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Inheritance of seedlessness in grapevine (*Vitis vinifera* L.)

by

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S u m m a r y : Despite considerable efforts made by breeders for over 70 years, inheritance of seedlessness in grapevine is not clearly defined. None of the numerous hypotheses proposed until now is satisfying, whether they are based on recessive or dominant genes. We measured precisely the phenotypic expression of the seeded/seedless character in a progeny obtained by crossing two partially seedless selections and using *in ovulo* and *in vitro* culture to rescue embryos. We propose the hypothesis that inheritance of seedlessness in grapevine is based on a complex system whereby the expression of three independently inherited recessive genes is controlled by a dominant regulator gene. This hypothesis was compared to other results published in the scientific literature and appeared coherent enough to be used as a theoretical basis for further work on seedlessness inheritance in grapevine. Attempts to explain the control of seedlessness involve interactions with endogenous gibberellins. The consequences of such a model for the development of breeding programs for seedless table grapes, and particularly for the use of molecular biology techniques, are discussed.

K e y w o r d s : grapevine, seedlessness, inheritance, Vitis vinifera.

Introduction

Seedless varieties of *Vitis vinifera* L. have been cultivated and prized as dried fruit for hundreds of years. Sultanina is by far the most commonly grown variety for this use. The seedlessness type is called «stenospermocarpic» (Stout 1936), and must be clearly distinguished from the «parthenocarpic» type of the variety Black Corinth. In Sultanina, fertilization occurs but seed development fails soon after (Nitsch *et al.* 1960; Barritt 1970) leaving small-sized or undetectable seed traces. Size of the berries is smaller than in normally seeded grapes, but can be improved by chemical treatments of gibberellic acid (Weaver 1958; Mavrikios 1977) or by genetic selection.

As seedless grapes are generally preferred for fresh consumption, breeding table grapes for seedlessness is an important field of research in many viticultural areas, particularly in California (Ledbetter and Ramming 1989). Classical breeding methods are based on hybridization between seeded and seedless varieties. However the proportion of seedless plants in the progenies is generally low and depends considerably on the choice of parents (Weinberger and Harmon 1964; Olmo and Baris 1973; Loomis and Weinberger 1979). By using *in ovulo* and *in vitro* culture technique, it is now possible to rescue viable embryos from seedless by seedless crosses (Cain *et al.* 1983; Emershad and Ramming 1984; Spiegel-Roy *et al.* 1985; Goldy and Amborn 1987; Gray *et al.* 1987; Barlass *et al.* 1988; Bouquet and Davis 1989; Tsolova 1990; Gribaudo *et al.* 1993). With this technique, a higher proportion of seedless plants can be recovered in the progenies (Barlass *et al.* 1988; Ramming *et al.* 1990; Spiegel-Roy *et al.* 1990 b). Attempts have also been made to use plant growth regulators as an alternative to *in ovulo* embryo culture (Ledbetter and Shonnard 1990).

Despite this progress and considerable work made by breeders for over 70 years, the inheritance of seedlessness in grapevine is not clearly understood, as shown by Tab. 1. None of the numerous hypotheses proposed until now is completely satisfying, even if the three dominant genes theory by Ledbetter and Burgos (1994) or the five dominant genes theory by Sato *et al.* (1994) seem to be viable hypotheses. Any hypothesis concerning the inheritance of seedlessness must take into account the fact that the seedless/seeded character is subject to mutation. Seedless berries have been observed on mutant canes (sports) at least nine times in the 20th century (Loomis and Weinberger

Table 1

The different hypotheses proposed for seedlessness inheritance in grapevine

1 recessive gene	Constantinescu *et al.* 1972 Dudnik and Moliver 1976
2 recessive genes	Bozhinova-Boneva 1978 Spiegel-Roy *et al.* 1990
Several recessive genes	Weinberger and Harmon 1964 Loomis and Weinberger 1979 Pospisilova and Patenik 1988
Quantitative factors	Sandhu *et al.* 1984 Golodriga *et al.* 1986
5 dominant genes	Sato *et al.* 1994
3 dominant genes	Ledbetter and Burgos 1994
1 dominant gene	Stout 1937 and 1939 Khachatryan and Martirosyan 1971

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1979). Likewise, there are reports of seeded grapes on seedless cultivars (KRIMBAS 1933). That tends to rule out the hypothesis based on quantitative factors where additional doses of genes might increase or decrease seed trace weight in a stepwise manner. Inheritance based on recessive genes cannot explain the occurrence of seeded phenotypes in seedless by seedless progenies or in selfings of seedless genotypes (BARLASS *et al.* 1988; RAMMING *et al.* 1990; SPIEGEL-ROY *et al.* 1990 a), nor the absence of seedless phenotypes in progenies obtained by self-pollination or crosses between seeded varieties having no seedlessness in their parentage. Inheritance based on dominant genes cannot explain the very low percentages of seedlessness often observed in seeded by seedless progenies (WEINBERGER and HARMON 1964), nor the occurrence of seedless phenotypes in progenies obtained by self-pollination of seeded varieties having seedlessness in their parentage (LOOMIS and WEINBERGER 1979).

This failure to know the inheritance of seedlessness in grapevine could limit considerably the exciting possibilities provided by the use of molecular biology techniques in breeding, and particularly the development of early selection procedures using molecular genetic markers (STRIEM *et al.* 1994). So, other hypotheses need to be proposed that explain, if not all, at least the majority of the results obtained by grape breeders. Inheritance studies are particularly difficult as the classification «seeded» or «seedless» is not clear cut, and the progenies of crosses range from completely seedless to normally seeded, with all the degrees of seed development. Sensory evaluation is too subjective to be used for seedlessness determination (LEDBETTER *et al.* 1994). Detectability of the seed traces, independently of their number and size, may be influenced by the degree of integumentary sclerification or by factors associated with the berry itself, as berry size or pulp texture. Several methods have been proposed to determine more precisely the level of seedlessness of varieties and selections. Seedless plants may be distinguished from seeded ones by having frequencies of less than 1 sinker per berry (LEDBETTER and SHONNARD 1991). Recent work presented by STRIEM *et al.* (1992) suggests that the seedless character can be divided into separate subtraits - hardness of the seed coat and degree of endosperm development - for a more accurate definition of phenotypic expression. Chemical methods have also been proposed: The total polyphenol content of berries separated from their skins was measured to quantify differences between seeded and seedless cultivars (MERIN *et al.* 1983). The inhibition of luciferase activity by whole berry extracts was also used as a quantitative measure of seed trace size (PERL *et al.* 1989).

Our study was conducted to determine as precisely as possible the phenotypic expression of the seeded/seedless character in a progeny obtained by crossing two partially seedless selections. Using the results of this analysis, we proposed the hypothesis that the inheritance of seedlessness in *Vitis vinifera* is based on a complex system whereby the expression of three independently inherited recessive genes is controlled by a dominant regulator gene. This hypothesis was tested on other results of seedless by seedless and seeded by seedless crosses, obtained in our laboratory, but also on the results of similar crosses, obtained and published by other grape breeding laboratories.

Materials and methods

The progeny (Mtp 3140) was obtained by crossing two partially seedless selections:

- Mtp 2223-27 (Dattier de Beyrouth x Sultana moscata)
- Mtp 2121-30 (Alphonse Lavallee x Sultanina)

Using embryo rescue by *in ovulo* and *in vitro* culture, 170 seedlings were cultivated in test tubes, acclimatized in greenhouse, grown in nursery, grafted and planted in the vineyard. 136 fruitful seedlings were analysed in 1994. On each seedling, 1 to 3 fruit clusters were harvested at full ripeness. Seeds or seed traces were extracted from 100 large uniform selected berries. The number, total fresh weight (TFW100) and total dry weight (TDW100) of seeds and seed traces were measured. Average fresh weight (AFW) and dry weight (ADW) per seed or seed trace were calculated, as the percentage of dry matter (% DM) which gives a quantitative estimate of the integumentary sclerification. Presence or absence of endosperm in the seeds and seed traces was also observed.

According to the distribution of the seedlings of the progeny in terms of TDW100 and % DM of their seed and seed traces (Fig. 1), 4 phenotypic classes have been characterized:

Class 1 (S): Seedless or small-sized seed traces with unsclerified integuments (% DM <40). Average number of remnants per berry <1. TDW100 <0.5 g. Endosperm always missing.

Class 2 (NST): Noticeable seed traces with unsclerified integuments (% DM <40). Average number per berry >1. TDW100 between 0.5 and 2.5 g. Endosperm very often missing.

Class 3 (HST): Large-sized hard seed traces with partially sclerified integuments (% DM between 40 and 50). Average number per berry >1. TDW100 between 0.5 and 5 g. Endosperm sometimes present, but poorly developed.

Class 4 (N): Normally developed seeds with totally sclerified integuments (% DM >60). Average number per

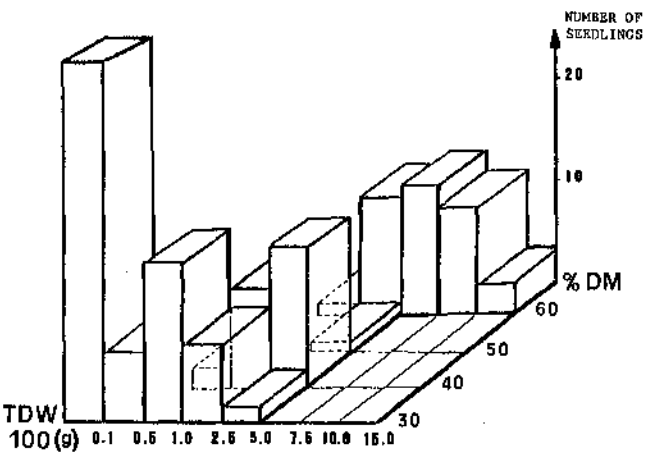

Fig. 1: Distribution of the seedlings of the progeny Mtp 3140 in terms of the total dry weight and % dry matter of seeds and seed traces in 100 berries.

berry >1. TDW100 between 2.5 and 15 g. Endosperm always present.

In addition to the progeny Mtp 3140, four other seedless by seedless progenies have also been studied and the seedlings were classified in the phenotypic classes previously defined:

- Mtp 3141: Mtp 2223-8 x Mtp 2121-30
- Mtp 3142: Mtp 2223-60 x Mtp 2121-30
- Mtp 3143: Mtp 2212-17 x Mtp 1993-15
- Mtp 3144: Mtp 2212-5 x Mtp 1993-15

Parents of the seedless by seedless progenies have been selected in seeded by seedless progenies obtained and observed 20 years ago (TRUEL and RENNES, unpublished results). Five of these progenies have been included in this study:

- Mtp 1993: Dattier de Beyrouth x Sultanina
- Mtp 2223: Dattier de Beyrouth x Sultana moscata
- Mtp 2121: Alphonse Lavallee x Sultanina
- Mtp 2212: Alphonse Lavallee x Sultana moscata
- Mtp 2298: Alphonse Lavallee x Perlette

At the time when these progenies were studied, the notation of the seeded/seedless character was made with only two phenotypes: Seedless and noticeable seed traces (S + NST), hard seed traces and normally developed seeds (HST + N).

Results

Progeny Mtp 3140: The distribution of the 136 seedlings in the 4 phenotypic classes and their average characteristics are shown in Tab. 2. The values of AFW and ADW are included, but not used to classify the seedlings, considering the difficulty to extract and weigh small remnants. It appears that some phenotypes classified as seedless on the base of TDW100, %DM and average number of seed traces per berry, present remnants whose AFW is varying from 7.8 to 16.9 mg. But the great majority of these phenotypes is characterized by AFW <10 mg. Similarly, phenotypes classified as NST present seed traces whose AFW is varying from 6.4 to 27 mg. Phenotypes of the parents are at the limit of the classes 2 and 3. The female and the male parent have 2.18 and 1.57 g TDW, with 39.3 and 40.3 % DM, respectively. Average numbers of seed traces per berry are 3.2 and 3.0. AFW of seed traces are 17.5 and 12.7 mg, respectively.

Table 2

Distribution of the seedlings of progeny Mtp 3140 in the four phenotypic classes and average characteristics

Phenotypes	1 (S)	2 (NST)	3 (HST)	4 (N)
Number	44	27	25	40
TFW 100 (g)	< 0.42 (0 - 1.4)	3.38 (1.6 - 7.3)	4.98 (1.1 - 9.8)	9.85 (3.5 - 23.1)
AFW (mg)	10.4* (7.8 - 16.9)*	14.8 (6.4 - 27.3)	20.2 (7.5 - 49.4)	54.3 (29.8 - 78.0)
TDW 100 (g)	< 0.15 (0 - 0.48)	1.25 (0.52 - 2.6)	2.30 (0.8 - 4.1)	6.40 (2.1 - 16.0)
ADW (mg)	3.4* (2.6 - 5.6)*	5.2 (2.3 - 10.4)	9.1 (4.6 - 21.6)	35.2 (19.2 - 54.1)
DM (%)	35.7* (31.8 - 39.5)	36.9 (30.6 -40.3)	46.1 (41.5 - 51.5)	64.9 (60.2 - 70.0)

* measured only on phenotypes with seed traces that could be extracted and weighed

We have tested a hypothesis based on a system of three complementary recessive genes a1 a2 a3 independently inherited, with incomplete dominance of the alleles driving normal expression of the seeded character. This system is placed under the control of a completely dominant regulator gene I. When this gene is homozygous recessive (i-/i-), the expression of the seedlessness genes is inhibited and all the phenotypes are N (class 4) whatever are the genotypes. When the gene is heterozygous (I+/i-) or homozygous dominant (I+/I+), there is expression of the seedlessness genes, and the observed phenotypes correspond to the different possible genotypes (Tab. 3). Expression of the S (seedless) phenotype requires a minimum of two genes to be homozygous recessive. When only one gene is homozygous recessive, there is presence of seed traces and the distinction between the phenotypes NST and HST depends on the homozygous or heterozygous condition of the dominant alleles of the two other genes. If none of the three genes is homozygous recessive, the plants are normally seeded (N). If we consider that the parents

Table 3

Correspondence between observed phenotypes and possible genotypes for the seeded/seedless character

Phenotypes	Genotypes (I +/I -) or (I +/I +)						Genotypes (I -/I -)
Class 1 (S)	a1a1	a2a2	a3a3	A1A1	a2a2	a3a3	no genotype
	a1a1	A2a2	a3a3	a1a1	A2A2	a3a3	
	a1a1	a2a2	A3a3	a1a1	a2a2	A3A3	
	A1a1	a2a2	a3a3				
Class 2 (NST)	a1a1	A2a2	A3a3				no genotype
	A1a1	a2a2	A3a3				
	A1a1	A2a2	a3a3				
Class 3 (HST)	a1a1	A2A2	A3a3	A1A1	a2a2	A3a3	no genotype
	a1a1	A2A2	A3A3	A1A1	a2a2	A3A3	
	a1a1	a2A2	A3A3	A1A1	A2a2	a3a3	
	A1a1	A2A2	a3a3	A1A1	A2A2	a3a3	
	A1a1	a2a2	A3A3				
Class 4 (N)	A1a1	A2a2	A3a3	A1A1	A2a2	A3a3	all genotypes
	A1a1	A2A2	A3a3	A1A1	A2A2	A3a3	
	A1a1	A2A2	A3A3	A1A1	A2a2	A3A3	
	A1a1	A2a2	A3A3	A1A1	A2A2	A3A3	

Mtp 2223-27 and Mtp 2121-30 are phenotypically NST and have the same genotype A1a1 A2a2 a3a3 (I+/i-), the cross will give a 21:12:15:16 expected ratio (Tab. 4). The observed distribution fits this ratio (chi-2 = 2.65).

Other seedless by seedless progenies: The female parents of the progenies 3141 and 3142 are descended from the cross Dattier de Beyrouth x Sultana moscata, as the female parent of the progeny 3140, but their phenotypes must be classified as HST (BOUQUET and DAVIS 1989) and we can attribute to them the genotype A1A1 A2a2 a3a3 (I+/i-). The male parent is the same as in the progeny 3140 and we have attributed to it the genotype A1a1 A2a2 a3a3 (I+/i-). The cross will give a 6:6:12:8 expected ratio that fits the observed distributions of the phenotypes in the progenies (Tab. 5).The female parents of the progenies 3143 and 3144 are descended from the cross Alphonse Lavallee x Sultana moscata, and the male parent from the cross Dattier de Beyrouth x Sultanina. Their phenotypes can be classified as NST (BOUQUET and DAVIS 1989). If we attribute to the female parents the genotype

Table 4

Gametic segregation and rearrangements corresponding to a phenotypic ratio 21 (1) : 12 (2) : 15 (3) : 16 (4)

gametes 2223-27			gametes 2121-30	A1 A2 a3 (I+)	A1 a2 a3 (I+)	a1 A2 a3 (I+)	a1 a2 a3 (I+)	A1 A2 a3 (i-)	A1 a2 a3 (i-)	a1 A2 a3 (i-)	a1 a2 a3 (i-)
A1	A2	a3	(I+)	3	3	3	2	3	3	3	2
A1	a2	a3	(I+)	3	1	2	1	3	1	2	1
a1	A2	a3	(I+)	3	2	1	1	3	2	1	1
a1	a2	a3	(I+)	2	1	1	1	2	1	1	1
A1	A2	a3	(i-)	3	3	3	2	4	4	4	4
A1	a2	a3	(i-)	3	1	2	1	4	4	4	4
a1	A2	a3	(i-)	3	2	1	1	4	4	4	4
a1	a2	a3	(i-)	2	1	1	1	4	4	4	4

Genotype Mtp 2223-27 and Mtp 2121-30 : A1a1 A2a2 a3a3 (I+/i-)

A1a1 A2a2 a3a3 (I+/i-) and to the male parent the genotype A1a1 a2a2 A3a3 (I+/i-), we obtain the expected ratio 18:15:6:25 that fits the observed distributions of the phenotypes, despite high values of the chi-2 which can be attributed to the smaller size of the progenies and the less precise notation than the one made on the progeny Mtp 3140.

Seeded by seedless progenies: Sultana moscata (75 Pirovano) was obtained from the cross Muscat d' Alexandrie x Sultanina. Perlette was obtained from the cross Muscat Reine des Vignes x Sultanina. As the three seedless varieties are phenotypically very close, we can assume they have the same genotype A1a1 a2a2 a3a3 (I+/i-), and group together the progenies Mtp 1993 and 2223 on the one hand, the progenies Mtp 2121, 2212 and 2298 on the other hand. If we attribute the genotype A1a1 A2a2 A3a3 (i-/i-) to the variety Dattier de Beyrouth and the genotype A1A1 A2a2 A3a3 (i-/i-) to the variety Alphonse Lavallee, the observed distributions of the phenotypes fit closely the expected ratios (Tab. 6).

Progenies studied in other laboratories whose results have been published: We tested our model on the results obtained and published by the Department of Fruit Breeding and Genetics, ARO, Volcani Center, Israel (SPIEGEL-ROY *et al.* 1990 a and b). Progenies from various seedless by seedless crosses segregated into 192 seedless without noticeable seed traces (S), 12 seedless with noticeable seed traces (NST) and 65 normal seeded (N). That fits a 3:1 ratio (chi-2 = 0.01), if we group together the two categories of seedless phenotypes. Such a ratio is expected in crosses between seedless cultivars with genotypes A1a1 a2a2 a3a3 (I+/i-). Progenies from various seeded by seedless crosses segregated into 140 seedless without noticeable seed traces, 391 seedless with noticeable seed traces and 1457 normal seeded. That fits a 1:3:12 ratio (chi-2 = 3.70) if we consider that the phenotypes rated as normal seeded (N) by SPIEGEL-ROY *et al.* (1990 a) include also phenotypes rated as hard seed traces (HST). Such a ratio is expected when a seeded variety with genotype A1a1 A2a2 A3A3 (i-/i-), is crossed by a

Table 5

Correspondence between observed and theoretical distributions of phenotypes in 5 seedless by seedless progenies

Progenies	Phenotypes 1	2	3	4	Total	Parental genotypes Female (I+/i-)			Male (I+/i-)			Ratio	Chi-2
Mtp 3140	44	27	25	40	136	A1a1	A2a2	a3a3	A1a1	A2a2	a3a3	21:12:15:16	2.65
Mtp 3141	14	12	15	9	50	A1A1	A2a2	a3a3	A1a1	A2a2	a3a3	6: 6:12: 8	3.87
Mtp 3142	7	3	17	10	37	A1A1	A2a2	a3a3	A1a1	A2a2	a3a3	6: 6:12: 8	2.95
Mtp 3143	12	14	3	10	39	A1a1	A2a2	a3a3	A1a1	a2a2	A3a3	18:15:6:25	3.67
Mtp 3144	21	26	10	22	79	A1a1	A2a2	a3a3	A1a1	a2a2	A3a3	18:15:6:25	4.84

Critical value of Chi-2 (p = 0.05; 3 d.f.) : 7.81

Table 6

Correspondence between observed and theoretical distribution of phenotypes in 5 seeded by seedless progenies. (Results from TRUEL and RENNES, unpublished)

Progenies	Phenotypes 1+2	3+4	Total	Parental genotypes Female (i-/i-)			Male (I+/i-)			Ratio	Chi-2
Mtp 1993 + 2223	44	80	124	A1a1	A2a2	A3a3	A1a1	a2a2	a3a3	11:21	0.07
Mtp 2121 + 2212 + 2298	58	167	225	A1a1	A2a2	A3A3	A1a1	a2a2	a3a3	1:3	0.07

Critical value of Chi-2 (p = 0.05; 1 d.f.) : 3.84

seedless variety with genotype A1a1 a2a2 a3a3 (I+/i-). High value of the chi-2 is probably due to the fact that these results include numerous crosses with different seeded and seedless varieties. Probably, some of these varieties do not have the same genotypes.

Likewise, this inheritance model was tested with the results obtained and published by the Horticultural Crops Research Laboratory, USDA/ARS, Fresno, California (LOOMIS and WEINBERGER 1979; RAMMING *et al.* 1990; LEDBETTER and BURGOS 1994). Results concerning seedless by seedless crosses (RAMMING *et al.* 1990) are particularly interesting as the authors distinguished between two levels of seedlessness, based on the average fresh weight of seed traces: 25 mg FW was selected as the best division between seeded and seedless phenotypes, but 10 mg FW was selected as the maximum seed size for consumer acceptance as seedless. 10 and 25 mg FW correspond approximatively to the upper limits of our classes 1 (S) and 2 (NST). According to our results and the seedling distributions in the histograms presented by the authors, we have selected 45 mg FW as the best division between our classes 3 (HST) and 4 (N). Tab. 7 shows that we can attribute to the 14 seedless varieties or selections used by these authors, different genotypes corresponding to the phenotypic expression of seedlessness, and that the nine progenies fit the expected ratios.

Tab. 8 presents 16 progenies obtained at the Fresno USDA/ARS laboratory, by crossing 3 seeded and 10 seedless varieties or selections (LEDBETIER and BURGOS 1994). In this case, only two phenotypes were observed (S + NST) and (HST + N). As previously, 25 mg FW for seed traces was selected as the best division between seedless and seeded phenotypes. 12 progenies fit the expected ratios, according to the genotypes attributed. The unexpected result obtained with Sultanina is difficult to explain. The heterozygous genotype (I+/i-) we attributed to Kishmiski is uncommon for a seeded variety. It is justified by the fact that LOOMIS and WEINBERGER (1979) observed 11 % seedlessness in a 28 plants progeny obtained by crossing the seeded varieties Nunakasia (syn. Kishmiski) and Cardinal. That fits closely (chi-2 = 0.07) the 6:58 expected ratio, if we attribute the genotypes A1a1 A2a2 A3A3 (I+/i-) and A1a1 A2a2 A3a3 (i-/i-) to these varieties. However, the unexpected presence of seedless plants in the abovementioned seeded by seeded progeny could be unsignificant or due to errors of notation. In this case, if we attribute to Kishmiski a more classical genotype a1a1 A2a2 A3A3 (i-/i-), the 3 progenies listed in Tab. 8 fit the 1:3 expected ratio.

More evidence supporting our hypothesis may be obtained from the high levels of seedlessness (up to 83%) observed by LEDBETTER and BURGOS (1994) in progenies involving the seeded variety A81-110 and 9 seedless selections from *in ovulo* embryo culture of seedless by seedless crosses. Tab. 9 shows that among the 9 progenies, one fits a 1:7 phenotypic ratio, four fit a 3:1 ratio, two fit a 1:1 ratio and two fit a 3:5 ratio. The 1:1 and 3:5 ratios are expected if we attribute the genotype a1a1 A2a2 A3a3 (i-/i-) to the seeded variety A81-110, the genotypes a1a1 a2a2 a3a3 (I+/i-) and A1a1 a2a2 a3a3 (I+/i-) to the seedless selections. As for the 3:1 and 7:1 ratios, they are expected only with seedless genotypes A1A1 a2a2 a3a3 (I+/I+) and A1a1 a2a2 a3a3 (I+/I+), which can be obtained only in seedless by seedless crosses.

Our model can explain the very low percentages, or the absence of seedless plants observed in numerous seeded by seedless crosses (WEINBERGER and HARMON 1964). The crosses between the seedless genotypes A1a1 a2a2 a3a3 (I+/i-) or A1A1 a2a2 a3a3 (I+/i-), and the seeded genotypes A1A1 A2A2 A3A3 (i-/i-) or A1a1 A2A2 A3A3 (i-/i-) will give progenies without seedless plants, or at best with 12.5 % seedless plants with noticeable seed traces. Seeded sports on seedless varieties (I+/i-) can be explained by the mutation of the allele I+. In this case, Sultanina monococco, which is considered as a seeded mutation of Sultanina (KRIMBAS 1933), must have the genotype A1a1 a2a2 a3a3

Table 7

Correspondence between observed and theoretical distributions of phenotypes in 9 seedless by seedless progenies. (Results from RAMMING *et al.* 1990)

Progenies	Phenotypes 1	2	3	4	Total	Parental genotypes (I+/i-) and phenotypes * Female			Male			Ratio	Chi-2
C85-82 x C20-149	21	7	3	5	36	A1a1	A2a2 (10,6) *	a3a3	A1a1	a2a2 (5.6)	a3a3	15:6:3:8	2.87
P60-58 x Sultanina	28	5	5	9	47	A1a1	A2a2 (13,8)	a3a3	A1a1	a2a2 (3.2)	a3a3	15:6:3:8	4.51
B46-112 x C18-36	16	0	0	8	24	A1a1	a2a2 (5.7)	a3a3	A1a1	a2a2 (5.9)	a3a3	12:0:0:4	0.89
A71-185 x C32-68	21	15	7	21	64	A1a1	A2a2 (19.9)	a3a3	a1a1	A2a2 (14.2)	A3a3	18:15:6:25	0.67
P79-101 x C32-68	16	4	2	2	24	A1a1	A2a2 (25.0)	a3a3	a1a1	A2a2 (14.2)	A3a3	18:15:6:25	4.65
P79-101 x C33-199	19	7	3	5	34	A1a1	A2a2 (25.0)	a3a3	A1a1	a2a2 (0.0)	a3a3	15:6:3:8	2.11
P79-101 x Flame Sd	58	24	0	29	111	A1a1	A2a2 (25.0)	a3a3	a1a1	a2a2 (5.9)	A3A3	15:8:0:11	3.11
P79-101 x B31-164	7	6	1	10	24	A1a1	A2a2 (25.0)	a3a3	a1a1	A2a2 (13.8)	A3a3	18:15:6:25	0.75
P79-101 x C35-33	19	15	21	23	78	A1a1	A2a2 (25.0)	a3a3	A1a1	A2a2 (14.0)	a3a3	21:12:15:16	2.83

Critical value of Chi-2 (p=0.05; 3 d.f.) : 7.81

( )* : Seed trace weight (mg FW) determined by weighing the largest seed from the 10 largest berries

Table 8

Correspondence between observed and theoretical distributions of phenotypes in 16 seeded by seedless progenies. (Results from LEDBETTER and BURGOS 1994)

Progenies		Phenotypes 1+2	Phenotypes 3+4	Total	Male genotype (I+/i-)	Ratio	Chi-2
Kishmiski	x C32-129	17	80	97	a1a1 a2a2 A3A3	3:13	0.10
	x C35-33	17	66	83	A1a1 A2a2 a3a3	15:49	0.41
	x Fresno Seedless	13	51	64	a1a1 A2a2 A3a3	15:49	0.34
P45-98	x Sultanina	57	164	221	A1a1 a2a2 a3a3	7:9	26.90 S
	x Perlette	22	42	64	A1a1 a2a2 a3a3	7:9	2.28
	x Autumn Seedless	20	51	71	a1a1 a2a2 A3a3	7:9	6.90 S
	x Flame seedless	90	189	279	a1a1 a2a2 A3A3	3:5	3.25
	x C32-145	16	68	84	a1a1 a2a2 A3A3	3:5	12.29 S
	x Black Monukka	43	93	136	A1a1 A2a2 a3a3	11:21	0.47
	x Fresno seedless	92	159	251	a1a1 A2a2 A3a3	11.21	0.57
C15-133	x Autumn seedless	72	81	153	a1a1 a2a2 A3a3	7:9	0.66
	x Fiesta	93	135	228	a1a1 a2a2 A3a3	7:9	0.76
	x C32-145	27	49	76	a1a1 a2a2 A3A3	3:5	0.13
	x Black Monukka	89	174	263	A1a1 A2a2 a3a3	11:21	0.03
	x C35-33	20	51	71	A1a1 A2a2 a3a3	11:21	1.21
	x Fresno seedless	36 (*)	29 (*)	65	a1a1 A2a2 A3a3	11:21	12.80 S
					Critical value of Chi-2 (p = 0.05; 1 d.f.) : 3.84		
Female genotypes		Kishmiski :			A1a1 A2a2 A3A3 (I+/i-)		
		P45-98 and C15-133 :			a1a1 A2a2 A3a3 (i-/i-)		

(*) Possibility of error : RAMMING *et al.* (1990) give 26:39 for the cross C15-133 x C32-68 (Fresno seedless). In that case, chi-2 value is 0.59.

Table 9

Correspondence between observed and theoretical distributions of phenotypes in 9 seeded by seedless progenies. Male parents are obtained from seedless by seedless crosses and *in vitro* embryo rescue. (Results from LEDBETTER and BURGOS 1994)

Progenies	Phenotypes (1+2)	Phenotypes (3+4)	Total	Parental male genotypes		Ratio	Chi-2
A81-110 x A32-167	54	11	65	A1a1 a2a2 a3a3	(I+/I+)	7:1	1.15
A81-110 x A32-140	103	29	132	A1A1 a2a2 a3a3	(I+/I+)	3:1	0.64
A81-110 x A32-107	45	17	62	A1A1 a2a2 a3a3	(I+/I+)	3:1	0.19
A81-110 x A32-151	45	12	57	A1A1 a2a2 a3a3	(I+/I+)	3:1	0.47
A81-110 x A32-122	44	18	62	A1A1 a2a2 a3a3	(I+/I+)	3:1	0.53
A81-110 x A32-171	24	30	54	a1a1 a2a2 a3a3	(I+/i-)	1:1	0.66
A81-110 x A32-173	37	35	72	a1a1 a2a2 a3a3	(I+/i-)	1:1	0.06
A81-110 x A32-152	16	24	40	A1A1 a2a2 a3a3	(I+/i-)	3:5	0.14
A81-110 x A32-133	13	23	36	A1A1 a2a2 a3a3	(I+/i-)	3:5	0.03
				Critical value of Chi-2 (p = 0.05; 1 d.f.) : 3.84			
Female genotype (A81-110) :				a1a1 A2a2 A3a3	(i-/i-)		

(i-/i-). Sultanina monococco has been crossed with different seedless or partially seedless varieties or selections (OLMO and BARIS 1973). The progenies, grouped together, fit a 7:9 ratio (chi-2 = 0.16), expected if we attribute the genotype a1a1 A2a2 A3a3 (I+/i-) to the seedless parents.

Discussion

Seedless sports on a seeded variety (i-/i-), by reverse mutation of the allele i-, would be possible only if the seeded variety has two genes of the system a1 a2 a3 homozygous recessive. That excludes *a priori* the possibility of seedless mutation on varieties like Cardinal, Alphonse Lavallee or Dattier de Beyrouth, taking into account the genotypes we attributed to them in this study. The varieties Chasselas, Muscat de Hambourg, Concord and Emperor, on which seedless sports have been observed (BAILEY 1887; STURTEVANT 1890; STOUT 1936; OLMO 1940), could have genotypes like A1A1 a2a2 a3a3 (i-/i-) or A1a1 a2a2 a3a3 (i-/i-), and give consequently high seedless progenies when crossed with seedless parents. The cross Muscat de Hambourg x Perlette gave effectively high seedless progeny (DOAZAN and OTTENWÄLTER, unpublished results). Seedless progeny was also obtained with the cross Concord x Sultana (RAMMING, pers. comm.), but on the other hand, the cross Emperor x Perlette gave no seedless progeny (WEINBERGER and HARMON 1964). Further work is needed to examine the characteristics and inheritance of seedlessness in Emperor seedless. No report of crosses between Chasselas and seedless varieties is available.

Our model implies that some normally seeded plants in seeded by seedless or seedless by seedless progenies could have very different genotypes, for instance a1a1 a2a2 a3a3 (i-/i-) and A1a1 A2a2 A3a3 (I+/i-). So, in the search

for molecular markers linked to genes of seedlessness, much caution will be needed in using simplified methods like «Bulk Segregant Analysis» (MICHELMORE *et al.* 1991) where a great number of RAPD primers will be tested with only two samples made with the DNAs extracted from plants showing the extreme phenotypes seedless and normal seeded. Possibly, analysis of individual plants will be necessary.

The model is based on three independently inherited recessive genes whose expression is controlled by a dominant regulator gene. Inheritance of characters based on triplicate factors is not uncommon in grapevine (MULLINS *et al.* 1992) and supported by the work of PATEL and OLMO (1955), which shows the genome of *Vitis* to be probably made up with three sets of chromosomes. On the other hand, our model would be the first example of the control of a character of agronomic importance in grapevine, by a regulator gene. It is possible to propose different hypotheses to explain the regulated expression of seedlessness in grapevine. One of the more plausible involves the gibberellin effect on the developmental biology of the flower and young berry. Application of the growth retardant Cycocel (2-chloro-ethyl-trimethylammonium chloride), 18 days before bloom, significantly reduces the expression of seedlessness in a stenospermocarpic grapevine selection (LEDBETTER and SHONNARD 1990). As Cycocel typically acts as an inhibitor of the biosynthesis of gibberellins, we can suppose that the expression of the seedless/seeded character is linked to the concentration of endogenous gibberellins in the flowers and young berries, during anthesis and the post-bloom stage.

We can hypothesize a genetic system in which normal development of the seeds is governed by three sets of structural genes whose expression is controlled by the regulator gene. The allele I+ produces a repressor which is activated in presence of gibberellins (co-repressor), and block the transcription of the structural genes through the three genes a1 a2 a3, which act as operator genes (Fig. 2). When 1, 2 or 3 operator genes are homozygous, there is partial or total expression of seedlessness. As seeds are known as the main source of endogenous gibberellins in the berries, expression of seedlessness will lower the concentration of the co-repressor. The repressor becomes inactive and development of the seed resumes. The system is regulated and lead to seed traces whose size and hardness are in inverse ratio to the number of homozygous alleles, as these alleles are the receptive sites of the activated repressor. If the concentration of co-repressor is lowered by treatments with inhibitors of gibberellin biosynthesis, as Cycocel, there will be development of noticeable and hard seed traces in seedless genotypes A1a1 a2a2 a3a3 (I+/i-) or A1a1 A2a2 a3a3 (I+/i-), as observed by LEDBETTER and SHONNARD (1990).

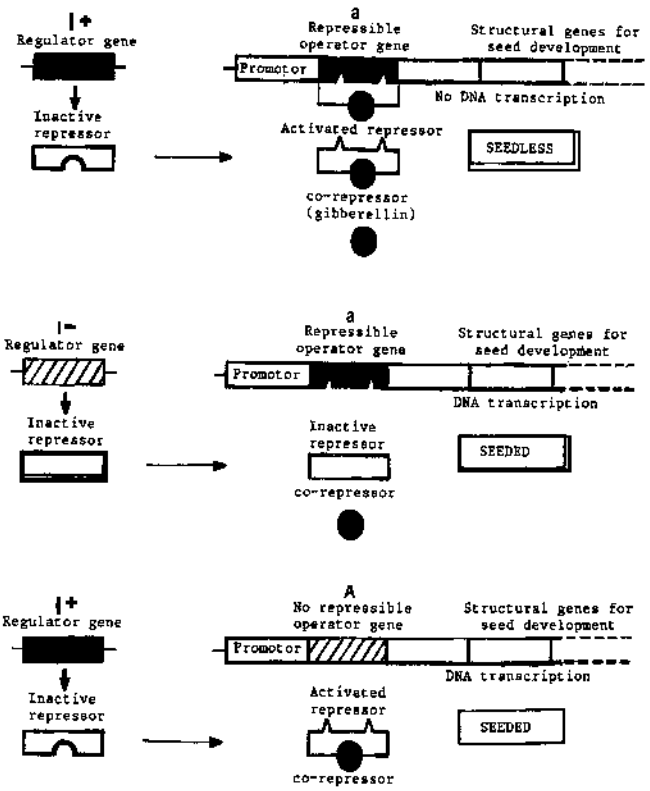

Fig. 2: Hypothesis for a regulated genetic expression of seedlessness in grapevine.

A1 A2 A3 correspond to allelic forms of the operator genes which are insensitive to the repressor: Even in the presence of the repressor (genotypes I+/I+ or I+/i-) and co-repressor (gibberellins) in the berries, there will be no expression of seedlessness if all three genes are homozygous AA or heterozygous Aa. The allele i- produces a structurally modified repressor which cannot be activated by endogenous gibberellins. The operator genes a1 a2 a3 cannot be repressed and there is constitutive expression of seededness when the regulator gene is homozygous (i-/i-). It is obvious that this attempt of explanation is only one hypothesis among others and can appear highly speculative. But gibberellins are likely to take a prominent part in seedlessness, possibly in association with other growth substances, like auxins (WEAVER 1953; NITSCH *et al.* 1960), or ethylene (KENDER and REMALY 1970). That could explain some environmental or rootstock effects observed on seedlessness (CHRISTENSEN *et al.* 1983). Further work is needed, including comparative determination of endogenous gibberellins in seeded and large-berried seedless varieties, and experimental treatments with gibberellins and inhibitors of gibberellin biosynthesis. The confirmation of the existence of the regulator gene by further studies could open exciting prospects for a better knowledge of the developmental biology of the berry in grapevine.

Conclusions

The model of inheritance we propose can explain the observed distributions of the seedless character in numerous seeded by seedless and seedless by seedless progenies. According to the phenotypic expression of seedlessness, different genotypes have been attributed to the seedless varieties, selections and seedlings. In the great majority of the cases, the progenies fit the expected ratios. Nevertheless, in some cases, results are difficult to explain. Possibly, the expression of seedlessness is influenced by modifying factors present in seeded and/or seedless varieties. This influence, connected with the effect of environment,

is certainly more pronounced at middle levels of seedlessness (noticeable or hard seed traces) than at extreme levels (seedless and normally seeded). The validation of our model needs further work with clearly defined crosses and precise evaluation of seedlessness in the progenies. In particular, our model implies the existence in seedless by seedless progenies, of completely homozygous genotypes a1a1 a2a2 a3a3 (I+/I+) and a1a1 a2a2 a3a3 (i-/i-), which have phenotypes being seedless and normal seeded, respectively. Identification of such plants would be of considerable value for breeders, as their cross should produce 100 % seedless progenies.

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