

Seedless x seedless grape progeny: Technique, results and perspectives

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S u m m a r y : Recent refinements in the *in vitro* embryo rescue technique employed to raise progenies from abortive ovules of seedless x seedless cultivars include addition of 0.2 ppm NAA (naphthaleneacetic acid) to the medium, inducing highly improved root systems and faster plant development. Selfing seedless *Vitis vinifera* yielded seedless progeny only. Open pollinated Perlette and Flame Seedless gave rise to a high percentage (75 and 87 %, respectively) of seedless progeny. Progeny from various crosses between seedless cultivars segregated into 65 normal seeded and 204 seedless. From the totality of 204 seedless progeny 192 bore fruit with very slight seed traces. Progeny from crosses between seeded and seedless segregated only 7.5-8 % individuals with comparably slight seed traces, amounting to $\frac{1}{3}$ of the progeny rated as seedless. Fresh weight determinations of aborted seeds per berry showed a pronouncedly lower weight in progenies from seedless x seedless crosses. Reduction in average berry size in the seedless fraction of seedless x seedless progenies compared to midparent values was of a similar order of magnitude as that obtained in seedless progeny derived from seeded x seedless crosses.

Key words: table grape, berry, stenospermocarp, seed trace, selfing, crossing, genetics, technique, tissue culture.

Introduction

Breeding new seedless cultivars has been performed in the past by selecting from progenies of crosses between seeded (female parent) and seedless (male parent) genotypes. Only about $\frac{1}{4}$ of the progeny, on average, proved seedless (SPIEGEL-ROY, unpublished). Moreover, only 6.5-9.5 % of the totality of the hybrids bore fruit without noticeable seed traces (SPIEGEL-ROY *et al.* 1986). Recently, *in vitro* methods for culturing abortive ovules and seeds from seedless cultivars and selections have been described (EMERSHAD and RAMMING 1984, SPIEGEL-ROY *et al.* 1985). These methods enable to raise progeny from crosses between two seedless parents and also from selfed seedless genotypes. The present paper deals mainly with the inheritance of the seedless trait in selfed progeny and crosses between seedless genotypes. New developments in technique and breeding potential of seedless x seedless crosses are also discussed.

Materials and methods

Crosses have been made between seedless parents and their progeny analyzed for seed content, berry size and colour. Progeny from open pollinated Perlette, Flame Seedless and Sultanina was also examined for the same characteristics, as well as progeny obtained from selfing seedless genotypes. A panel consisting of 3 persons classified progeny in 3 categories; normal seeded (N); with noticeable seed traces and texture of the seed deviating from that of the pulp (B); practically seedless, with barely noticeable seed traces (S).

In certain progenies seed trace content has also been evaluated by determining number of aborted seeds per berry and fresh weight of each aborted seed in the berries. On average, 20 berries of each hybrid or genotype have been thus sampled. Berry weight determinations were based on 25 berries. *In vitro* culture was based essentially on the technique developed in our laboratory (SPIEGEL-ROY *et al.* 1985).

Results

Several modifications have been made in the *in vitro* protocol for embryo rescue developed by us (SPIEGEL-ROY *et al.* 1985), successfully adopted since also elsewhere (GRAY *et al.* 1987). At the 4-leaf stage plantlets are transferred from Petri dishes to Magenta vessels (7.6 cm L x 7.6 cm W x 10 cm H). The fresh agar medium contains neither IAA nor GA₃. However, NAA (naphthaleneacetic acid) 0.2 ppm is being added to the solid medium, resulting in a much more profuse root system. As the top of the Magenta vessel is reached, plantlets are transferred to Jiffy pots (No. 7) and these are being placed inside Magenta vessels. Jiffy pots have been moistened with water containing salts of the Nitsch medium (1/2 strength), but no vitamins or amino acids are added. NAA 0.2 ppm is again included. As soon as roots emerge from the Jiffy pot, plants are transferred to an unsterilized volcanic tuff-peat mixture in the greenhouse for hardening. We have found no advantage by adding BA (benzyladenine) to the solidified medium. A further factor influencing success of culture is size of seed traces; cultivars and selections with at least moderate seed traces (e.g. Flame Seedless, Ruby Seedless) yield a much higher proportion of developing embryos and plants than cultivars with very low ovule size and very small seed traces (e.g. Sultanina).

Table 1: Distribution of normal seeded (N), seedless with seed traces (B), and seedless without noticeable traces (S) in selfed and open pollinated progeny of seedless cultivars and in crosses made between seedless *V. vinifera* parents

female parent	male parent	normal seeded (N)	seedless seed traces (B)	seedless v. slight traces (S)
Perlette	O.P.	3	1	8
Flame Seedless	O.P.	6	1	40
Sultanina	O.P.	17	1	15
L 12	O.P.	1	0	1
Perlette	Perlette	0	0	8
L 12	L 12	0	0	2
Perlette	Beauty Seedless	0	0	3
Perlette	Flame	9	2	6
Perlette	L 12	5	0	12
L 12	Perlette	0	1	17
L 12	Sultanina	0	0	4
L 12	Flame Seedless	6	0	36
L 12	Ruby	11	1	30
L 12	Black Kishmish	1	2	0
Ruby	L 12	6	2	27
Ruby	Sultanina	21	1	35
Flame Seedless	Perlette	3	1	17
Flame Seedless	L 12	0	1	0
Sultanina	Flame Seedless	1	0	2
Sultanina	L 12	0	0	1
Centennial	Flame Seedless	1	0	2
Centennial	Sultanina	1	1	0

So far, 279 plants from seedless x seedless crosses including 10 developed from selfing of seedless parents have fruited. In addition open pollinated progeny of several seedless cultivars have also fruited. Results are summarized in Table 1. All 10 selfed progenies have been seedless without noticeable traces (S). Results from open pollinated progeny show a large difference between Sultanina, in which a close to 1 : 1 distribution between seedless and seeded was noted, and Flame Seedless progeny in which 41 seedless individuals were found compared to 6 normal seeded ones. These results point to a high degree of selfing under natural conditions with Flame Seedless and

Table 2: Distribution of normal (N), seedless with noticeable traces (B), seedless without noticeable traces (S) in progenies a) from crosses between seeded and seedless parents, b) crosses between seedless parents only

Year of observation	Type of cross	Normal seeded (N)	Seedless	
			B	S
1988	Seeded X seedless	1457	391	140
1988	Seedless X seedless	20	5	71
1989	Seedless X seedless	65	12	192

Table 3: Average fresh weight of single aborted seed and of total of aborted seeds per berry and their average number per berry in Ruby Seedless and L 12, in progenies from their reciprocal cross and in crosses between two seeded cultivars and Ruby Seedless*

Genotype and progenies	Avg. weight of single aborted seed mg	Avg. weight of aborted seed berry mg	Avg. no. aborted seed per berry	Avg. berry weight g	Range berry weight
Ruby Seedless	6.25	16.55	2.65	3.26	
L 12 (seedless)	11.00	37.30	3.40	4.19	
Ruby Seedless X L 12	7.64	19.51	2.52	2.44	1.27-3.94
L12 X Ruby Seedless	8.67	24.94	2.85	3.20	1.92-4.72
M. Alexandria X Ruby Seedless	19.99	56.34	2.70	3.08	1.57-4.54
Italia X Ruby Seedless	24.16	61.30	2.50	2.44	1.40-3.34

* 20 hybrids examined in each progeny. Only progenies rated as seedless have been included.

probably also with Perlette. Of the 269 hybrids derived from various crosses between seedless cultivars, 204 could be rated as seedless, while close to a quarter, 65 were rated as having normal seeds (Table 1). Normal seeded progeny appeared in crosses with all 6 seedless parents used as a female parent, including Perlette which was also shown to segregate only seedless progeny from selfed flowers. Another striking result is the preponderance of seedless progeny with very slight seed traces (S), 192 in number, by far exceeding those with noticeable seed traces (B), 12 only. The significance of this distribution will be even more apparent in comparison with the distribution between seedless (S) and seedless with traces (B) in progenies derived from crosses between seeded cultivars and selections (as a female parent) and seedless cultivars (male parent). Data are given in Table 2. Perusal of the table shows that in progeny from crosses between seeded x seedless only 26.4 % of the individuals rated as seedless were without noticeable traces (S), making out only close to 7.1 % of the total progeny. In contrast, seedless progeny derived from two seedless parents consisted nearly completely of individuals with barely noticeable seed traces (S). These constituted 93.4 % in 1988, 94.1 % in 1989 of the total progeny rated as seedless (B + S) (Table 2). The proportion of individuals devoid of noticeable seed traces in the total progeny (including seeded and seedless) amounted in 1988 to 74.0 %, in 1989 to 71.4 %.

In order to examine nature and size of aborted seed vestiges in seedless progenies by a method other than that of organoleptic determination, fresh weight of each aborted seed was determined in seedless parents and progenies from some crosses between seedless genotypes made in 1989. Similarly, fresh weight of each aborted seed was also determined in seedless hybrids derived from two crosses in which the female parent was a seeded cultivar and the male parent Ruby Seedless. Results are summarized in Table 3. No large differences were noted in the number of aborted seeds per berry, except a larger number with L 12. Average weight of a single aborted seed in the cross between seedless genotypes was between the average weight of the two parents. Average weight of a single aborted seed (column 1, Table 3) was much higher in the progeny classified as seedless derived from crosses of seeded x seedless (19.99 mg and 24.16 mg, respectively). This was reflected also in the much higher proportion of progeny rated as seedless with noticeable seed traces from the M. Alexandria x Ruby Seedless and Italia x Ruby Seedless cross (data not given). The average weight of all aborted seeds, taken together per single berry (column 2, Table 3), showed a similar trend and was nearly 3 times higher in progeny of crosses between seeded x seedless parents compared to the cross between the two seedless parents. Ruby Seedless has figured as a male parent in both types of crosses involved. Only progeny rated as seedless (with and without noticeable traces) has been included in the table. Rather similar berry weights and ranges in the progeny have been obtained in both types of cross. In the reciprocal seedless x seedless cross a rather large difference in berry weight has been obtained, pointing to an effect that could be due to cytoplasmic factors.

Further examination of the inheritance of fruit weight in seedless x seedless progenies has led us to try to compare fruit size in seedless progeny (normal seeded progeny has not been included in the comparison also because of lack of full data) from seedless x seedless crosses with seedless progeny derived from crosses between 4 seeded cultivars and 5 seedless parents. The comparison is somewhat incomplete as no hybrids or only a small number are available from certain crosses. Results are given in Table 4, presented in 4 columns. The 1st column shows progeny mean. The 2nd column represents the midparent value, while the 3rd column gives the percentage decrease of the progeny mean from the midparent value. Number of progeny in which berry weight of the seedless progeny has been determined is given in brackets in the 4th column. No definite conclusions concerning parental contribution can be inferred, though mean decrease in fruit weight with Sultanina seems indicated; however, only 2 crosses are involved. In progeny from selfed Perlette a similar berry weight decrease has been noted as that observed with Sultanina crosses (average of selfed Perlette 1.6 g against 2.56 g in Perlette, a 37.5 % decrease). On the whole, the 7 seedless x seedless populations averaged a similar decrease to the 11 progenies from seeded x

Table 4: Average berry weight in seedless fraction of progenies between seedless parents and between four seeded parents with seedless male parents. 1st column: Average berry weight (g) of progeny (seedless progeny only). 2nd column: midparent berry weight (g). 3rd column: percent berry weight decrease of seedless fraction of progeny compared to midparent. In brackets: number of seedless hybrids in the progeny

Female/male parent	Ruby Seedless	Perlette	L 12	Flame Seedless	Sultanina
Seedless:Ruby Seedless			2.44		1.50
			3.66		2.38
			33.3		37.0
			(20)		(27)
" Perlette			2.70	2.00	
			3.40	2.61	
			20.6	23.4	
			(9)	(16)	
" L 12	3.20	2.20		2.70	
	3.66	3.40		3.33	
	12.6	35.3		18.9	
	(20)	(10)		(30)	
Seeded Early Muscat		2.35	2.89		
		2.87	3.58		
		18.1	19.3		
		(33)	(12)		
" Muscat Hamburg	2.34	1.95		2.44	
	3.50	3.24		3.17	
	33.1	39.8		31.0	
	(62)	(19)		(69)	
" Italia	2.61		3.55	2.60	
	4.10		4.58	3.80	
	36.7		22.5	31.5	
	(25)		(17)	(41)	
" Muscat Alexandria	2.75			2.93	1.84
	3.85			3.52	3.03
	28.6			16.8	39.3
	(40)			(26)	(9)

seedless crosses, also displaying a similar range. While the lack of realization of a partial diallele setup does not allow the drawing of final conclusions, results seem to point to an essentially similar mode of inheritance of berry weight in other types of crosses, and as already noted with a much

better distribution of desirable seedless types (without noticeable seed traces) in progeny derived from seedless x seedless crosses.

Discussion

269 hybrids from crosses between stenospermocarpic *V. vinifera* parents, as well as a small selfed progeny and a sizable open pollinated progeny have fruited so far. *In vitro* method used by us for the rescue of embryos and aborted seed (SPIEGEL-ROY *et al.* 1985), Ga_3 and IAA proved to be necessary additions to the solid medium during the first phase of culture. While the addition of NAA was highly effective in enhancing at an early stage the development of a large root system of the *in vitro* grown hybrids, contrary to other findings (GRAY *et al.* 1987), no further benefit accrued from the addition of a cytokinin (BA). Though results obtained from selfing the seedless Perlette genotype do confirm the recessive nature of the seedless trait postulated by WEINBERGER and HARMON (1964) and LOOMIS and WEINBERGER (1979) and strongly indicated by results of our seeded x seedless crosses, the results obtained by us from crosses between seedless cultivars seem much more difficult to interpret. Such crosses segregated into 65 normal seeded and 204 seedless individuals. Contamination by airborne pollen during emasculation could not possibly account for such a relatively high rate of normal seeded progeny. Not taking into account the very small progenies from certain crosses between seedless parents (Table 1), only 1 cross, between L 12 and Perlette, did yield 100 % seedless progeny, while of 7 other crosses all gave rise to a certain number of seeded individuals in the progeny. These results contrast with the postulated 3 : 1 seeded x seedless ratio obtained in most seeded x seedless populations examined by us during 5 nearly consecutive years (SPIEGEL-ROY, unpublished results).

Analysis of results obtained from open pollinated progeny of seedless genotypes discloses some data of interest. With Flame Seedless and to a lesser extent with Perlette, the bulk of the open pollinated progeny was found to be seedless and, moreover, belonging to type S, with very slight seed traces. This suggests the possibility of selfing having occurred on a large scale, possibly before anthesis. This might not have been the case with Sultanina as in its progeny from open pollination a close to 1 : 1 ratio between seeded and seedless was found.

A distinct feature of the seedless progeny derived from seeded x seedless crosses is the preponderance of individuals with very slight seed traces. While in progeny of crosses from seeded x seedless genotypes only 6.5-9.5 % bore fruit with barely noticeable seed traces (SPIEGEL-ROY *et al.* 1986), constituting less than $\frac{1}{3}$ of the total progeny rated as seedless, analysis of seedless progeny from seedless x seedless crosses yielded over 94 % hybrids rated as seedless with slight seed traces. Thus, while crosses between seeded and seedless yield only less than 8 % progeny with slight seed traces, a total 71 % of the progeny from seedless x seedless crosses have borne seedless fruit without noticeable seed traces. This would amount in selection blocks from seedless x seedless crosses about 9 times more truly seedless progeny (from an identical number of hybrids) compared to that to be obtained from seeded x seedless progeny. Methods for measuring seed traces more objectively have been developed (MERIN *et al.* 1983; PERL *et al.* 1989). We have reported here on another simplified approach, namely determining fresh weight of aborted seed in individual berries. Differences between progenies of seedless x seedless crosses and progenies of seeded x seedless crosses are rather large with much smaller weight of aborted seed in progenies sampled from crosses between two seedless parents. Ruby Seedless has been a common parent in the progenies analyzed, including those with a seeded female parent.

Lower berry weight and perhaps also some inbreeding effect could have been anticipated in at least certain seedless x seedless crosses. It was therefore of interest to follow the inheritance of berry weight in seedless progeny from both seeded x seedless and seedless x seedless crosses. Berry size is known to be quantitatively inherited (SPIEGEL-ROY *et al.* 1981). Seeded parents used in crosses with seedless cultivars often have large berries. As we are interested primarily in seedless progeny

from seeded x seedless and seedless x seedless crosses, the analysis of berry size or weight from that portion of the progeny only will obviously give rise to a biased distribution. This will amount to a pronouncedly lower average size or weight compared to midparent values, as normal seeded fruit is potentially larger (MÜLLER-THURGAU 1898; WINKLER 1932). Analyzing, however, seedless progeny from seedless x seedless crosses and from seeded x seedless crosses, we found wide variation but no substantial difference in fruit size diminution compared to midparent values between the two groups. Moreover, in the latter group a much larger part of the progeny had noticeable seed traces, and still no better average berry weight was manifested in comparison to progenies originating from seedless x seedless crosses. In one case examined by us notable differences especially as to berry weight occurred in a reciprocal cross between two seedless genotypes, pointing to a cytoplasmic factor or factors in addition to that of nuclear genes. The development of the *in vitro* technique for embryo rescue will allow in the future, though more laborious, to test also the effect of reciprocal crosses between seeded and seedless genotypes. First selections with satisfactory berry size and with negligible traces have been already made by us from seedless x seedless progenies.

As transmission of the fruit size trait does not seem to differ essentially in crosses between seedless parents from that found in seeded x seedless crosses, use of two different seedless genotypes with large sized berries as male and female parents in a cross should prove effective in obtaining individuals with good fruit size in the progeny. Interspecific crosses between seedless types (GRAY *et al* 1987) may perhaps enhance plant vigour of hybrids along with some further contribution to fruit size.

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