Occurrence and cytogenetic development of unreduced pollen in *Vitis*

by

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S u m m a r y : Percentages of unreduced pollen (>40 µm for tetraploids, >30 µm for diploids) produced in 13 tetraploid *V. labruscana* x *V. vinifera* hybrid cultivars were higher than those in 11 of 14 diploid cvs and species (0.18-1.27 and 0.06-0.37 %, respectively). The production of 2x pollen was positively correlated to dyad formation during microsporogenesis, the frequency of dyad formation varied among cvs and flowers for 4x genotypes. Cytogenetic studies showed that the predominant mechanism of unreduced pollen production was first division restitution (FDR) accompanied by a lower proportion of co-orientation of second division spindles. The prospects of utilization are briefly discussed.

K e y w o r d s : Vitis, unreduced gametes, pollen, cytogenetics.

Introduction

The ploidy varies among varieties of *Vitis*. There are some disadvantages when interploidy hybridizations are carried out in breeding programs. Polyploidization of interspecific as well as interploidy are usually desirable but neither spontaneous nor colchicine-induced polyploids have attained economic importance (Einset and Pratt 1975).

The presence of unreduced gametes offered opportunities for manipulating the genome easily as previously reported for many plant species (Veilleux 1985, Bretagnolle and Thompson 1995). Jelenkovic and Olmo (1969) were the first who obtained an allotetraploid hybrid; they observed the corresponding cytological mechanism in the progeny of *V. vinifera* x *V. rotundifolia*. Martens *et al.* (1989) scanned the variability of pollen grains of 157 genotypes from 1983 to 1987, 18 2x and all 11 4x genotypes produced distinct large grains (≥ 30 µm). The objectives of this study were to screen for 2n pollen production in different varieties and to determine its cytogenetic mechanism.

Material and methods

Pollen of 27 cvs, species and genotypes was sampled by tapping inflorescences to a culture dish and by microscopical (OLYMPUS BH2) observation in 1 % aceto-carmine. Diameters were measured according to Martens *et al.* (1989), pollen grains of each genotype were scanned for unreduced pollen with 3 replications.

At meiosis inflorescences were collected and kept for 24 h in a $FeCl_3$-saturated Carnoy fixative. Anthers were squashed and stained with carbol fuchsin. Meiotic disturbance and abnormality of cytokinesis were observed microscopically. The frequency of dyad formation was calculated from more than 3000 PMCs in total with 3 replications for each of 4 flowers of a genotype (exception: cv. Niunai with 268 PMCs). Data were computed for analysis of variance with a STATISTIX 3.1 software (NH Analytical Software Co.).

Results

F r e q u e n c y o f u n r e d u c e d p o l l e n p r o d u c t i o n : For tetraploid varieties, unreduced pollen would refer to that of 4x in comparison to 2x pollen grains for diploids. Within tetraploid varieties, diameters of pollen grains ranged in 3 size categories, large, 4x (mean: 40.7 µm), normal, 2x (31.3 µm) and small (25.6 µm) and were normally distributed. Diameters of pollen grains of diploid genotypes were measured at large, 2x, 31.3 µm and normal, 1x, 25.6 µm in average.

Data are listed in the Table, all 13 4x *V. labruscana* x *V. vinifera* cvs produced 0.18-1.27 % 4x pollen grains (Figure, i) including 2 cvs with higher percentages up to 1.2 %, 7 with lower (less than 0.4 %) and 4 intermediate. The expected 2x pollen in tetraploids varied from 71.66 to 98.27 % according to the cvs. Eleven of 14 diploids were distinctive for 0.06-0.37 % 2x pollen (Figure, g, h), but all less than 1 %, the higher percentages of 2x pollen occurring in samples of Christmas Rose, Bolgar and Niunai.

F r e q u e n c y o f a b n o r m a l s p o r a d f o r m a t i o n : In tetraploid hybrid varieties, the sporad abnormality was higher than in diploids. The frequency of dyads was 0.32-3.27 and 0-0.21 % in tetraploids and diploids, respectively (Table; Figure, e, f). Highly significant variations were shown among genotypes and flowers but not among anthers of tetraploids; this was tested by analysis of variance and agrees with results of Veilleux (1985). Percentage of viable unreduced pollen was significantly (1 % level) correlated to the frequency of dyad formation($r=0.7902^{**}$) tested with 25 4x and 2x types.

C y t o g e n e t i c d e v e l o p m e n t : Cytogenetic observations were conducted with several tetraploid varieties. From late prophase I to metaphase I of male meiosis,

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Table

Percentages of 4x, 2x and x pollen, and frequency of sporads

Genotypes	Percentage of pollen				Frequency of sporads		
	Total	4x %	2x %	x %	Total	Tetrad%	Dyad%
4x *V. labruscana* x *V. vinifera* hybrids							
Guixiangyi	1774	0.21	88.42	11.36	4439	99.24	0.40
Honey Red	1041	1.12	71.66	27.13	3763	98.55	0.73
Jingya	1262	0.72	96.59	2.69	4197	97.48	1.96
Kyoho	1217	0.29	97.66	2.05	4710	99.52	0.36
Molixiang	1549	0.31	95.61	4.08	7840	99.01	0.33
Red Queen	1769	0.26	96.25	3.49	3117	98.85	0.81
Seedling of Campbell's Early	1053	1.27	74.48	24.25	3520	95.80	3.27
Takasumi	1659	0.63	96.64	2.73	5021	98.98	0.91
VF-1	1528	0.23	85.94	13.83	4612	99.64	0.32
W3N3-4	2052	0.18	98.27	1.55	5470	99.00	0.90
W3S1-2	1031	0.35	71.66	27.98	3776	98.71	1.12
W1S1-2	1020	0.69	93.45	5.86	7063	99.08	0.88
Xiyanghong	1325	0.54	98.13	1.34	6024	99.05	0.79
2x genotypes							
Baco-22a	1401	—	0.13	99.87	4360	99.87	0.13
Bolgar	3144	—	0.31	99.69	—	—	—
Cabernet franc	1093	—	0.10	99.90	6217	99.95	0.05
Christmas Rose	1155	—	0.37	99.63	7816	99.82	0.18
Fenghuang 12	—	—	—	—	1830	99.80	0.10
Krimisk	1200	—	0.09	99.91	4647	99.90	—
LN-33	1160	—	0.10	99.90	7600	99.96	0.04
Mission	1112	—	—	100	5088	99.91	0.09
Muscat Hamburg	4484	—	0.10	99.90	7030	99.93	0.07
Muscat of Shaling	1313	—	0.08	99.92	6511	99.95	0.05
Niunai	268	—	0.36	99.64	—	—	—
Phzamat	—	—	—	—	4350	99.92	0.08
Ruby Seedless	1132	—	—	100	7621	99.77	0.20
Venus	1336	—	—	100	9235	99.87	0.12
VF-3	3347	—	0.06	99.94	5792	99.90	0.10
V. rupestris	1307	—	0.17	99.83	5815	99.73	0.21

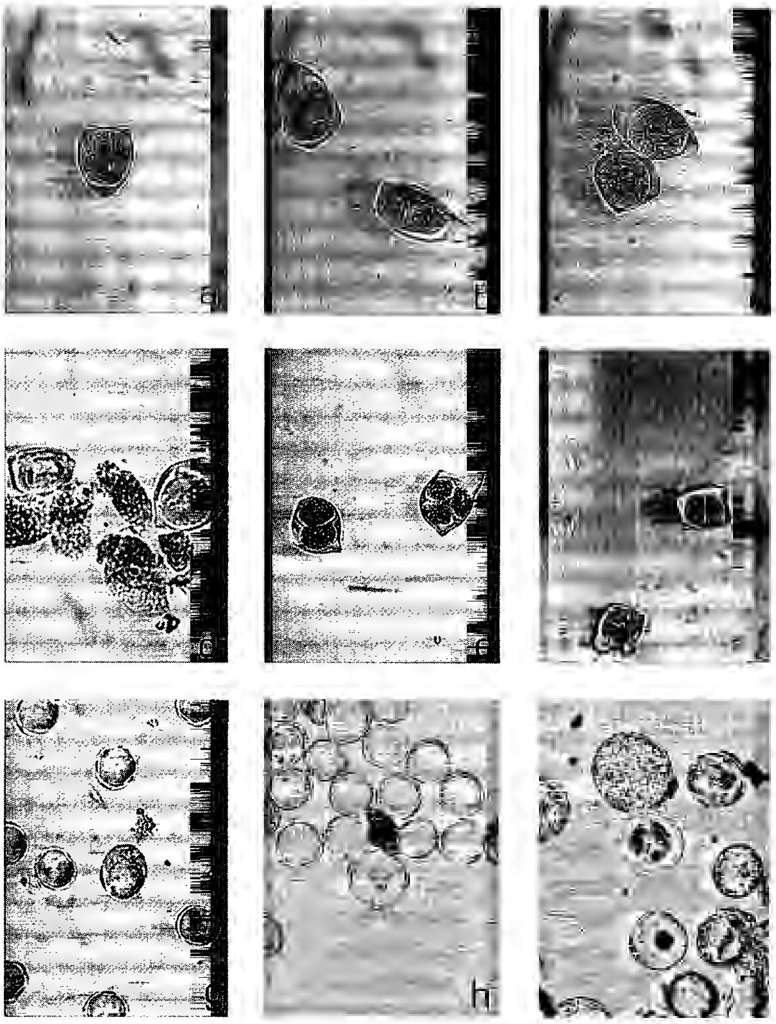
incomplete synapsis with multivalent and univalent was observed, which led to subsequent failure or partially failure in segregation of homologous chromosomes at telophase I with laggards (Figure, a) , restituted nuclei (Figure, b) or acentric nuclei (Figure, c, d). Laggards could be found as micronuclei at sporad stage, acentric nucleus dyads were equal to that of first division restitution. Hence, first division restitution was the main cytogenetic mechanism of 4x pollen production. Beside this feature, co-orientation of second division spindles also occurred in a lower proportion by either parallel or tripolar spindles, confirming the triads formation.

Discussion

Christmas Rose, Bolgar and Niunai produced over 0.3 % of 2x stainable pollen grains compared with several cvs in which no large grains were observed. The frequency of 2x pollen observed was relatively lower than that observed by MARTENS *et al.* (1989).

For tetraploid varieties, 4x pollen might indeed be characterized by a size of >40 μm in diameter. Unreduced pollen of tetraploids containing 4 chromosome sets occurred in higher percentage than diploids, probably due to unsuccessful meiotic pairing and bivalent formation of the 4 homologues (DYER 1979; KODURU *et al.* 1981).

Small chromosome size, high chromosome number, low frequency of unreduced gametes and long reproduction cycles are essential problems of cytogenetic studies of unreduced gametes in fruit crops. The cytological mechanism of spontaneous unreduced gametes in *Vitis* is still unknown, despite of the first division restitution (FDR) mechanism observed in an aneuploid by JELENKOVIC and OLMO (1969). In this study the cytological investigation of the mechanism of unreduced pollen production in diploid



Figure, **a**: Honey Red, telophase I with 3 lagging chromosomes. **b**: Honey Red, nucleus restitution at telophase I. **c**, **d**: Honey Red, acentric nuclei at anaphase I. **e**: Jingya, a dyad with micronucleus. **f**: Muscat Hamburg, a dyad. **g**: Bolgar, normal and unreduced pollen. **h**: Muscat Hamburg, normal and unreduced pollen. **i**: Seedling of Campbell's Early, normal and unreduced pollen (x 400).

cvs and species was also unsuccessful. But in tetraploid hybrid varieties, the FDR mechanism was conclusive. With regard to the lower percentage of spontaneous unreduced pollen, mechanical separation by sieving (EIJLANDER 1988) is available for efficient utilization without decreasing fertility in (1) breeding for polyploids, (2) breeding for disease resistance, and (3) interploidy hybridization without interferring sexual fertility.

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