.3	T
10	Cardinal	A	14	1.58	4.0	T
	Perlette	B	24	1.76	3.3	T
12	Cardinal	A	5	0.62	3.7	T
	Perlette	B	8	0.66	3.4	T
14	Cardinal	A	3	0.38	2.9	T
	Perlette	B	15	1.08	2.7	T
31	Dabouki	A	4	0.68	3.1	T (small)
	Sultanina	B	5	0.62	1.7	T
32	Oz X Sultanina	A	2	0.58	3.6	T (variable)
		B	7	1.35	2.4	T
34	Oz X Sultanina	A	1	0.17	4.3	S
		B	6	1.12	2.0	T
35	Queen of Vineyards X	A	8	1.53	3.8	T
	Flame Seedless	B	15	2.20	2.3	T
38	Selection 14 X	A	3	0.95	2.0	T
	Sultanina	B	5	1.05	1.9	T
41	Shani X	A	2	0.52	3.0	S
	Flame Seedless	B	6	1.66	2.2	T
42	Alphonse x	A	20	1.65	3.9	T
	Flame Seedless	B	26	2.53	3.2	T
58	Cardinal X	A	6	1.37	3.7	T (mixed)
	Perlette	B	10	2.05	2.6	T with S)
62	Queen of Vineyards X	A	12	2.03	4.3	T
	Flame Seedless	B	17	2.44	3.2	T

xx S = Seedless

T = Noticeable traces

N = Normal seeded

x A2 Gibberellin sprays

B Unsprayed check

VII. Inheritance of seedless trait:

a) Analysis of progeny from seeded X seedless genotypes.

ARO - Progeny of crosses with Sultanina, Perlette and Flame Seedless as pollen parent were analyzed. Seedlings that have fruited so far have been classified into 3 categories: a) seedlings with stenospermocarpic fruit; b) seedlings bearing fruit with perceptible seed traces; c) seedlings with seeded fruit. Results are given in Table 27.

Table 27. Relative proportion of seedlings bearing stenospermocarpic fruit, fruit with seed traces, seeded fruit in progenies of Sultanina, Perlette and Flame Seedless in Bet Dagan, Israel, 1983. (ARO).

Cultivar	Number progeny analyzed	a	b	c
		Number with fruit seeded	Number with seed traces	Stenospermocarpic (seedless fruit) and percentage
Sultanina	198	138	28	13(6.5%)
Perlette	230	168	47	18(7.8%)
Flame Seedless	41	30	7	4(9.7%)
	469	356	82	34
Ratio of seeded (a) to seedless (b + c) 356:117 + 3.04.				

Recent analysis of fruiting progeny (1985) from crosses between seeded and seedless progeny, as well as analysis of fruit from a small progeny from crosses between seedless genotypes, reared in-vitro and established in the vineyard has permitted us to postulate a genetic theory on the inheritance of the seedless trait. Again, seedless progeny has been classified into 2 groups (with noticeable traces and without traces), both groups have been included in the seedless group, while progeny with normal seeded fruit has been classified as seeded.

Male parents were Sultanina, Perlette, L12, L13, Black Kishmish, Loose Perlette and Flame Seedless. Fourteen seeded parents were used in these crosses and so far 768 hybrids from combinations bore fruit. Classification of total progeny from crosses between seeded parents and stenospermocarpic male parents is given in Table 28.

Table 28. The number of seedless bearing stenospermocarpic fruit, fruit with seed traces, and seeded fruit in progeny of Sultanina, Perlette, L 12, L 13, Flame Seedless and Black Kishmish in Bet Dagan, Israel, 1985. (ARO).

	<u>Normal Seeded</u>	<u>Traces</u>	<u>Seedless</u>
Sultanina	289	54	38
Perlette	132	43	6
L 12	116	19	15
L 13	7	2	1
Flame Seedless	15	3	3
Black Kishmish	<u>21</u>	<u>4</u>	<u>0</u>
Total	580	125	63

We have already indicated in a previous report, that seedlessness is probably determined by two complementary recessive genes. The results obtained during 1985 show that our assumption is correct. The seeded genotype is Aa Bb, while seedless one (both with and without traces) have the genotype aa bb. Numbers obtained in each class deviate only very slightly from the expected genetic rates of 3 seeded to 1 seedless.

	<u>Seeded</u>	<u>Seedless</u>	
Expected	192	576	
Obtained	188	580	$\chi^2 = 0.1111$

The ratio of progeny with traces is about 2/3 of the total progeny considered seedless (stenospermocarpic). However, with Perlette ratio of seedless to seedless with traces is much lower than with Sultanina.

Another conclusion that can be drawn is that use of seeded progeny from a seeded X seedless cross somewhat increase chances of seedlessness. Seeded genotypes in such crosses should segregate into Aa Bb in equal proportions. Though the genotype will not be known before crossing, use of genotypes from such a cross as seeded parent would increase expectations for seedless progeny (when crossed with seedless as male parents) from 25% (1:3 ratio) to 37.5% (3:5 ratio); two out of three would yield seedless progeny in the 1:1 ratio (Aabb x aaBb x aabb) and only one out of three would replicate the original seeded parent ratio (AaBb x aabb) yielding 3:1 seeded:seedless.

USDA - In 1977 and 1978 three seeded grapes were crossed with ten seedless genotypes with different size seed traces. Number seed per berry, % good seed, seed weight and % floaters were evaluated to see if the pollen source could cause seed abortion and the expression of seedlessness in the zygote and endosperm. In 1977 the pollen genotype caused significant differences in the % good seed developed and weight of good seed, but not in 1978. The number floaters and weight of floaters was significantly different for both years. This may explain why Loomis and Weinberger (1978) found different % seedless progeny from different years of crossing. There were significant differences between the female parents for all measurements taken for both years. This shows that seeded grapes have a characteristic number of good and floater seed per berry.

The number of seedless progeny from the 1977 crosses averaged 30-35% by the seedless male except for Thompson Seedless and Black Monukka, which averaged 18 and 15.5% seedless progeny (Table 29). This seems to indicate

that Thompson Seedless and Black Monukka have a different genetic make-up than the other seedless parents. This contradicts the theory that all seedless are homozygous recessive with one or two genes. When compared to the seed trace size of the seedless parent, Thompson Seedless had the smallest seed trace, 0.8 mg fresh weight, and had the next to lowest % seedless progeny (Table 30). Black Monukka, on the other hand, had the next to largest seed trace and the lowest % seedless progeny. Therefore, seed trace size does not appear to affect the % seedless progeny a genotype will produce. When seed size of the seeded females is compared to the % seedless progeny there appears to be some correlation as C15-133 had the largest trace and lowest % seedless progeny and P45-98 had the smallest seed size and the greatest % seedless progeny although these were not significantly different.

Hybrids have also been made between Emperor X Emperor Seedless (mutation of Emperor), Tokay X Tokay Seedless (mutation of Tokay) and Seeded Thompson X Thompson Seedless. These crosses were made in 1983, 1984 and 1985 to try to get large enough populations to work with (Table 31). The population will be analyzed for % seedless progeny and for size of seed to gain a better understanding of the seedless gene by matings with seedless mutations.

Table 29. The number of seeded^z and seedless^y progeny from seeded X seedless crosses made in 1977. (USDA).

Data from 2 years on 1977 crosses									
	P-45-98		Kishmiski		C15-133		$\bar{x}$		
	Seeded	Seedless(%)	Seeded	Seedless(%)	Seeded	Seedless(%)	Seeded	Seedless(%)	Seeded
T.S.	40	9(18.4)	9	2(18.2)	5	1(16.7)	54	12(18.2)	
Perlette	32	17(34.7)			1	9(0)	33	17(34.0)	
C32-68	62	37(34.7)			33	8(19.5)	95	45(32.1)	
C58-22	25	13(34.2)	4	4(50)	52	25(32.5)	81	42(34.1)	
C32-129	38	21(35.6)			10	3(23.1)	48	25(34.2)	
C32-145	14	11(44.0)	4	0(0)	4	1(20.0)	22	12(35.3)	
Fiesta	7	2(22.2)	1	2(66.7)	33	15(31.3)	41	19(31.7)	
Flame Seedless	73	41(36.0)	4	0(0)	84	37(30.6)	161	78(32.6)	
Black Monukka	5	2(28.6)	11	2(15.4)	109	19(14.8)	125	23(15.5)	
C35-33	4	4(50)	3	2(42.9)	20	5(20.0)	28	12(30)	
$\bar{x}$	300	157(34.4)	36	12(26.0)	351	114(24.5)	688	285(29.8)	

^z Seeded = seeds average greater than 20 mg fresh weight.

^y Seedless = seeds average less than 20 mg fresh weight.

Table 30. Fresh weight (mg) of seeds from the seeded and seedless parents used in the seedless inheritance study. (USDA).

1982 Average fresh weight (mg) of seed	
<u>Seedless</u>	
T.S.	0.8
Perlette	3.8
C32-68	7.0
C58-22	6.0
C32-129	9.0
C32-145	6.0
Fiesta	4.8
Flame Seedless	5.3
Black Monukka	10.1
C35-33	13.0
<u>Seeded</u>	
P45.98	55.3
C15-133	83.6
Kishmiski	60.1

Table 31. The number of emasculations, seed and plants from seeded genotypes Emperor and Tokay crossed with their seedless mutations and a seeded form of Thompson Seedless with Thompson Seedless. (USDA).

Parents	No. Emasc.	No. Seed	No. Germin.	In field
Emperor x Seedless Emperor				
1983	800	4	0	-
1984	700	23	12	7
1985	2,130	378	*	
Tokay x Seedless Tokay				
1983	1,223	37	21	18
1984	40	7	4	4
Seeded Thompson x Thompson Seedless				
1983	1,121	21	2	2
1984	2,745	792	*	

* Not germinated yet.

b) Analysis of progeny from seedless X seedless genotypes.

ARO - Analysis of 10 hybrids that fruited so far out of seedless X seedless crosses show 9 seedless, 1 normal seeded, thus substantiating our theory that seedlessness is due to recessive gene(s). At least 40 more hybrids resulting from crosses between seedless X seedless are expected to bear fruit in 1986. Hybrids that have fruited so far came mainly from reciprocal crosses between Perlette and Flame Seedless hybrids raised in vitro (our publication in J. Amer. Soc. Hort. Sci. 1985).

USDA - In 1983, 89 seedlings from open pollinated Thompson Seedless and 42 seedlings from P60-58 open pollinated seedless ovules were planted in the field. The seedlings started to fruit in 1985 and the color of the fruit and size of seed trace was recorded (Table 32). Many of the seedlings are out crosses because the fruit in many of the seedlings from Thompson Seedless are colored. Assuming all are out crossed to seeded genotypes, Thompson Seedless produced 65% seedless progeny and P60-58 produced 62%.

In 1983 Thompson Seedless and P60-58 were hybridized with black fruited seeded and seedless genotypes. The black fruit color of the pollen parent allows us to be sure of the hybrid nature of the seedlings as we test the genetic nature of the seedless trait.

Diallel crosses have been made between 3 seedless and 3 seeded genotypes (Table 33). This will give us good information for more inheritance studies.

Table 32. The number of white, red, and black seeded and seedless fruiting seedlings in 1985 from open pollinated ovules of Thompson Seedless and P60-58 seedless. (USDA).

Genotype	No. seedlings in field	% Fruiting	No. seeded		No. seedless		% seedless
			White	Red	White	Red	
Thompson Seedless (white)	89	19	5	-	1	2 ^z	65%
P60-58 (red)	42	48	3	1	4	7	62%

^z The seed traces were undetectable in these seedlings.

Table 33. Diallel crosses made in 1984 and 1985 between 3 seedless and 3 seeded genotypes. (USDA).

		Seedless		Seeded	
T.S.	Fl. S.	C85-81(85)	A81-110	C78-53	C15-162
1984	2973/200/0 ^z	2400/600/0	2564/407/3	2400/238/0	2186/189/0
1985	2018/698/(24)- ^x	2617/698/(34)-	1900/494/(9)-	1908/36/(2)-	2200/510/(2)-
Fl. S.					
1984	2618/300/14	2097/662/3	2800/927/26	2424/242/1	1916/308/9
1985	2035/546/(165)-	2276/868/(181)-	1739/448/(83)-	1930/584/(105)-	2076/223/(30)-
C85-82					
1984	2101/80/0	2228/93/0	1950/38/0	2027/202/1	2264/16/0
1985	1591/264/(104)-	1982/808/(375)-	1793/383/(122)-	1880/311/(192)-	1463/434/(248)-
A81-110					
1984	1398/0/0	1775/132/69		2532/9/1	1280/112/50
1985	155/360/-	1600/441/-	-/478/-	1850/322/-	1100/332/166
C78-53					
1984	1420/365/0	1802/356/6	1450/174/107		1591/408/0
1985	1790/246/-	1825/245/-	Enough '84	-/211/-	1543/118/-
C15-162					
1984	1450/96/36	1480/134/75	1230/14/5	1348/106/59	
1985	1453/262-	1700/861-	1530/203/-	1300/132/-	-/105/-

^z Emasculations/# ovules or seed/# plants produced.

^y Clusters were bagged without emasculating to produce selfed seed.

^x Seed have not germinated yet. () = numbers of embryos subcultured.

^w Enough plants were produced in 1984 so the cross was not repeated.

c. Enviromental induced variation in seedlessness.

USDA - Temperature has been observed to influence the seed trace development in the field. It has been noted that the second crop that develops in warmer temperatures on the vine have larger seed traces than the first crop. Fruiting cuttings of P60-58 (small seed trace), C35-33 (seed trace with a soft seed coat and some endosperm) and Red Malaga (seeded) were grown for 30 days at a high temperature (90°F day, 65°F night) and a low temperature (80°F day, 55°F night). The temperature was increased 5°F for the second 30 days. Berry weight, ovule length and weight for 5 berries in each treatment was evaluated 30 and 60 days after bloom. The berries, after the first 30 days in the high temperature, averaged 833.2 mg and the ovules were 5.4 mm long and 24.5 mg. In the low temperature the berries averaged 850.8 mg and the ovules were 4.22 mm long and 14.66 mg. The ovules developed faster at the higher temperature in similar size berries for C35-33. When all genotypes were evaluated for both dates there was no significant difference between temperatures (Table 34). Seed weight appeared to be less in the low temperature for the seedless genotypes although final berry size was larger. The seed trace size from the second crop in the field may be influenced more by cytokinin levels in the plants than by temperature, explaining the small differences found under controlled conditions.

Table 34. Berry weight, ovule length and ovule weight (mg) from P60-58, C35-33 and Red Malaga, 30 and 60 days after bloom at 80-55°F and 90-65°F (day-night). (USDA).

		Berry weight		Ovule length		Ovule weight(mg)	
		Days		Days		Days	
		30	60	30	60	30	60
P60-58	High	654	978.9	3.20	4.18	21.35	13.65
	Low	718	1,371.1	4.30	4.37	18.43	17.48
C35-33	High	601	705.7	5.07	5.38	23.38	25.37
	Low	561	1,129.3	4.72	5.27	14.10	20.72
Red Malaga	High	582	765.0	5.56	5.70	41.42	45.60
	Low	545	755.0	6.07	5.98	47.29	49.56
				NS	NS	NS	NS

d) Inheritance of muscat flavor.

Analysis of progeny of three distinctly muscat flavored seeded parent (Early Muscat, Muscat of Hamburg and Muscat of Alexandria) with several seedless cultivars used as male parents is given in the Table 35. Progeny has been classified into 1. Neutral; 2. Muscat flavor.

Table 35. The number progeny classified as having neutral or muscat flavor. (ARO).

<u>Progeny</u>	<u>Neutral</u>	<u>Muscat flavor</u>
Early Muscat x Sultanina	1	0
Early Muscat x Perlette	15	4
Early Muscat x L 12	38	27
M. Hamburg x Sultanina	88	17
M. Hamburg x Perlette	128	40
M. Hamburg x L 12	72	26
M. Alexandria x Sultanina	87	27
M. Alexandria x Black Kishmish	150	14
	579	155

The ratio of neutral flavored to muscat flavored is 3.735, when only Sultanina, Perlette and L 12 are considered, the ratio is close to 3.1 (439 ag. 141). A detailed analysis was performed as to proportion of normal seeded stenospermocarpic with traces and seedless stenospermocarpic in each of the two classes (neutral and muscat flavored). Results indicate similar proportions of neutral and muscat flavored in seeded progeny (462:124 = 3.726) and in the combined progeny for stenospermocarpic fruit (117:31 = 3.774).

Main achievements of the research related to original proposal.

ARO - One of the aims of the project was to test the efficiency of preselection for seedlessness in the greenhouse. However, only a small proportion of the progeny attained the flowering stage during the first year in the greenhouse even with applications of cytokinins and antigibberellin. This may have been due partly because of limiting light in the dense stands and, perhaps, lack of CO₂ enrichment.

On the other hand, a considerable shortening of the period from seed to flowering was obtained in a series of experiments dealing with earlier seed germination, seedling development and field application of cytokinin + anitgibberellins. In field plantings of hybrids during May and June (from seed germinated in December) close to 40% of progeny from seeded X seedless crosses fruited in the year subsequent to planting, with a total of about 78% fruiting during the second year. Field application during the first season by a combination of cytokinin and antigibberellin cause up to 50% of progeny to flower and set fruit during the same season. Clusters in this case were atypically small, enabling observations on color and seedlessness only. Soil application of paclobutrazol to early ripening female seed parents in the spring most significantly enhanced endosperm and seed development, increasing percentage of "sinkers" in these seed lots from 20-25%, in the check to 80%. Seed treatment with cyanamide enabled satisfactory seed germination immediately after harvest, with several genotypes tested. With early ripening cultivars as seed parent, planting in the field of hybrids during December and January (8-9 months after making the cross) can be achieved in the future.

Our studies on the inheritance of seedlessness have shown that seedlessness (both completely seedless and progeny with seed traces should be

included in this category stenospermocarpy) can be attributed to two complementary recessive genes. We can thus expect, on the average, 25% seedless progeny (about 2/3 of these may be noticeable seed traces) and in backcrosses to seedless genotypes, between 25-50% seedless progeny. Furthermore, as a result of work performed, the significantly improved germination in-vitro of seed from early ripening cultivars, will allow use of seeded parents in breeding with undeveloped embryos and only 65-70 days from flowering to fruit ripening, as female parents.

A further method which may allow up to 100% seedless progeny and thus increase efficiency of breeding for seedlessness to an appreciable extent, has been developed. An in-vitro method for culture and plant formation from grape cultivars with ovules and seeds has been developed and published, and will be probably of wide use by breeders. Establishment of plants from crosses between seedless parents has already allowed to confirm our theory on the recessive character of seedlessness, and the fruiting of further such progenies will allow other important conclusions concerning the application of the practice. We do not know yet whether there is an adverse effect as to ultimate fruit size in seedless X seedless progenies.

A further contribution of our study concerns the possibility of further exploitation of the seedless character. A considerable proportion of seedless hybrids bear fruit with noticeable seed traces, making such a selection worthless for fresh fruit and raisins. Moreover, this characteristic is known to fluctuate considerably, but slighter seed traces have been of no consequence in the important cultivar, Flame Seedless. In our work two new chemical assessment methods for defining the extent of seed traces have been developed and published. One is based upon polyphenol content of berries and the other on luciferase enzyme activity. As a further

contribution to the problem, application of gibberellin has been tested on 16 of our hybrids with seed traces. This work will be completed in 1986, allowing a wider year to year replication. Though achievements, so far, have not allowed general conclusions, we were able to transfer 4 hybrids (out of a total of 16) from the 'with traces' to the 'seedless' subclass. It seems number and weight of traces cannot be depended upon as criteria for predicting success of gibberellin treatment in obliterating seed traces.

Summing up, the present work has elucidated the genetics of seedlessness. It may substantially increase the efficiency of breeding for seedlessness by allowing us to obtain progenies from seedless X seedless crosses. It will help to shorten the breeding cycle, increase efficiency of prediction of distribution of characters in breeding programs and allow for a more efficient use of early-ripening cultivars in breeding.

Results will also contribute to a better definition of seed traces in stenospermocarpic genotypes by objective methods and may aid in the evaluation.

USDA - The aim to develop greenhouse preselection techniques has been unsuccessful. Only indications of flower formation resulting from hormone treatments occurred. Better methods of applying growth regulators and optimum conditions to cause flowering and fruiting within 6 months in the greenhouse are needed.

The goals to develop in-vitro methods to produce plants from seedless genotypes and early-ripening seeded genotypes has been accomplished. These techniques are now being used in breeding programs to shorten the generation of hybridizing seedless genotypes because an intermediate seeded female is no longer needed. It also allows the hybridization of very early-ripening genotypes, increasing the possibility of producing good early-ripening

hybrids.

Characterization of the seed trace in seedless grapes is still difficult because seedlessness is described differently in the botanical sense than it is from the consumer's stand point. There is a gradient in size of the seed trace in seedless genotypes as well as in seeded genotypes. Better evidence about the seedless trait will be obtained when more seedless X seedless progeny fruit.

Understanding what causes the seed to abort, the time of abortion and the size of the seed trace was not an objective of this study, but is important to achieve a better understanding of the seedless trait.

Determining the influence of environment on the seedless trait was another goal of the project. From the one study it appeared that temperature had only a small effect on seed trace development. Since plant hormones seem to play an important part in abortion of the seed and production of smaller seed traces, it may be logical to assume the endogenous levels of hormones are different when the plant produces the first and second crop. This produces the different seed trace sizes more than the effect of temperature.

The present study has been marked throughout by excellent cooperation between researchers in USDA in California and ARO in Isreal.

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List of Publications

ARO:

Merin, U., I. Rosenthal and U. Lavi. 1983. A chemical method for the assessment of grapes according to their seed content. *Vitis* 22: 306-310.

Perl, M., U. Lavi and R. P. Spiegel. 1985. Inhibitory effect of grape seed extracts on luciferase activity. A possible tool for detecting seed traces in grape berries. *South Africa Soc. for Enol. and Viti.* 5(1): 1-7.

Spiegel, P. R., N. Sahar, J. Baron and U. Lavi. 1985. In vitro culture and plant formation from grape cultivars with abortive ovules and seeds. *J. Amer. Soc. Hort. Sci.* 110(1): 109-112.

Spiegel, P. R., N. Sahar, I. Baron and U. Lavi. 1985. Ovule and seed culture from early ripening seedless and seeded grape cultivars. *Proc. Internatl. Symposium on Grapewine Breeding, Verona.* pp. 13-18. In press.

USDA:

Ramming, D. W. and R. L. Emershad. 1984. Embryo culture of early-ripening seeded grape genotypes. *HortSci.* 19(3): 594. (Abstr. 535)

Emershad, R. L. and D. W. Ramming. 1984. In-ovulo embryo culture of Vitis vinifera L. c.v. 'Thompson Seedless'. *Amer. J. Bot.* 71(6): 873-877.

Cooperation

The entire period of the study was marked by excellent and friendly cooperation between Dr. D. W. Ramming, Coordinating Principal Investigator and Dr. P. Spiegel-Roy, Cooperating Investigator.

Cooperation involved exchange of genetic material, up-to-date information, joint planning of next year's research, evaluation of results. Dr. Ramming visited Isreal (Bet-Dagan) in June 1983, and Dr. P. Spiegel-Roy visited Fresno in September 1984. A joint publication on the subject is envisioned.

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Ovule and seed culture from early ripening seedless and seeded grape
cultivars

P. Spiegel-Roy, N. Sahar, I. Baron and U. Lavi

Agricultural Research Organization, Volcani Center, Bet Dagan, Israel.

ABSTRACT. Breeding stenospermocarpic seedless cultivars by crossing seeded and seedless cultivars yields a low proportion of seedless hybrids. In vitro culture of abortive ovules and seeds of 'Perlette', 'Flame Seedless', 'Sultanina' and of a further seedless genotype (L12) was developed, using a solid medium (Nitsch's or Orchid agar) with the addition of IAA and GA3. Progeny from selfed 'Perlette' and from reciprocal crosses between 'Perlette' and 'Flame seedless' has been established in the field and will yield genetic information in 1986. In vitro embryo rescue from seed of early ripening seeded cultivars ('Cardinal', 'Sivan') has also been developed. Percentage of plants recovered varies between 24-34%, according to genotype, compared to 8-9% germination in vivo.

The development of seedless *Vitis vinifera* cultivars is a major goal of the grape breeding program at the Volcani Center, A.R.O. Considerable interest is also shown in the development of both seeded and seedless early ripening cultivars. In pursuing these aims, certain difficulties arise. Only a relatively small percentage of progeny from crosses between seeded and seedless cultivars yields stenospermocarpic fruit without noticeable seed traces (between 6.5 to 9.5% in our breeding program). There is scarce genetic knowledge

concerning the inheritance of the character for seedlessness. It seems that seedlessness is controlled by recessive genes (5,13) although the claim of dominant control has also been raised (8,12), this does not seem to be borne out by the data obtained so far by us.

Raising selfed progeny or progeny from crosses between stenospermocarpic cultivars might therefore yield a higher proportion of seedless progeny. Nitsch et al (7) and later Barritt (2) reported that embryo and/or endosperm breakdown occur about 3 weeks after bloom, in stenospermocarpic fruit. Embryos and plants have been cultured from a grape hybrid with a large abortive ovule by, Cain (3) and in-ovulo embryo culture of Thompson Seedless has been recently described by Emershad and Ramming (4). Our work with 3 leading cultivars of stenospermocarpic *Vitis vinifera* in 1982 and 1983 has been reported recently (11). Main findings and work performed during 1984 will also be reported here, as well as recent results obtained with the culturing of immature embryos from seed of early ripening seeded *Vitis vinifera* cultivars.

MATERIALS AND METHODS. Ovules from open pollinated Perlette were first cultured successfully in 1982. Details of the method have been described by us (11). Ovules were cultured in petri dishes on either Nitsch medium (2) or Orchid agar. The addition of 10^{-6} M GA3 + 10^{-5} M IAA to either medium proved essential for successful culturing. In 1983, another medium in agar (Murashige and Skoog) was also tried. This medium has been also supplemented with 10^{-6} M GA3 and 10^{-5} M IAA. 200 Ovules were cultured from each of the 3 cultivars. 'Perlette', 'Flame Seedless' and 'Sultanina'. Another variable examined was time

after anthesis (31 and 52 days for 'Perlette', 28-49 days for 'Flame Seedless', 25-46 days for 'Sultanina'). These ovules were cultured from open pollinated flowers. In addition ovules were cultured from selfed 'Perlette' and from a reciprocal cross between 'Perlette' and 'Flame Seedless'. Germinating embryos were transplanted at the 4 leaf stage into a plastic container with a sterilized mixture of volcanic tuff, peat and perlite. A further transfer was made into 1 gallon containers placed in the greenhouse. Well established plants were then planted in the field during May-July 1984 about 10-12 months after starting the ovule culture. In 1984, a further seedless selection without noticeable traces, L 12 was also successfully cultured. Plants developed in 1984 were mainly from selfed flowers and from crosses between the 4 seedless genotypes mentioned. A complete diallel between the 4 cultivars has not yet been established, but selfed progeny from 3 cultivars have been raised and hybrids from several combinations established.

During 1983 and on a larger scale in 1984, sterile culture in agar of immature embryos has been performed with two seeded early maturing cultivars, 'Sivan' and 'Cardinal'. Cultures have been established 85 days after pollination with 'Sivan' and 89 days after pollination with 'Cardinal'. A simple medium (Orchid agar) without the addition of growth substances has been employed. Transplanting procedures have been similar to those described for culturing abortive ovules of seedless cultivars: development of plants proceeded, however at a somewhat faster rate with immature embryos of seeded cultivars than with abortive ovules from seedless cultivars.

RESULTS. Table 1 summarizes results obtained in 1983 and 1984 by culturing ovules of 'Perlette', 'Flame Seedless' and 'L 12'. Only results for ovules from self pollinated flowers and from crosses between seedless cultivars are given in the table. Nitsch's medium with the addition of IAA and GA3 was used. Percentage of viable embryos from selfed flowers was somewhat lower than that obtained from certain crosses of the same female parent. Ovules cultured from 'Flame Seedless' with 'Perlette' as male parent yielded a higher number of viable embryos than ovules cultured from the reciprocal cross ('Perlette x 'Flame Seedless'). About 70% of the viable embryos from this reciprocal cross were successfully established in the field during 1984. About 50% of the embryos successfully cultured during 1984 from seedless L 12 have already been transplanted into a plastic container (10.5 cm high, 8 cm in diameter) by the last week of February 1985 and will probably achieve planting size in the field by June 1985.

Satisfactory results in establishing embryos from ovules of open pollinated 'Perlette', 'Flame Seedless' and 'Sultanina' have been achieved in 1983 and likewise embryos from ovules of open pollinated L 12 have been established in large numbers in 1984.

A comparison of different media with ovule culture from open pollinated flowers of 3 cultivars was conducted in 1983. The results are summarized in Fig. 1. 400 ovules of each cultivar were cultured on 3 different media (2 media only with 'Sultanina'). Ovules were cultured June 13 (25-31 days after anthesis according to cultivar) and on July 4 (46-52 days after anthesis). 10^{-5} M IAA + 10^{-6} M GA3 were added to each of the 3 media. With 2 of the 3 cultivars Nitsch's

medium gave better results than Orchid agar. Murashige and Skoog medium gave poor results with 'Perlette'. On the basis of these results, Nitsch's medium was used in subsequent cultures.

So far, we have established plants in the field from selfed 'Perlette' and from a reciprocal cross between 'Perlette' and 'Flame Seedless' as well as from open pollinated flowers of the two cultivars and from 'Sultanina'. Embryos cultured during 1984 will enable us to plant in addition to selfed L 12 progeny and open pollinated progeny of L 12, also hybrids from several combinations between the seedless L 12 as a female parent with other seedless cultivars (a male parent). We plan to achieve a complete or near complete diallel between the four cultivars by July 1986; however establishment of embryos and plants from 'Sultanina' is slower and more difficult than with the three other cultivars mentioned.

Culturing ovules of stenospermocarpic cultivars thus extends the type of combinations that can now be performed. However in breeding for early ripening another obstacle encountered is the poor germination of undeveloped embryos (usually below 1mm in size) common to early ripening seeded, table grape cultivars. We have, therefore, attempted to culture undeveloped embryos from seed of 2 early ripening cultivars, 'Cardinal' and 'Sivan' (the latter is a named selection from a 'Dabouki' x 'Cardinal' cross). Seed collected was derived from flowers pollinated by either 'Flame Seedless' or 'L 12'. In addition to the cultured control (Orchid agar) additions of either GA3 or daminozide (butanedioic acid mono 2,2 dimethylhydrazide) to the Orchid agar medium were also tried. Seed was also collected, at the same date (corresponding to the approximate ripening date) for an in vivo

germination comparison. These seed lots were germinated in a mixture composed of volcanic tuff and peat. Results are given in table 2. Differences in favour of in vitro germination are highly pronounced. Moreover, while the in vivo germinated seed included only seed with comparatively developed embryos (sinkers), the in vitro germination percentage is based on embryos irrespective of size, as no attempt has been made to discard floaters before culturing*. Analysis of the results of in vitro germination show that 'Sivan' germinated in higher percentage than 'Cardinal'. There was also a significant difference in favour of seed from flowers pollinated by 'Flame Seedless' compared to seed from pollination by 'L 12'. As to the effect of the addition of daminozide or GA3 to the medium the differences compared to the control (Orchid agar without additions) are not statistically significant, in spite of a tendency for higher germination in media with daminozide in 3 out of 4 of the genotype x male parent combinations (table 2).

DISCUSSION. Our work has shown that it is possible to recover viable embryos and establish hybrid plants from three seedless *Vitis vinifera* cultivars and from a further seedless selection, all devoid of noticeable seed traces. Cain (3) reported good results in culturing a seedless selection with a large abortive ovule and seed trace. Emershad and Ramming (4) reported culturing, by a different method, in -ovulo embryos of 'Thompson Seedless'. While we have been successful in culturing ovules from open pollinated 'Sultanina', it seems now that the culture of embryos of this cultivar (known also as 'Thompson Seedless') is somewhat more difficult than culturing other

stenospermocarpic genotypes, such as 'Perlette', 'Flame Seedless', 'L 12'. The addition of IAA and GA3 to the agar medium used by us proved essential to success in our experiments (11). Selfed plants have been raised so far from three cultivars and several combinations between seedless genotypes have given rise to plants in sufficient number to allow genetic analysis in the near future: it seems probable that first results for analyzing the inheritance of the seedless trait will be forthcoming in 1986. A higher percentage of seedless progeny can be expected from crosses between seedless progeny than from crosses between seeded and seedless genotypes.

We have also been able to demonstrate that a simpler medium (Orchid agar), without phytohormones will allow germination of embryos from seed of early maturing table grape cultivars in substantial numbers. Similar results have been indicated by Ramming and Emershad (9). Balthazard (1) has shown that embryos attaining a size of only 1mm or less germinate very poorly if at all in vivo. As a result of our work and progress achieved elsewhere (3,4,9) very early seedless cultivars can now be crossed with very early seeded ones, in both directions, thus exploiting the high heritability found for days from bloom to ripening in the grape (10).

In conclusion, ovule culture by producing viable embryos and plants in normally seedless cultivars, will facilitate breeding programs, and enable genetic analysis of seedlessness, while the embryo rescue technique with early ripening seeded genotypes will enable use of such cultivars as female parents.

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Table 1. Percentage of viable embryos produced by cultured ovules from selfed seedless 'Perlette' and seedless selection 'L 12' and from different crosses with seedless male parents using 'Perlette', 'Flame Seedless' and 'L 12' as seed parents. Ovules cultured on Nitsch's medium + 10^{-6} M GA3 + 10^{-5} M IAA.

	No. of ovules cultured	Viable embryos (%)
Perlette, selfed	220	17.2
L 12, selfed	205	13.7
Perlette x Flame Seedless	280	15.3
Flame Seedless x Perlette	300	25.6
L 12 x Perlette	93	21.5

L 12 x Flame Seedless	118	10.2
L 12 x Sultanina	102	17.6

Table 2. Percent germinating embryos from culturing undeveloped seed of early ripening table grape cultivars 'Sivan' and 'Cardinal'. Orchid agar medium. Sivan pollinated 2.5.84, seed cultured 25.7.84; Cardinal pollinated 29.4.84, seed cultured 25.7.84.

Genotype	Male parent(x)	Treatment(y)	Germinating embryos (%) (z)
Sivan	1(x)	a	42
Sivan	1	b	46
Sivan	1	c	26
Sivan	2	a	28
Sivan	2	b	27
Sivan	2	c	32
Cardinal	1	a	38
Cardinal	1	b	20
Cardinal	1	c	28
Cardinal	2	a	21
Cardinal	2	b	16
Cardinal	2	c	17

(x) - 1=Flame Seedless, 2=L 12

(y) - a=daminozide 4×10^{-3} b=GA3 10^{-6} M c=check

(z) - germination in vivo (%) Sivan 80 Cardinal 90