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The degrees of development of the seed-coat and the endosperm as separate subtraits of stenospermocarpic seedlessness in grapes[1])

by

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Die Entwicklungsgrade von Kernhülle und Endosperm als separate Untereigenschaften von stenospermokarper Kernlosigkeit bei Reben

Zusammenfassung: Die Entwicklungsgrade von Kernhüllen und Endosperm stenospermokarper *Vitis vinifera*-Sorten wurden untersucht. Die Kerne kernloser und kernhaltiger Beeren wurden visuell in 4 Größenkategorien eingeteilt: normal entwickelte Kerne, große, mittlere und kleine Kernspuren. Es wurde festgestellt, daß Kerne mit erhärteten, sklerifizierten und vollentwickelten Kernhüllen mit Endosperm unterschiedlicher Entwicklungsgrade auftreten. In Kernen mit weichen oder wenig entwickelten Hüllen wurde auch vollentwickeltes Endosperm gefunden.

Wir fanden, daß die Härte der Kernhülle und der Entwicklungsgrad des Endosperms den Nachkommen getrennt vererbt werden. Zwei kernhaltige Rebsorten (Oz und Early Muscat) wurden als weibliche Eltern gewählt, weil sie sich klar unterscheiden: die normalen Kerne von Oz sind härter und enthalten weniger entwickeltes Endosperm als die von Early Muscat. Jede Rebsorte wurde mit demselben Pollenspender (Flame Seedless) gekreuzt. 23,7 % der Oz, aber nur 1,2 % der Early Muscat hatten normale Kerne mit teilweise unentwickeltem Endosperm.

Unsere Resultate deuten darauf hin, daß Kernlosigkeit in Weintrauben besser analysiert werden kann, wenn man Untereigenschaften (Härtegrad der Kernhülle und Entwicklungsgrad des Endosperms) benutzt.

Key words: grape breeding, Vitis vinifera, seedlessness, stenospermocarpy, grapes.

Introduction

Seedlessness in table grapes is considered a trait of increasing importance to the consumer. The existence of several developmental stages of seed traces in stenospermocarpic grapes was described in detail by STOUT (1936). A whole range of sizes of seeds and seed traces, with a continuous nature, is found in grape progenies resulting from crosses. The existence of small-but-noticeable, and large-but-undetected seed traces, makes it difficult to distinguish between offsprings, usually divided into groups (seeded versus seedless) according to their seed content. Different parameters correlated to the seed content of the berries were suggested (PERL *et al.* 1989), a threshold size in fresh weight of the seeds (RAMMING *et al.* 1990), and also the use of the frequency of sinkers (LEDBETTER and SHONNARD 1991). A three category classification was practiced by SPIEGEL-ROY *et al.* (1990 b): (I) normal seeds (N); (II) with noticeable seed traces and texture of the seed deviating from that of the pulp (B): (III) practically seedless, without noticeable seed traces (S). In referring to the seeded:seedless ratio, from the genetic stand point, the (B) group is also considered 'seedless'. On the other hand, the detection of the presence (perceptibility) of the less developed seed traces is also affected by the firmness and crispness of the berry (LEDBETTER and SHONNARD 1991),

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thus, an organoleptic determination cannot be considered as a sufficiently valid classification for the degree of development of the seeds, and a genetic analysis of seedlessness, based on these observations might be biased to some extent.

Numerous theories for the inheritance of the stenospermocarpic character have been proposed (STOUT 1936; WEINBERGER and HARMON 1964; OLMO and BARIS 1973; LOOMIS and WEINBERGER 1979; SPIEGEL-ROY 1979; SANDHU *et al.* 1984; LEDBETTER and RAMMING 1989; SPIEGEL-ROY *et al.* 1990 a; RAMMING *et al.* 1990). No wholly adequate inheritance scheme, taking into full account the variable degrees of development of the seed traces, has yet been proposed.

In our studies we have noted that the perceptibility of the seed traces is not necessarily correlated to the size of the seed, but rather to the hardness of the seed coat. Furthermore, in progenies of crosses between 'seeded × seedless' cultivars, we have noticed several combinations of the hardness of the seed coats with different degrees of development of the endosperm. These observations led us to conduct the present study, to examine whether assessing the degrees of development of the seed components (i.e. seed coat, endosperm and, possibly, embryo) as separate traits, could contribute to a better understanding of the seedless character in stenospermocarpic grapes.

Material and methods

Plant Material. Clusters from 27 cultivars, as well as from 84 offsprings of Early Muscat × Flame Seedless progeny, and 118 offsprings of Oz × Flame Seedless progeny, were sampled at fruit maturity, in the vineyard at Bet Dagan. 2—5 samples/cultivar were gathered and one sample for each hybrid offspring. The largest 20 berries in each sample were carefully removed for evaluation and classification of the seeds. Berries classified as 'shot berries' were not included.

Classification and evaluation of seeds. Seeds and seed traces from each sample were removed from the berries by longitudinal sectioning with a razor. In each sample (20 berries), and sometimes, even in the same berry, several kinds of seeds/seed traces were found to be present. Normal seeds were clearly distinguishable from less developed seed traces. When normal seeds were not present, and other sizes of seed traces were present, all were visually classified into four categories of seeds: (1) normal seeds, (2) large traces, (3) medium and (4) small traces, which were later found to be significantly different in parameters such as fresh weight (mg) and length (mm). The samples from cultivars and progenies were also classified into four groups of seed content according to the largest seeds in each sample: genotypes bearing normal seeds (e.g. Italia, Cardinal), large traces (Flash Seedless, 57), medium traces (Perlette, Flame Seedless) and small traces (Sultanina, Ruby Seedless).

Evaluation of the seed components. 5—10 seeds and seed traces in each category of size, were longitudinally dissected. Using a stereoscopic 40 × magnification, the degree of development of the seed coat, the endosperm and the embryo, were visually evaluated into three degrees of development. The degree of hardness of the seed coat ranged from soft = 1 to hard = 3, and was evaluated separately from the colour of the seed coat (light = 0 and dark = 1). These grades were summed up, resulting in a range of 1—4, describing the degree of development of the seed coat. The reddish colour of seeds, located in the outer integuments, was not affecting the hardness of the seed coat, hence only the dark or light colour of the inner integuments was evaluated.

The endosperm development ranged also from fully developed endosperm, which filled the whole volume of the seed (grade '3'), to undeveloped endosperm (grade '1').

Fully developed embryos (torpedo-shaped) were found when enough supporting endosperm tissue was present. Less developed embryos as described by STOUT (1936), which range from a few cells to the globular stage (roembryos) were classified as 'undeveloped' (= grade '1').

For the evaluation of the potential of a genotype to develop seeds, the group of the largest seeds in each sample was chosen to represent the degree of development of the seed components: seed coat, endosperm and embryo.

Results

The differences between the cultivars, classified into four groups according to the largest seeds, were found to be more significant in the fresh weight of the seeds (total fresh weight of all seeds per berry, and mean fresh weight of one seed), than in the number of seeds per berry (data available). When analysing the relation between the size of the seeds and the degree of development of seed components, it was found that the smaller seeds had softer seed coats and less developed endosperm (Tab 1). However, several combinations of hardness of the seed coat with different degrees of development of the endosperm were observed.

Table 1

The relation between of the cultivar group and the degrees of development of the seed components of 27 grape cultivars classified according to the largest seeds observed in the samples; mean separation within column by Duncan's multiple range test, P = 0.05.

Beziehungen zwischen Rebsortengruppen, eingeteilt nach den größten Kernen ihrer Beeren, und Entwicklungsgraden der Kernkomponenten von 27 Rebsorten.

Cultivar group	Number of seeds per berry mean	Weight (mg) of seeds per berry mean	Degree of colour seed-coat mean	Degree of hardness seed-coat mean	Degree of endosperm development mean
normal seeds	1.37 B	67.70 A	0.94 A	2.80 A	2.98 A
large traces	1.51 B	18.82 B	0.30 B	1.00 B	1.80 B
medium traces	1.58 B	12.13 BC	0.03 C	1.00 B	1.05 C
small traces	2.43 A	0.90 C	0.00 C	1.00 B	1.00 C

In order to examine the inheritance of the traits affecting the degree of development of the seeds (i.e. seedlessness), two progenies were chosen: Oz × Flame Seedless, and Early Muscat (= E.M.) × Flame Seedless. The seeded cultivars were chosen because they differ in the phenotypic appearance of their seeds, mainly in the number of normal seeds and large traces per berry, and in the degree of development of the seed coat and the endosperm. The berries of Oz bear, on the average, more normal seeds than the berries of E.M. (2.0 vs. 1.5 seeds/berry respectively), however fewer large traces were present in Oz berries than in E.M. berries (0.1 vs. 0.6 traces/berry respectively). Oz seeds were dark-brown coloured, compared to the light-green E.M. seeds. In the large traces of Oz, endosperm and embryos were not detected, while in the large traces of E.M. fully developed endosperm and embryos were found. Thus, it seems that Oz seeds had more developed seed coats, but less developed endosperm compared to E.M. seeds.

When grouping these progenies according to the size of the largest seed in the berries of their offsprings, only slight differences were found: in the Oz progeny there were only slightly more offsprings with normal seeds (61 %) and slightly less large traces (27 %) than in the E.M. progeny (46 % and 35 % respectively) (Tab. 2).

Table 2

Segregation of progenies into categories according to the largest seed in the offspring. The mean segregation of each of the two progenies is not significantly different from the mean segregation of these two progenies by chi-square test: $X^2 = 2.88, 0.5 > P > 0.1$ (d.f. = 3).

Aufteilung der Familien in Kategorien nach den größten Kernen des Nachkömmlings.

Progeny		Normal seeds	Large traces	Medium traces	Small traces	Total
Early-Muscat ×	N	39	29	15	1	84
Flame-Seedless	%	46.5 %	34.5 %	17.8 %	1.2 %	100 %
Oz × Flame-	N	72	32	12	2	118
Seedless	%	61.0 %	27.1%	10.2 %	1.7 %	100 %
Mean segregation		53.75 %	30.80 %	13.95 %	1.45 %	

However, when examining the segregation of the hardness of the seed coat and the degree of development of the endosperm in the progenies as separate traits, major differences between the two progenies were found (Tab. 3). In the Oz progeny more offsprings (23.7 %) having normal seeds with undeveloped or partially developed endosperms were observed than in the E.M. progeny (1.2 %). Furthermore, only 46.3 % of the Oz progeny had soft and semi-soft seed coats, compared to 60.7 % of the E.M. progeny.

Discussion

The consumer's practical definition for 'seedless' is based on the perceptibility of the seeds upon eating and is affected also by berry characteristics such as flesh crispness, and not only by the degree of development of the seed, its size and the sclerification of integuments (LEDBETTER and RAMMING 1989). We evaluated the size of the seeds and seed traces visually in order to avoid organoleptic tests.

The obvious difference in the ability of some of the fertilized ovules to become fully developed seeds, could mean that the largest seeds in berries, under normal conditions, represent the genetic capability as to seed development. Our classification of cultivars and offsprings into groups, according to the largest seeds in the berries, was based on this assumption, and the difference between these groups was found to be significant (Tab. 1), in parameters of seed content and degree of development of the seed components. PEARSON (1932) already noticed that the degree of sclerification of the integumentary tissues is not necessarily linked to the stenospermic trait, and should be regarded as a separate sub-trait. CAIN *et al.* (1983) observed that the seedless pollen parent influences the frequency of viable embryos in crosses between seedless grapes.

We conducted this study in order to seek a better definition of stenospermocarpic seedlessness by evaluating the degrees of development of separate seed components. In spite of the continuous nature of the fresh weight of the seeds, we found that significant morphological differences could be observed in the appearance of the different groups of size of the seeds. Our results suggest that it would be worthwhile to describe

Table 3

The segregation (in percents) of offsprings from two progenies according to the degrees of development of seed-coat and endosperm. The offsprings were classified by their largest seeds into 4 groups: normal seeds (N), large traces (L), medium (M), and small traces (S).

Aufspaltung (in Prozenten) von Nachkömmlingen zweier Familien gemäß der Entwicklungsgrade von Kernhülle und Endosperm. Die Nachkömmlinge wurden nach den größten Kernen ihrer Beeren in 4 Größenkategorien eingeteilt: normal entwickelte Kerne (N), große (L), mittlere (M) und kleine Kernspuren (S).

Degrees of development of seed components				Percent of offsprings in progenies											
				Oz × Flame-Seedless						Early Muscat × Flame-Seedless					
Seed-coat		Endosperm		S	M	L	N	total		S	M	L	N	total	
Soft	(1)	undeveloped	(1)	1.7	8.5	5.1	1.7	17.0	26.3	1.2	13.1	8.3		22.6	45.1
		partial	(2)			5.1	0.8	5.9			4.7	10.7		15.4	
		full	(3)			3.4		3.4				4.7	2.4	7.1	
Semi-soft	(2)	undeveloped	(1)		0.8	6.0	3.4	10.2	22.0			1.2		1.2	15.6
		partial	(2)		0.8	2.5	3.4	6.7				3.6		3.6	
		full	(3)			1.7	3.4	5.1				6.0	4.8	10.8	
Semi-hard	(3)	undeveloped	(1)			0.8	3.4	4.2	11.0						11.9
		partial	(2)			0.8	4.3	5.1							
		full	(3)			1.7		1.7					11.9	11.9	
Hard	(4)	undeveloped	(1)				2.5	2.5	40.7						27.4
		partial	(2)				4.2	4.2					1.2	1.2	
		full	(3)				34.0	34.0					26.2	26.2	
Segregation according to endosperm development:															
Undeveloped endosperm			(1)	1.7	9.3	11.9	11.0	33.9 %		1.2	13.1	9.5		23.8 %	
Partially developed endosperm			(2)		0.8	8.4	12.7	21.9 %			4.7	14.3	1.2	20.2 %	
Fully developed endosperm			(3)			6.8	37.4	44.2 %				10.7	45.3	56.0 %	
Total number of offsprings				2	12	32	72	118		1	15	29	39	84	
Percentage				1.7	10.1	27.1	61.0	100 %		1.2	17.8	34.5	46.5	100 %	

the progenies, as well as parents, in terms of seed coat hardness and endosperm development, and possibly also embryo development. Our results (Tab. 3) confirm that the seeded cultivars differed in their contribution to their progenies. Soft seed coats and more endosperm were inherited from Early Muscat, while a large percentage of Oz's offspring received hard seed coat and less endosperm.

Detailed results from the two progenies described here and additional results from further progenies examined (not reported here) suggest that the seed coat and the endosperm, in different combinations of their various degrees of development, result in a range of seed size and development. The observed existence of small but noticeable seed traces, can now be explained by the hardness of the seed coat and the degree of development of the endosperm. The same holds for large seed traces undetected due to their soft seed coat and undeveloped endosperm.

Our results, even if limited to a small sample of progenies, demonstrated that the separate evaluation of subtraits (hardness of seed coat and degree of development of endosperm) may explain more precisely the variable phenotypic composition of F1 populations. The now prevalent use of 'seedless' cultivars as female parents, by making use of embryo and ovule culture (EMERSHAD and RAMMING 1984; SPIEGEL-ROY *et al.* 1985), and as pollen donor parents, makes it even more important to evaluate the genotypic contribution of the parents to the development of the seeds in the progeny. Parents contributing to sclerified seed coats and fully developed endosperm may give less favorable results than those who inherit more developed embryos and less developed seed traces. From our own experience (as well as from personal communications by RAMMING (1991) and EMERSHAD (1991)) it seems that the size of the seed vestiges, and their endosperm development, are not related to their germinability. Viable embryos were obtained from aborted seeds even when endosperm abortion or break down does occur (EMERSHAD *et al.* 1989). Moreover, in future use of genetic molecular markers for assessing linkage with traits of economic importance, such as seedlessness, the degree of development of the seed components should be included in the matrix of data, along with data on organoleptic evaluation, and fresh weight (mg) of seeds per berry.

The use of subtraits, namely the hardness of the seed coat and the degree of development of the endosperm and embryo, should contribute to a better understanding of the inheritance of stenospermocarpic seedlessness in grapes.

Summary

The degree of development of the seed components, viz. seed coat and endosperm, were evaluated in seeds and seed traces of stenospermocarpic grapes of *Vitis vinifera*. The seeds in seedless and in seeded berries were classified visually into four categories of size: normal seeds, large traces, medium traces and small traces. Seeds with fully developed and sclerified seed coats were observed to bear endosperm at various developmental stages, and in seeds with soft and less developed seed coats also fully developed endosperms were observed.

We found that the hardness of the seed coat and the degree of development of the endosperm were transmitted as separate traits to the progenies. Two seeded cultivars (Oz, Early Muscat) were chosen as female parents because they differed in seed hardness and in degree of development of their endosperm. The normal seeds of Oz are comparatively harder and contain less developed endosperm than those of Early Muscat. Each cultivar was crossed with the same pollen donor parent (Flame Seedless). 23.7 % of the Oz progeny were normally seeded offsprings bearing undeveloped or partially developed endosperm, while only 1.2 % of the Early Muscat progeny had such a

composition of seed components. This suggests that seedlessness in grapes could be more precisely analysed using the hardness of the seed coat and the degree of development of the endosperm as subtraits of seedlessness.

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