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Cytogenetics of Vitis

V. Allotetraploids of V. vinifera L. × V. rotundifolia Michx.[1]

by

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Introduction

Crosses of distantly related species belonging to different sections, subgenera or genera have been performed in a number of cultivated plants. Such F_1 hybrids are highly sterile but fertility is restored after usually doubling the chromosome number. The first report of this kind is the classical work of Karpechenko (1927). Crossing the species *Raphanus sativus* L. (2n = 18) × *Brassica oleracea* L. (2n = 18), he obtained an F_1 generation in which only a few plants were partially fertile. Some pollen mother cells exhibited no chromosome pairing, so that 18 univalents were found. In the allotetraploids, fertility was considerably increased in comparison with the diploid F_1 hybrids, but was much lower than the parental species. Data for only two plants were presented; fertility was between the parental species. The allotetraploids revealed two to four univalents at MI and the rest of the chromosomes were bivalents. Karpechenko noted that when *Brassica oleracea* var. *capitata* L. was used in the crosses, higher fertility was obtained than with *Brassica oleracea* var. *gemmifera* D. C.

Sears (1941, 1959) crossed ten different species of the three genera: *Triticum, Aegilops* and *Haynaldia,* all with n = 7, and produced twenty-four different highly sterile F_1 hybrids. From 18 of these F_1 hybrids the corresponding allotetraploids were obtained that ranged in fertility from completely to "almost fertile". Microscopial examination showed that the pollen of but one allotetraploid exceeded more than 80 percent good. Ovule fertility was slightly higher but followed the same range. Sears concluded that there was no relation between the chromosome pairing in the F_1 hybrids and chromosome pairing and fertility of the derived tetraploids. As an example, Sears cited crosses of *Aegilops umbellulata* × *Haynaldia villosa* where the F_1 exhibited an average of 12.60 univalents and in the allotetraploids only 5.48 bivalents. Most of the rest of the chromosomes were univalents. The hybrid showed complete ovule sterility and from 13 to 30 percent good pollen.

More extensive work has been done on hybridization of *Agropyron* and *Triticum,* of interest because of the possibility of producing a perennial wheat. F_1 hybrids and allotetraploids have been produced in Russia, Canada and the United States (Stebbins 1950, 1958; Pope and Love 1952). Cytological analysis and fertility tests in some of these hybrids were reported by Pope and Love (1952). The three species of *Triticum,* namely *durum* (2n = 28), *timopheevi* (2n = 28) and *macha* (2n = 42) were crossed with *A. trichophorum* (2n = 42). The diploid F_1 hybrids set seeds only occasionally and had less than one percent functional pollen. In the corresponding allotetraploids the pollen viability varied from 56 to 95 percent and

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the average frequency of univalents was from 3.2 to 11.5. Hence, the fertility in the allotetraploids is increased, but is much lower than the parental species.

A similar situation is encountered in the intergeneric crosses of *Secale* and *Triticum,* in attempts to combine the grain quality of common wheat with the hardiness of rye. The F_1 diploid hybrids are sterile and the maximum pairing for any wheat-rye hybrid is about four to five bivalents (O'Mara, 1953). The allotetraploids between these two genera, called *Triticale,* should have 28 pairs, of which 21 would be wheat and 7 would be rye. However, the pairing of the chromosomes in *Triticale* is not complete and univalents are often found. The number of univalents varies depending on the strain. The average frequency of univalents, according to O'Mara (1953), ranged from 1.88 to 6.10 per cell. Fertility of *Triticale* varies considerably but according to Muntzing (cited by Allard, 1960), the raw amphiploid yields only about half of the standard wheat varieties. After fifteen generations of selection, the yield was increased to 90 percent of the standard wheat varieties.

In *Nicotiana* (Greenleaf 1941, 1942) crossed *N. sylvestris* (2n = 24) × *tomentosiformis* (2n = 24), belonging to different sections of the genus, and produced F_1 hybrids that showed a high degree of asynapsis and complete sterility. When the chromosome number of these hybrids was doubled, the resulting amphidiploid showed an average of 24 bivalents, but occasionally 2 to 4 univalents were present. Pollen averaged 90 percent good in appearance. Ovule fertility varied depending upon the strains used in the initial cross. Some combinations showed complete ovule sterility, others were partially fertile. Examination of the ovules of the sterile amphidiploids disclosed that megasporogenesis was quite regular, but the embryo sac did not develop beyond the two or four cell stage, when disintegration occurred.

Dermen (1954, 1958, 1962, 1964) published a series of papers dealing with hybridization of *V. vinifera* L. × *V. rotundifolia* Michx. and the fertility of diploid and allotetraploid hybrids. Using the diploid F_1 hybrids NC6-15 and NC6-16 that Detjen produced in 1917, which exhibited almost complete ovule and pollen sterility, he produced allotetraploids by colchicine treatment and described them as "fully fertile hybrids". Unfortunately, he has not presented experimental data to judge what is meant by "fully fertile". In reciprocal crosses of autotetraploid clones of *V. vinifera* and *V. rotundifolia*, he failed to obtain set even when *V. vinifera* was used as a female parent (1964). He concluded that the cross incompatibility reaction between the two species is strengthened at the tetraploid level. The cause of the difficulties in obtaining the hybrids at the diploid level, and the subsequent sterility, he attributed mainly to the different chromosome numbers of the two species.

The present paper describes meiotic behaviour of the chromosomes and fertility in allotetraploid *V. vinifera* × *V. rotundifolia* hybrids, obtained by colchicine treatment of completely sterile diploid F_1 hybrids.

Materials and Methods

The allotetraploid seedlings were obtained by colchicine treatment of diploid hybrids from the cross 'Almeria' × 'Trayshed' (Patel and Olmo, 1956). They are designated the N53-series. From this series five seedlings were chosen for further cytogenetical analysis. They proved to be, in regard to stratification of the growing point, completely tetraploid (Olmo, unpublished). In this group is also included the diploid seedling N53-32, because it originated from the same parents and which, because of anomalous meiotic division, breeds as a tetraploid, i. e. produces functional diploid pollen. The diploid pollen grains originated from a restitution nucleus

Table 1

List of the tetraploid clones used in the present investigation

Clone	Genomic formula	Parentage	Flower type
Allotetraploid F_1 progeny			
N53-1	VVRR	Almeria × Trayshed	♂
N53-2	VVRR	Almeria × Trayshed	♀
N53-3	VVRR	Almeria × Trayshed	♂
N53-8	VVRR	Almeria × Trayshed	♀
N53-56	VVRR	Almeria × Trayshed	♀
N53-32	VR	Almeria × Trayshed	♂
Selections used as testers			
V. vinifera			
M15-35	VVVV	Muscat of Alexandria (4n) × Alicante Bouschet (4n)	♀
V. rotundifolia			
G. H. I	RRRR	Introductions from the North	☿
G. H. II	RRRR	Carolina Agricultural Experiment Station	☿
V. rupestris metallique	RuRuRuRu		♂
V. vinifera × *rupestris*			
M24-1	VVRuRu	Muscat of Alexandria (4n) × *V. rupestris* (4n)	♀

in the first meiotic division and are hybrid in nature, namely VR. The cytogenetics of N53-32 will be published elsewhere.

The tetraploid clones used in the present investigation are listed in Table 1. Pollination and other techniques used in the present investigation have been described in a previous paper (JELENKOVIĆ and OLMO, 1968).

Results

Fertility studies of the F_1 allotetraploids

Ovule fertility test: For the ovule fertility test the three male-sterile sibs were used, namely N53-2, N53-8 and N53-56. Pollen of 7 autotetraploid *V. vinifera* varieties were applied on the receptive flowers of N53-2 (Table 2, Fig. 1). Pollination with Carignane and Sauvignon blanc failed to produce a berry set. Other backcrosses with autotetraploid clones of *V. vinifera, V. rupestris* and *V. rotundifolia* produced berry and ovule sets, the average number of seeds per berry ranged from 1.1 to 2.8. Under selfing, berry sets were nil.

N53-8 failed to set fruit with pollen of Zinfandel (Table 2). Pollination with Muscat of Alexandria failed in 1962, but repeated pollination with the pollen of the same variety produced fruit set in the 1963 season.

From selfing 6,800 ovules only 8 seeds were obtained and these were small in size and all were floaters. Most of the seeds collected from this plant, resulted from open pollination and were floaters. The flowers of N53-8 shed their calyptras very irregularly. Some berries reached maturity with the calyptra still attached, so that some of the pollen grains may be functional and cleistogamy could be a factor

Table 2

Ovule fertility of the male-sterile F_1 allotetraploids

| Parents (4n)	Pollinated	Set		Seed		
	Flowers (clusters)	Berry %	Ovule %	Average/ berry	Total	Floaters %
VVR × VVVV						
N53-2 × Folle blanche	355(6)	4.8	1.5	1.3	22	4.5
N53-2 × Zinfandel	325(5)	6.8	3.8	2.3	50	2.0
N53-2 × Saint-Emilion	330(7)	2.4	1.2	2.0	16	0.0
N53-2 × Gamay	360(6)	6.4	4.5	2.8	65	4.6
N53-2 × Sauvignon blanc	295(6)	0.0	0.0	0.0	0	0.0
N53-2 × Muscat of Alexandria	580(11)	16.0	4.0	1.6	153	2.4
N53-2 × Carignane	415(6)	0.0	0.0	0.0	0	0.0
N53-8 × Muscat of Alexandria	220(5)	14.1	3.5	1.0	31	0.0
N53-8 × Zinfandel	289(6)	0.0	0.0	0.0	0	0.0
N53-56 × Gamay	128(4)	0.0	0.0	0.0	0	0.0
N53-56 × Carignane	212(6)	0.0	0.0	0.0	0	0.0
N53-56 × Muscat of Alexandria	142(4)	0.0	0.0	0.0	0	0.0
VVRR × RRRR						
N53-2 × V. rotundifolia G. H. II	208(5)	13.9	3.8	1.1	31	3.2
N53-8 × V. rotundifolia G. H. I	208(4)	2.4	0.7	1.2	6	0.0
VVRR × RuRuRuRu						
N53-2 × V. rupestris metallique	425(9)	5.4	2.4	1.8	41	9.7
VVRR × VVRR						
N53-2, Selfed	834(14)	0.0	0.0	0.0	0	0.0
N53-8, Selfed	1700(38)	0.3	0.1	1.6	8	100.0
N53-8, Cleistogamic	4	—	—	1.5	6	100.0
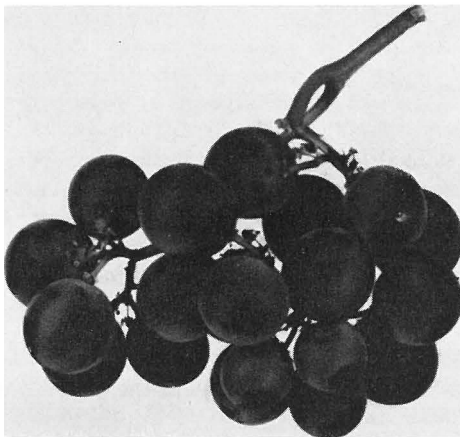
Fig. 1   Fig. 2
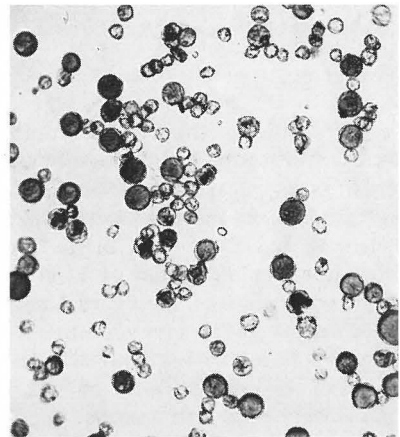
Fig. 1: Berry set on N53-2 when pollinated with tetraploid *V. vinifera "Folle blanche"*
(× 0.67).

Fig. 2: Pollen of N53-32 (× 230).

in producing the berry and seed setting. Four flowers with adherent calyptras were labeled and followed through development to maturation of the berry. The cap fell off before the skin of the berry began to turn red. When the berry skin became completely red, the seeds were extracted. They were small, all floaters, and none germinated. No attempt was made to dissect the seeds and to determine if embryos were present. The presence of pollen without germ-pores on the stigma most likely stimulated the ovule to form partly developed seed coats.

The third clone, N53-56, has shown complete sterility (Table 2). Of fourteen clusters isolated and pollinated with the pollen of 3 *V. vinifera* autotetraploids, none bore fruit. Under the conditions of open-pollination, the ovaries of the flowers began to grow, but abscissed when they reached about 0.5 cm in diameter. Over a three year period not a single berry persisted to maturity. The calyptra did not shed and adhered to the young berries. At flowering time, it is strongly attached to the receptacle, and emasculation is difficult without damaging the ovary. When the calyptra did shed naturally, it did so very late, so that the stigmas of the flowers were not receptive. The lack of synchronization of the two processes, abscission of the calyptra and receptivity of the stigma, is related to the complete failure of pollination to produce berry set.

Table 3

Pollen fertility of the male F_1 allotetraploid hybrids

Parents (4n)	Pollinated	Set		Seed		
	Flowers (clusters)	Berry %	Ovule %	Average/berry	Total	Floaters %
VVVV × VVRR						
Husseine × N53-1	172(3)	20.9	5.2	1.0	36	50.0
Husseine × N53-32	285(4)	0.0	0.0	0.0	0	0.0
Muscat of						
Alexandria × N53-32	123(1)	13.8	3.4	1.0	17	0.0
M24-1[1]) × N53-32	394(4)	4.6	2.5	2.2	40	0.0
M15-35 × N53-32	505(5)	26.5	7.8	1.2	158	31.6
RRRR × VVRR						
G. H. I. × N53-1	410(7)	0.0	0.0	0.0	0	0.0
G. H. I. × N53-32	162(3)	0.0	0.0	0.0	0	0.0

[1]) M24-1 = VVRuRu.

Pollen fertility test: Measurements of pollen fertility are presented in Table 3. The berry and ovule set is highly variable (from 0.0 to 26.5 and 0.0 to 7.8 per cent respectively). The failure of setting in Hunisa × N53-32 can be explained by low germinability of the pollen. The cross with the pollen of the same seedlings with N24-1 resulted in more than two seeds per berry. In combinations where *V. rotundifolia* was used as the female parent, pollens of the allotetraploid hybrids were ineffective. Pollination with N53-1 induced development of seven berries (in one flower cluster) which enlarged to about 0.5 cm in diameter, then shriveled and died. Aborted seeds were found in these berries, with the exception of one fully developed seed, but it was hollow.

The results of pollen germinability and stainability tests are summarized in Table 4. The seedlings N53-1 and N53-3 have more than 80 percent stainable pollen. Non-stainable grains were different in size and shape. Microcytes are very com-

Table 4

Pollen appearance and germinability of F_1 allotetraploids

Vine	Total No. of pollen grains	Stained %	Non-stained %	Shriveled %	Germination %
N53-1	1605	85.9	4.92	9.15	0.85
N53-3	1518	81.35	3.16	15.48	1.10
N53-32	Large 1934	11.11	3.87	9.87	0.62
N53-32	Small	0.67	13.75	60.70	0.00

mon in these seedlings. The germinability test revealed very low germination. The diploid seedling N53-32, as described previously, yields diploid and haploid pollen grains. The diploid pollen is much larger and has four germ pores. Of 1900 pollen grains counted, 11.1 percent were stainable (Fig. 2). The haploid pollen grains are mostly unstainable and in germination tests proved inviable. Diploid pollen grain germination in vitro was 0.62 percent.

Test of crossability among the seedlings: The results of crossing male-sterile and male allotetraploid seedlings inter-se are presented in Table 5. The berry and ovule set are very low (0 to 12.6 and 0 to 3.4 percent respectively). The cross of N53-2 with pollen of N53-32 under field conditions produced berry setting. In the greenhouse, however, in the 1962 and 1963 seasons, 78 flower clusters were pollinated without a single berry setting. N53-8 pollinated with N53-32 in the vineyard was ineffective in 1963.

Table 5

Sib crosses of the F_1 allotetraploid hybrids

Parents	Pollinated Flowers (clusters)	Set Berry %	Set Ovule %	Seed Average/ berry	Seed Total	Seed Floaters %
VVRR × VVRR						
N53-2 × N53-1	350(8)	12.6	3.4	1.1	48	8.3
N53-2 × N53-3	220(5)	1.4	0.3	1.0	3	66.7
N53-2 × N53-32	643(12)	1.9	0.5	1.0	12	8.3
N53-2 × N53-32	2875(78)	0.0	0.0	0.0	0	0.0
N53-8 × N53-1	235(6)	1.7	0.5	1.2	5	0.0
N53-8 × N53-32	590(10)	0.0	0.0	0.0	0	0.0

Tests of crossability among the clones of different ploidy level and different genomic constitutions, are presented in Table 6. The interesting feature of these results is the setting of berries and seeds on male-sterile diploid V. rotundifolia clones with pollen of N53-32, i. e. RR × VVRR.

Setting was also obtained in the diploid F_1 hybrid (T6-42) with pollen of N53-32, i. e. VR × VVRR. However, all the seeds were classified as floaters and none of them germinated.

In the cross using N53-2 with the pollen of V. rotundifolia 'Trayshed', i. e. VVRR × RR, a relatively high berry and seed set was obtained and the seeds had less than 50 percent floaters. Pollen of the V. vinifera selection C22-24 with the same N53-2, i. e. VVRR × VV, produced good berry and seed sets, but more than 90

Table 6

Crosses of VR hybrids of different ploidy level and different genomic constitution

Parents	Pollinated Flowers (clusters)	Set Berry %	Set Ovule %	Seed Average/ berry	Seed Total	Seed Floaters %
VR × VVRR						
T6-38 × N53-32	112(3)	0.0	0.0	0.0	0	0.0
T6-42 × N53-32	665(12)	1.1	0.3	1.0	7	100.0
RR × VVRR						
Dulcet × N53-32	105(13)	4.7	1.5	1.3	25	100.0
Higgins × N53-32	145(6)	4.8	1.5	1.3	9	100.0
Hunt × N53-32	382(12)	2.6	0.6	1.0	10	100.0
VVRR × RR						
N53-2 × V. rot. Trayshed	213(4)	16.0	9.8	2.5	84	45.2
VVRR × VV						
N53-2 × G22-24	380(7)	11.8	5.4	1.8	82	92.7

percent of the seeds were floaters. The bearing of these data on the genetical mechanism of cross-incompatibility of the two species will be discussed later.

Tests of crossability between autotetraploid clones of the two species are presented in Table 7. Two autotetraploid hermaphroditic clones of *V. rotundifolia* were emasculated and pollinated with the pollen of autotetraploid clones of *V. vinifera*. The clone G.H.2 of *V. rotundifolia* was pollinated with haploid pollen of the *V. vinifera* variety Red Malaga. All pollinations failed to produce berry and seed

Table 7

Crosses between autotetraploid clones of *V. vinifera* and *V. rotundifolia*

Parents	Pollinated Flowers (clusters)	Set Berry %	Set Ovule %	Seed Average/ berry	Seed Total	Seed Floaters %
RRRR × VVVV						
V. rotundifolia GH 1 × Muscat of Alexandria	495(11)	0.0	0.0	0.0	0	0.0
V. rotundifolia GH 1 × Thompson Seedless	309(8)	0.0	0.0	0.0	0	0.0
V. rotundifolia GH 1 × Gamay	178(3)	0.0	0.0	0.0	0	0.0
V. rotundifolia GH 2 × Muscat of Alexandria	342(9)	0.0	0.0	0.0	0	0.0
V. rotundifolia GH 2 × Thompson Seedless	512(12)	0.0	0.0	0.0	0	0.0
VV						
V. rotundifolia GH 2 × Red Malaga	211(4)	0.0	0.0	0.0	0	0.0
VVVV × RRRR						
M24-1 × *V. rotundifolia* GH 1	350(2)	4.9	2.3	2.2	33	12.1
Muscat of Alexandria × *V. rotundifolia* GH 1	275(4)	1.4	0.4	1.0	4	0.0

Table 8

Chromosome associations at M I in allotetraploid hybrids

Vine	P. M. C. Analyzed	I	II	III	IV	V	VI
N53-1	29	2.72[1]	28.40	2.70	3.55	0.24	0.10
		1—6[2]	24—35	0—4	1—5	0—1	0—1
N53-2	32	0.54	31.10	1.65	1.80	0.18	0.06
		0—3	29—39	0—2	0—5	0—1	0—1
N53-3	20	2.84	25.00	2.45	3.80	0.10	0.05
		0—7	15—33	1—6	2—5	0—1	0—1
N53-8	12	3.20	26.40	4.20	1.82	0.25	0.16
		1—6	21—32	2—8	0—4	0—1	0—1
N53-56	17	1.40	31.00	2.15	1.90	2.11	0.00
		0—6	27—39	0—3	0—4	0—1	0.

[1]) Mean, [2]) Range.

sets. Male sterile *V. vinifera* 'M24-1' (4n) and emasculated flowers of Muscat of Alexandria (4n) were pollinated with autotetraploid clones of *V. rotundifolia*, resulting in berry and seed setting. Therefore the crosses RRRR × VVVV and RRRR × VV are unsuccessful, whereas the cross VVVV × RRRR is successful, though at a low level.
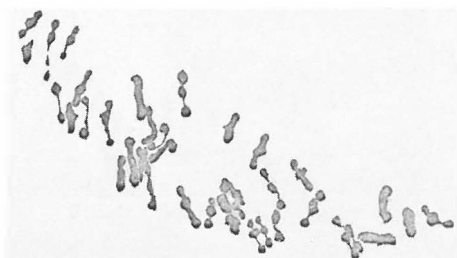
Fig. 3   Fig. 4

Fig. 3: First metaphase of meiosis of the allotetraploid N53-56, showing 39_{II} of chromomes ($\times$ 1400).
Fig. 3a (below): Drawing of same plate.

Fig. 4: First metaphase of meiosis of the allotetraploid N53-2, showing
$36_{II} + 1_{III} + 3_{1}$ ($\times$ 1400).
Fig. 4a (below): Drawing of same plate.

Chromosomal analysis of the F_1 allotetraploids

The somatic chromosome number of the allotetraploid N53 series was found to be the expected 2n = 78. The meiotic studies are summarized in Table 8. About two thirds of the chromosomes formed bivalent configurations; the range of bivalents was largest in clone N53-3 (15 to 33) and the lowest in the seedling N53-2 (from 29 to 39), see Fig. 3, 4. The highest mean bivalent formation was in the seedling N53-2 and the lowest in N53-3. The bivalents appear at MI (metaphase I) as small, round bodies and are completely paired. The highest mean number of univalents occurred in N53-8 (3.20) with a range from 1 to 6. From 0 to 3 univalents were found in N53-2, with average 0.54. Multivalent configurations are common in the squash prepara-tions. Only trivalents or quadrivalents can be recognised with certainty. With larger associations of chromosomes, especially if two of them are present in the same cell, the estimation of numbers of chromosomes in each association is difficult. The quadrivalents were loosely associated.

The common irregularity in A I (anaphase I) was lagging of the chromosomes. There were 14 PMC's studied in the seedling N53-1 at A I. Only one of them was without laggards, 7 with two laggards, 4 with one laggard and 2 with 4 laggards. In A II of the same plant, of 8 cells scored 2 displayed no laggards, 4 had 6 laggards and 2 had 7 laggards (Fig. 5, 6).

Fig. 5   Fig. 6

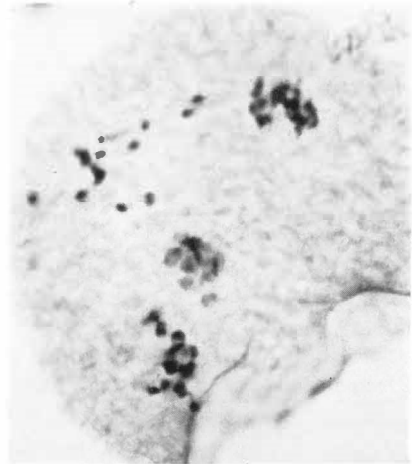Fig. 5: N53-1. Micronucleus (center) after telophase II ($\times$ 1400).

Fig. 6: N53-56. Lagging of the chromosomes in A II ($\times$ 1400).

Morphology of the F_1 allotetraploid VR hybrids

The allotetraploid population of seedlings displayed more variation in morpho-logy and vigor than did the uniform diploid F_1 hybrids of the T6 series and the diploid F_1 hybrids from which the allotetraploid hybrids originated (studies pre-viously by PATEL and OLMO, 1955). In growth rate the allotetraploid seedlings are poorer than diploid F_1 hybrids. The seedling N53-56 is extreme in this regard. Its trunk is very short, with few arms that bear shoots with very short internodes. The shoots are thin in diameter and do not extend more than 40 cm in a season. The

other seedlings of the N53 series grew somewhat better. During the growing season, shoots develop with comparable thickness to diploid shoots of moderately vigorous *V. vinifera* varieties, but they are much fewer in number and shorter in length.

In comparison with the autotetraploid clones of both species they differ in many respects. The autotetraploid clones of *V. vinifera* are on the average more vigourous, with fewer shoots per vine, shorter internodes, and thicker canes than the allotetraploids. The two autotetraploid clones of *V. rotundifolia* are weak vines of bushy habit, having many short shoots of 20—30 cm in length and very small in diameter. Hence, the VR allotetraploids are intermediate between autotetraploids of the two species in general appearance and growth rate.

Studies of some phenotypic characters revealed dominance of *V. rotundifolia* characters. The bark of the trunk was found to be fibrous only in the seedlings N53-1 and N53-56. In other seedlings of this group the bark adheres tightly to the trunk. The wood in all seedlings was of *V. rotundifolia* type, i. e. the specific gravity was above one. The lenticels were present on the canes although fewer in number than in diploid populations of the same cross. On the mature canes the diaphragm is absent and the pith is green in all seedlings.

The flower clusters are small, composed of about 45—50 flowers. The leaves of N53-2 are without lateral upper and lower sinuses and with completely overlapping petiolar sinus. The petiolar sinuses in other seedlings of this population are open and wide angled in N53-8 and N53-1 and square in N53-3. The surface of the leaf-blade is glabrous and even, occasionally the presence of some pubescence was noticed on N53-56, but not on the others.

The berry and seed studies disclosed presence of the *V. rotundifolia* characters exclusively. The berries are reddish-black, spherical, and easily detachable from the pedicel. The berry skin is tough and thick and the pulp mucilaginous, with a strong musky flavor. The seed body is oblong in shape and flat in cross section. The chalaza occupies the central part of the seed body and has radial striations. The ventral part of the seed is characterized by very shallow fossettes and the beak of the seeds is extremely short.

From the data presented, it is evident that in the allotetraploid population two characters appeared that had not been reported by Patel and Olmo (1955) in the diploid hybrid. These are the entire leaves of N53-2 and fibrous bark in N53-1 and N53-56. Such morphological changes induced by polyploidization may be explained in terms of differential dosage effect of some genetical factors.

In the diploid F_1 hybrid N53-32 which in the present investigation is grouped with allotetraploids (reasons are given in the section on materials and methods), the inheritance of morphological characters is identical to that reported by Patel and Olmo (1955) and our T6 series described in a previous paper (Jelenković and Olmo, 1968).

Discussion

In discussing fertility of the F_1 allotetraploids three points are of special interest: the wide variation among the seedlings, the erratic setting behavior and the high average number of seeds per berry.

In seedling N53-56, of 1928 ovules tested, none functioned. Davidis and Olmo (1964) reported very low set in the same clone (one seed from 1340 ovules). In two other clones the range of berry and ovule set is also very low. In comparison with the fertility of *V. vinifera* autotetraploids, the F_1 VR allotetraploids are very low (Randall, 1940 and Alley, 1957).

DERMEN (1964) reported that by chromosome doubling of sterile F_1 hybrids "fully fertile plants" were obtained. No doubt different genotypes of the F_1 hybrid respond differently after chromosome doubling, but as we have already pointed out, the lack of data prevents adequate comparison. He pointed out that stainability of the pollen grains varied between 50 and 90 percent in his allotetraploids. However, our results show that pollen viability was extremely low in the allotetraploids despite high stainability. These findings are in agreement with ALLEY (1957) that stainability of pollen grains in tetraploid *Vitis* is a poor criterion of pollen viability. The allo-tetraploids of Vitis are similar in behaviour to those in other plant genera, they are somewhat more fertile than the diploid hybrid, but do not regain the high fertility of the original parental species. A long period of selection of the raw allotetraploids appears necessary to attain the degree of fertility considered necessary in a commercial variety.

Another interesting feature of these hybrids is the erratic setting behavior. The flower clusters were usually pollinated when about 75 percent of the flowers had shed the calyptra. A typical result was to have one or two clusters with a relatively large number of berries (up to 30), and the remaining flower clusters failed to set. Clusters with only one to four berries rarely reached maturity. These variations in set occurred even with the same pollen sample.

Whenever pollination was successful more than one seed per berry was obtained. In the cross of N53-32 $\times$ Gamay the average seed number per berry was 2.82. This value is much higher than in autotetraploid crosses obtained by RANDALL (1940) and ALLEY (1957). The percent of floaters was lower than those reported by RANDALL (1940). Thus low fertility of the allotetraploid population cannot be interpreted simply on the basis of chromosomal irregularities during meiosis. Setting in each flower cluster is apparently largely influenced by the physiological conditions created by an unbalanced genetical system in these plants.

Chromosomal analysis has yielded evidence regarding the causes of sterility in VR hybrids. According to STEBBINS (1958) two classifications of hybrid sterility have been proposed. RENNER (1929) and MUNTZING (1930) (both cited by STEBBINS) distinguished haplontic or gametic sterility acting in haplophases, (i. e. in gametes or gametophytes), and diplontic sterility affecting diplophase i. e. diploid tissue in different stages of ontogenesis in the hybrid. DOBZHANSKY (1951) classified hybrid sterility as genic and chromosomal.

Chromosomal sterility results from the structural differences between the chromosomes in the hybrids. Such differences may reduce pairing. If the structural rearrangements are very small, then pairing may take place, but because of segregation and recombination gametes are formed with deficiency and/or duplication for chromosomal segments and sterility results. STEBBINS (1950) referred to the last phenomenon as "cryptic structural hybridity". Genic sterility is due to the presence of genetic factors causing disharmony either in the metabolic activity of the cells or in chromosome behaviour during meiosis.

DARLINGTON and MATHER (1950) suggested two categories of hybrid sterility: genotypic and segregational. These would correspond to the genic and chromosomal sterility of DOBZHANSKY.

Chromosomal analysis of diploid F_1 hybrids originated from *V. vinifera* 'Almeria' $\times$ *V. rotundifolia* 'Male' revealed an average of 11—13 bivalents at MI (OLMO, unpublished). They were completely sterile. Allotetraploids from the same diploid population of hybrids showed an average frequency of bivalents ranging from 25 to 31 and an average frequency of univalents ranging from 0.54 to 3.20 per

cell. Apparently most of the chromosomes were paired as bivalents, with a few trivalents and quadrivalents. Fertility tests established partial ovule and pollen fertility in four of the five seedlings, and complete sterility in N53-56. Chromosome doubling of the sterile F_1 hybrid was offered by Dobzhansky (1951) as the only direct operational approach to distinguish chromosomal from genic sterility. If the sterility is chromosomal in nature, then doubling of the chromosomes results in the presence at meiosis of a complete homolog for every chromosome, i. e. V^1V^1, $V^2V^2 \ldots V^{19}V^{19}$ and R^1R^1, $R^2R^2 \ldots R^{20}R^{20}$. Pairing and disjunctin of the chromosomes during meiosis should be expected to be completely normal and consequently fertility is restored. If the sterility is genic in nature then after doubling of the chromosome number, the quantitative balance of genetical factors causing sterility in diploid hybrids is the same in the tetraploid tissue (assuming absence of differential dosage effect) and therefore sterility should not be altered. Stebbins (1958) pointed out that a system of complementary gene pairs controlling the sterility in the F_1 diploid hybrids, by changing the segregation ratios which take place in the tetraploids may cause a rise in fertility. The assumption implies two conditions: first, normal chromosome pairing in the diploid F_1 hybrids; second, quadrivalent formation in most of the cells and consequently random chromosome segregation. It is clear that neither of these two conditions is fulfilled in our case. Complete sterility of N53-56, partial fertility in other seedlings of this population, and the presence of some chromosomal irregularities indicates that sterility in VR hybrids is both chromosomal and genic in nature.

In regard to crossing behaviour of the tetraploids, whenever tetraploid *V. rotundifolia* is used as female, pollination with *V. vinifera* fails to produce berry set. The reciprocal cross is successful. These results are not consistent with the findings of Dermen (1964) who reported complete failure of setting in all crosses at the tetraploid level. The allotetraploids revealed almost identical crossing patterns as the diploid hybrids reported in previous papers (Jelenković and Olmo 1968, 1969).

Unilateral failure in crosses is common in species that possess gametophytic or sporophytic incompatibility systems (Martin, 1964). The present case cannot be reconciled with either of these two systems. Self-sterilization which follows selfing has been reported in *Theobroma* (cited by Bateman, 1954). The possibility that *V. vinifera* pollen damages the *V. rotundifolia* egg is ruled out by double pollination technique (Olmo, unpublished).

A summary of the crossability reactions of the two species and hybrid derivatives at the diploid and tetraploid level is given in Fig. 7. The backcross progeny of VVVR genomic constitution were derived from the crosses of autotetraploid *V. vinifera* with N53-32. Fertility and cytology of these hybrids is not a part of this report. They are, however, included here in considering comparative crossability of diploid and tetraploid hybrid derivates.

Three essential facts are established concerning crossability of the two species:

1. Whenever the maternal diploid plant contains two chromosomal complements of *V. rotundifolia,* the generative nucleus of *V. vinifera* or VR hybrids fail to fertilize the *V. rotundifolia* egg. If the maternal diploid contains only one or a partial chromosomal complement of *V. rotundifolia,* fertilization succeeds with *V. vinifera* and VR pollen.

2. In allotetraploids at the ratio 2 : 2 of *V. vinifera-rotundifolia* complements in the maternal plant, pollen of autotetraploid *V. vinifera* and VVRR hybrids fertilize the egg. With 4 *V. rotundifolia* complements in the female no setting was obtained with tetraploid *V. vinifera* or VVRR hybrids.
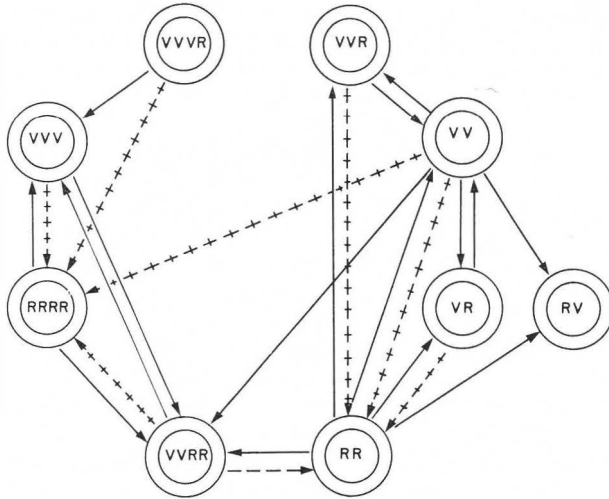
Fig. 7: Crossability of *V. vinifera* and *V. rotundifolia* and their hybrids.

——— Successful fruit set, +++ failure of fruit set, - - - some fruit set, but seeds inviable, ← indicates maternal parent. Cross-hatched circles = *V. rotundifolia* cytoplasm, clear circles = *V. vinifera* cytoplasm.

3. In a maternal plant with 2 : 2 VR complements, pollen of diploid *V. vinifera* fertilizes the egg. Pollen of VVR fertilizes diploid but not tetraploid *V. rotundifolia*. The cross RR × VVRR produced berry set but all seeds were found to be floaters and nonviable.

On the basis of these findings a chromosomal ratio hypothesis may be proposed. The hypothesis implies that the success by which: (a) *V. vinifera* pollen fertilizes eggs depends on the ratio of the chromosomes of the two species in the maternal parent, (b) *V. rotundifolia* eggs can be fertilized with pollen of a plant containing chromosomes of both species, but depends on the ratio of V : R in the pollen parent.

If the ratio of V : R in the maternal plant is 1 or larger, then *V. vinifera* pollen will fertilize the eggs. At a ratio of V : R less than 1, *V. vinifera* pollen probably will fail in fertilization. In a pollen parent with a ratio of 1 or less, pollen will fertilize *V. rotundifolia* eggs, but if the ratio is greater than 1, failure should be expected.

This hypothesis can be tested on the diploid level by backcrossing F_1 hybrids with *V. rotundifolia* pollen if random assortment of the V and R chromosomes is assured. The BC_1 progeny should display a range of crossability when tested to *V. vinifera* as a male parent or with *V. rotundifolia* as the female parent. A similar test can be carried out at the tetraploid level. Triploids might be used as well; but the extremely low recovery of balanced gametes would be a serious limiting factor.

Although the allotetraploids displayed the same crossability pattern as diploids, because of the erratic fruit setting and restricted genetic segregation interspecific hybridization and selection at the tetraploid level seems a less efficient breeding method than at the diploid level. However, if the F_1 hybrid is completely sterile, the use of tetraploids may be the only avenue to introduce the genes from one species to another.

Olmo (1942, 1952), found that autotetraploid *V. vinifera* varieties have many disadvantages compared to diploid parents. He pointed out that tetraploids derived from interspecific hybrids are less apt to have undesirable characters, yet comparatively few of them have been successful in commercial practice.

Summary

1. The allotetraploid hybrids obtained by colchicine treatment of the completely sterile F_1 diploid hybrids revealed very low and erratic fertility. A great variation in the fertility between seedlings was the rule.

2. There is no apparent relation between chromosome pairing at MI and fertility of the allotetraploid vines. Average bivalent formation in the allotetraploids varied between 25.0 and 31.1 per cell compared to the expected 39 if complete pairing occurred.

3. In all of the allotetraploid seedlings dominance of *V. rotundifolia* morphological characters was evident, although several exceptions were noted.

4. Autotetraploid clones of the two species could be hybridized only by using *V. vinifera* as a female and *V. rotundifolia* as a male parent.

5. Crossing behaviour of the allotetraploid F_1 hybrids is identical to diploid VR hybrids. Hybrids are crossable among themselves; they can be crossed successfully only as female parents to *V. rotundifolia* and as female or male parent to *V. vinifera*.

6. A chromosomal ratio hypothesis is proposed to explain the unilateral crossability pattern between the two species and their hybrid derivaties. The alternative breeding procedures are given for testing this hypothesis.

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