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Cytogenetical studies of three *Vitis* species

by

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S u m m a r y : The aim of this study was to determine the genomic relationship between *Vitis vinifera*, *V. rotundifolia* and *V. rupestris*. The hybrid between *V. vinifera* and *V. rotundifolia* (RT88-2) was almost sterile, whereas the hybrid between *V. vinifera* and *V. rupestris* (RP88-14) was fertile. A low percentage (0.52 %) of the F1 hybrid (RT88-2) seed germinated, provided that *V. vinifera* was the seed parent. The result of this one way ability to cross could possibly be attributed to the incompatibility between the cytoplasm of *V. rotundifolia* and the chromosomes of *V. vinifera*. The F1 hybrid RT88-2 had 39 chromosomes of which 19 were derived from *V. vinifera* and 20 from *V. rotundifolia*. The homology differs between the genomes of *V. vinifera* and *V. rotundifolia*. The sterility of the F1 hybrid was chromosomal and was reflected in the abnormal meiosis and lower chiasma frequency. The F1 hybrid RP88-14 had normal meiosis and a chiasma frequency similar to that of the parents. This could be attributed to the fact that the parents (*V. vinifera* and *V. rupestris*) have the same chromosome number and are closely related.

K e y w o r d s : chiasmata, chromosome, genome, grapevine, V. rupestris, V. rotundifolia, V. vinifera.

Introduction

Wild and cultivated grapevines belong to the family Vitaceae, which includes 16 living and two fossil genera and more than a thousand species (GALET 1988). The economically important genus *Vitis* is divided, according to chromosome numbers, into two subgenera. The subgenus *Euvitis* comprises 75 grape species and has a somatic chromosome number of 38, whilst subgenus *Muscadinia* comprises only three species (*V. rotundifolia* Michx., *V. munsoniana* Simpson and *V. popenoei* Fen.) and contains 40 somatic chromosomes (PATEL and OLMO 1955; PONGRÁCZ 1978). *Euvitis* is, furthermore, divided into 3 different groups: the European group with only one species (*V. vinifera*), the American group with 34 species and the Asiatic group with 40 species (PONGRÁCZ 1978). Before the discovery of North America, all cultivated grape varieties derived from *V. vinifera*. More than 10 000 cultivars were propagated from this species (PONGRÁCZ 1978; ALLEWELDT and POSSINGHAM 1988).

Although *V. vinifera* (2n=38) and *V. rotundifolia* (2n=40) are taxonomically distant, their hybrids are both of practical and academic interest. It is well known that *V. vinifera* varieties are susceptible to most grape diseases and pests, whereas *V. rotundifolia* varieties are mostly resistant (OLMO 1954). The failure to secure viable inter-specific hybrids is, often, a consequence of a well-defined barrier which operates at a specific stage of zygotic development. If it passes successfully through the critical stage, the hybrid will develop to maturity (MOAV and CAMERON 1961). Very little is known about the cytogenetics and genomic relationships of *Vitis* and related genera and species (NEBEL 1929; SAX 1929; PATEL and OLMO 1955; SHETTY 1958; SACERDOTE *et al.* 1981; ALLEWELDT and POSSINGHAM 1988). This is possibly due to the small-sized chromosomes of this genus which discouraged work in this field.

The purpose of this study was to investigate the genomic relationships between *V. vinifera* L. and *V. rupestris* Scheele which both have 38 chromosomes and between *V. vinifera* and *V. rotundifolia* Michx. with, respectively, 38 and 40 chromosomes.

Materials and methods

I n t e r s p e c i f i c c r o s s e s : *Vitis vinifera* (Barlinka), *V. rupestris*, *V. rotundifolia* and hybrids between *V. vinifera* and *V. rupestris* (RP88-14) and between *V. vinifera* and *V. rotundifolia* (RT88-2) were grown at the Nietvoorbij Institute for Viticulture and Oenology. The plants were first crossed during 1987 and the crosses were repeated during subsequent years.

The viability of seeds was determined by placing them in water for 24 hours. Floaters were classified as non-viable (YEO-DER *et al.* 1968). All the F1 hybrid seedlings were grown to maturity in the vineyard.

Pollen germination was tested by the hanging drop technique, using 20 % sucrose (WATKINS and CURTIS 1968). Grains that stained were considered viable and non-staining and shriveled grains non-viable.

M i t o s i s : Actively growing root tips were used for somatic chromosome counts. Cuttings from field-grown vines were rooted in water and root tips collected and placed in a tray containing cold water (2-4 °C) and ice cubes. The tray was placed in a refrigerator at 2-4 °C and the ice replenished every 6-10 h. After 24 h, root tips were removed and fixed overnight in a freshly prepared fixative (methanol:propionic acid, 3:1). After 18 h the fixative was drained and replaced by 5 ml distilled water (every 30 min over 2.5 h). The roots were then transferred to vials con-

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taining 1N HCl and incubated at 60 °C for 23 min. To stop hydrolysis of DNA, roots were transferred to vials containing distilled water for 1-2 min. The distilled water was then drained and replaced with 1 ml leuco-basic fuchsin (DARLINGTON and LACOUR 1976) and the vials placed in a refrigerator for 12 h. The leuco-basic fuchsin was then drained and the roots rinsed with a 0.2 M sodium acetate buffer ($CH_3COONa·3H_2O$ and CH_3COOH; pH 4.5). The buffer solution was replaced by 1-2 ml filtered 5 % (m/v) pectinase solution and incubated at 37 °C for 45 min (R. DE V. PIENAAR, unpublished data). The roots were rinsed in distilled water and placed on a clean slide. The stained root tip was placed in a droplet of 1 % (v/v) Rosner aceto-carmine and cut off.

A cover slide was gently pressed over the tissue and the slide lightly heated in a flame to ensure a more even distribution of the chromosomes (MCCLINTOCK 1929).

Meiosis: Inflorescences were collected at 12:00, fixed in freshly prepared fixative (ethanol: chloroform:glacial acetic acid, 6:3:1) for 48 h at room temperature, and stored in 70 % (v/v) ethanol at -10 °C. Anthers were dissected from the florets and placed on a slide in a small drop of 2 % Rosner aceto-carmine. The meiocytes were tapped out of the anther with the blunt end of a hardwood needle holder and the coarse remains removed. The slide was covered with a cover slide, dried for 1-3 s on a spirit flame and gently pressed in folded filter paper to remove the excess stain and to flatten the cells. To obtain an even distribution of cells, the slide was passed 5-6 times over a spirit flame and returned to the folded filter paper. The cells were flattened by pressing the cover glass until the meiotic configurations had spread out to form a single plane.

Permanent slides were made as follows: After freezing the slide, using liquid CO_2, the cover slide was removed and the slide dipped in absolute ethanol. A drop of Euparal was placed on the slide and a clean cover glass was pressed gently on top. The preparation was labelled and dried in an incubator at 37 °C.

Microscopy: Meiosis and mitosis studies were done using a Nikon Optiphot microscope fitted with a PlanApo 60/1.40 oil lens.

Results and discussion

Interspecific crosses: *Vitis vinifera* was crossed with both *V. rupestris* and *V. rotundifolia*. Seedset was obtained only when *V. vinifera* was used as seed parent. The cross between *V. vinifera* and *V. rotundifolia* produced no seeds in 1987 and only 3 seeds in 1988. The rest of the seeds were obtained in subsequent years (Tab. 1).

A surprisingly low percentage of viable plants were obtained in all cases, especially with the interspecific hybrids (Tab. 1). The very low percentage of viable seeds derived from crossing *V. vinifera* x *V. rupestris* probably resulted from the incompatibility between the cytoplasm of *V. vinifera* and the chromosomes of *V. rupestris* (PATEL and OLMO 1955). The extremely low percentage of viable seeds obtained when *V. vinifera* and *V. rotundifolia* were crossed can, therefore, be due to different chromosome numbers as well as cytoplasmic sterility occurring between the seed and pollen parent.

Pollen samples of individual plants were studied to determine the percentage of normal and of shrivelled grains. Pollen grains of *V. vinifera*, *V. rupestris*, *V. rotundifolia* and RP88-14 showed high percentages of normal pollen, but the pollen of RT88-2 was mostly non-viable (Tab. 2). Only 6.2 % of RT88-2 pollen was normal compared to 67-94 % for *V. vinifera*, *V. rupestris*,*V. rotundifolia* and

Table 1

Number of florets pollinated, number of seeded berries, seeds obtained and the percentage of viable plants, expressed as the number of seeds times the germination percentage, minus the number of inviable plants in *Vitis vinifera, V. rupestris,V. rotundifolia* and their hybrids

Cross ♀	Cross ♂	Florets pollinated	Seeded berries	Total seeds	Germination (%)	Weak plants	Viable plants (%)
V. vinifera	*V. vinifera*	7620	1549	4801	43.0	75	41.0
V. rupestris	*V. rupestris*	6312	927	1946	22.0	201	11.5
V. rotundifolia	*V. rotundifolia*	5940	1179	3537	39.5	187	34.2
V. vinifera	*V. rupestris*	4316	733	459	6.6	4	5.6
V. vinifera	*V. rotundifolia*	7012	562	640	2.2	2	1.9

Table 2

Pollen fertility of *Vitis vinifera, V. rupestris, V. rotundifolia* and their F_1 hybrids RP88-14 and RT88-2

Species/Hybrid	Number of pollen grains	Stained	Non-stained	Viable (%)
V. vinifera	1002	814	188	81.2
V. rupestris	911	608	303	66.7
V. rotundifolia	613	576	37	93.9
RP88-14	956	800	156	83.7
RT88-2	1172	73	1099	6.2

Table 3

Chromosome associations during metaphase I of *V. vinifera, V. rotundifolia, V. rupestris* and their F1 hybrids RP88-14 and RT88-2

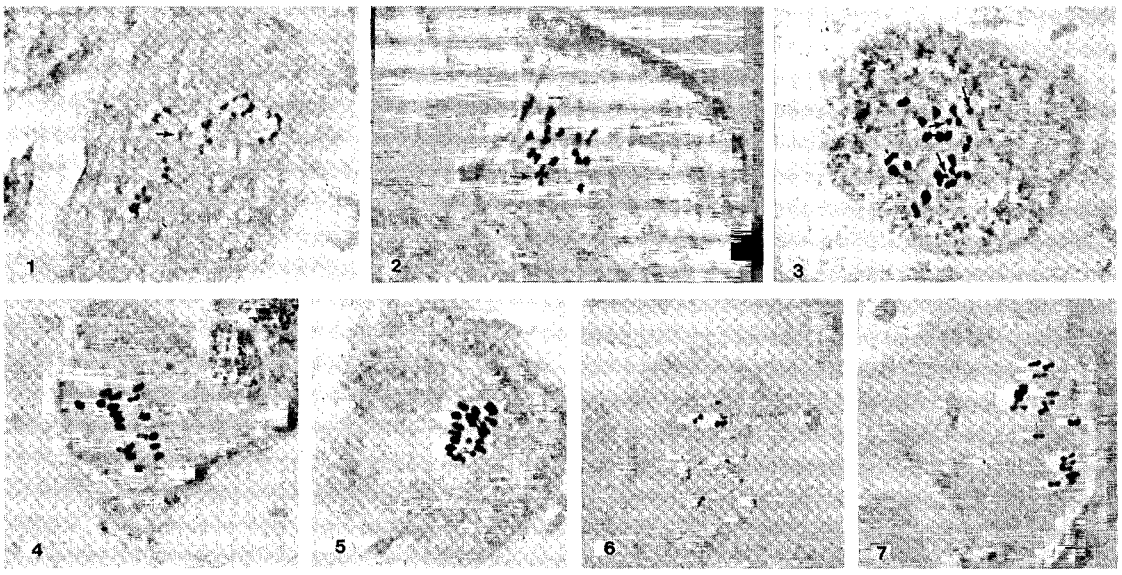
Species/ Hybrid	Pollen mother cells analysed	Univa-lent (%)	Bivalent (%)		Triva-lent (%)	Quadri-valent (%)
			Ring	Rod		
V. vinifera	20	1.8	20.8	76.6	0.8	0.0
V. rupestris	20	2.8	12.8	84.4	0.0	0.0
V. rotundifolia	20	0.0	6.0	93.0	0.0	1.0
RP88-14	20	1.6	21.6	76.8	0.0	0.0
RT88-2	20	19.0	5.6	73.8	1.0	0.2

RP88-14. These results concur with those of PATEL and OLMO (1955).

M i t o s i s : Due to the small sizes of the chromosomes, it was not possible to construct a kariogram and only the chromosome number could be determined. The somatic chromosome number is 38 for *V. vinifera*, *V. rupestris* and RP88-14, 40 for *V. rotundifolia*, and 39 for RT88-2. These results for *V. vinifera* x *V. rotundifolia* correspond with the results of PATEL and OLMO (1955) and of ALLEWELDT and POSSINGHAM (1988). These differences in chromosome numbers can be attributed to either aneuploidy or it can indicate totally different origins for the different sections of *Vitis*. In order to determine the origin of these species, their basic chromosome numbers must be known. Since too few specimens have been studied during this investigation to determine the basic chromosome number of the genus, it is necessary to determine the basic chromosome from existing literature. According to published chromosome numbers in chromosome atlasses GOLDBLATT (1981, 1983, 1985, 1988) and GOLDBLATT and JOHNSON (1990, 1994), two different basic chromosome numbers for the genus *Vitis* is possible, i.e. 19 and 20. However, GOLDBLATT (1979) suggested that all basic chromosome numbers higher than nine or ten are secondarily derived. Therefore, it seems that the genus *Vitis* has at least a secondary basic chromosome number. In his paper on the grapes, OLMO (1976) even proposed a tertiary basic chromosome number for *Vitis*. He suggested basic chromo-some numbers of 5, 6 and 7 for the Vitaceae, with three sets of genomes, in combination of (6 + 7) + 6 = 19, as the tertiary haploid number of the *Euvitis* species. The basic chromosome combination of the *Muscadenia* species is proposed as (6 + 7) + 7 = 20 (OLMO 1976).

M e i o s i s : The chromosome configuration varied between the three species and the hybrids (Tab. 3). The percentage of univalents varied from 0 % (*V. rotundifolia*) to as high as 19.03 % for RT88-2. The very low frequency of univalents observed in RP88-14 suggests that the genomic composition of *V. vinifera* and *V. rupestris* correspond greatly. The F1 hybrid RT88-2 showed a high frequency of univalents compared to RP88-14 and the three parental species. This may be the result of asynapsis, which is probably due to the lack of homology between chromosomes of different genomes. The number of univalents in RT88-2 varied between one and 15 per cell, which is less than those described by PATEL and OLMO (1955).

A very low percentage of multivalents were observed. Tab. 3 shows that only *V. vinifera* and RT88-2 had trivalents (Fig. 1), whilst *V. rotundifolia* and RT88-2 had quadrivalents (Fig. 2). This occasionally observed multivalents may be



Figs. 1-7: Meiotic chromosomes in the genus *Vitis*. **1-3**: Diakinesis of the F1 hybrid RT88-2 with one trivalent (**1**), one quadrivalent (**2**) or univalents (**3**) indicated by arrows. **4**: *Vitis vinifera*, late diakinesis, n = 19. **5**: *V. rotundifolia*, metaphase I, n = 20. **6**: *V. rupestris*, diakinesis, n = 19. **7**: The F1 hybrid, RP88-14, diakinesis, n = 19. All figures magnified ca. 2000 x.

attributed to the fact that the haploid chromosome number is at least secondarily derived and may include ancient homologies.

The chromosome associations are presented in Tab. 4. Only RT88-2 had univalents in all the studied cells, with 2-3 univalents in 40 % (Fig. 3) and 7-8 univalents in 10 % of the cells. The number of bivalents (Tab. 4) varied between 17 and 20 per cell in *V. vinifera* (Fig. 4), *V. rotundifolia* (Fig. 5), *V. rupestris* (Fig. 6) and RP88-14 (Fig. 7). RT88-2 had 12-19 bivalents per cell (Fig. 3). The number of chiasmata per cell were much lower in the F1 hybrid RT88-2 than in either parent. This low chiasma frequency may be the result of the high percentage of univalents in the specimen.

Table 4

The percentage of cells with different chromosome associations

Chromosome association	Percentage cells				
	V. vinifera	*V. rupestris*	*V. rotundifolia*	RP88-14	RT88-2
20_{II}	0	0	99	0	0
19_{II}	80	75	0	85	0
$19_{II}1_{I}$	0	0	0	0	10
$18_{II}1_{IV}$	0	0	1	0	0
$18_{II}2_{I}$	15	20	0	15	0
$18_{II}3_{I}$	0	0	0	0	30
$17_{II}1_{I}1_{III}$	5	0	0	0	0
$17_{II}4_{I}$	0	5	0	0	0
$16_{II}7_{I}$	0	0	0	0	10
$15_{II}5_{I}1_{IV}$	0	0	0	0	5
$15_{II}6_{I}1_{III}$	0	0	0	0	10
$15_{II}9_{I}$	0	0	0	0	10
$13_{II}13_{I}$	0	0	0	0	10
$12_{II}15_{I}$	0	0	0	0	15

Structural differences between chromosomes reduce the homology and chiasma frequency of such chromosomes (BURNS 1980). If the structural rearrangements are very small, pairing may occur, but segregation and recombination will result in gametes with deficiencies and/or duplications of chromosomal segments (JELENKOVIC and OLMO 1969). These structural differences in the chromosomes of the parental species will therefore result in reduced fertility of interspecific hybrids (BURNS 1980).

The very low fertility of the RT88-2 hybrid, as indicated by its seedset and pollen fertility, may be the result of the structural chromosome differences between the parental species, as well as of their different chromosome numbers and genome combinations. The higher seedset and pollen fertility, of the cross between *V. vinifera* and *V. rupestris* (RP88-14), can be attributed to the same somatic chromosome number (2n=38) of the parental species as well as to a low frequency of structural chromosome differences. The relative normality of meiosis in this hybrid supports this suggestion. These findings also support the current taxonomic positions of these species.

In this study, the criteria used to compare the relationship between the three species (*V. vinifera*, *V. rotundifolia* and *V. rupestris*), were their ability to cross and their meiotic chromosome pairing in the F1 generation. This study clearly indicated that the hybrid RT88-2 had irregular meiosis and low fertility, compared to RP88-14, which had regular meiosis and high fertility. The ability to cross different *Vitis* species and the fertility of the crosses could be determined by applying this method.

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