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Seed fertility in tetraploid grapes and their crosses with diploids

by

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In an attempt to produce triploids from the tetraploids of five varieties of grape evolved at this Institute (DAS and MUKHERIEE 1967), an unexpected feature, viz., total empty seededness in tetraploids and their crosses with diploids, was observed, which is discussed in this paper.

Materials and Methods

The tetraploid shoots were borne on the diploid bases from which they were produced by colchicine treatment. Diploid plants in the same plot were used as control.

Pollen stainability was determined from five microscopic fields on each slide, with acetocarmine stain. Pollen germination was tested by the hanging drop technique, using 15 and 20 percent sucrose solution with a trace of boric acid. Double cavity slides were used, one for diploid and the other for tetraploid, and kept moist at 25° C, for examination after 6 and 18 hours

Crosses: Studies were made on 5 categories of bunches, one each of (a) open pollinated diploid, (b) open pollinated tetraploid, (c) bagged tetraploid (not emasculated), (d) tetraploid×diploid and (e) diploid×tetraploid. The bunches were bagged with perforated butter paper bags which were removed ten days after anthesis.

Seed development was studied by collecting 5 ovaries each 0, 2, 4, 8, 15 days and 3 ovaries 25 days after anthesis. The ovaries were fixed in CRAF fixative, dehydrated through alcohol tertiary butyl alcohol mixtures and embedded in paraffin. Sections were cut at 10 μ and stained with iron-haematoxylin, and the length of ovules was measured in median sections. Ovules which increased in size over that of the previous stage and did not visibly show shrivelling of contents were scored as 'normal' and their percentage calculated on the basis of four ovules per ovary.

Seed size: Seeds were air-dried for one day after thorough washing after extraction. They were then separated into sinking or floating seeds in water, again air-dried for one day, and each class weighed separately. The nature of seeds tested through sinking in water was further checked by x-ray photography.

Results

Pollen stainability and pollen germination of the diploids and the respective tetraploids are presented in Table 1.

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	Pollen stainability %		Pollen germination	
	2n	4n	2n	4n
Bharat Early	99.00	98.33	82.00	36.60
Black Prince	68.22	92.79	67.80	64.60
Pearl of Csaba	99.70	92.00	74.50	30.00
Madeleine angevine	97.17	92.77	_	_
Madeleine royale	91.13	97.30	81.20	22.00

Table 1							
Comparative data on	pollen	stainability	and	germinability			

With the exception of Black Prince, there was little difference in pollen stainability, but the pollen germination was much lower in the tetraploids. The fruit and seed set in diploids, tetraploids and the crosses are presented in Table 2.

	Bharat Early %	Black Prince %	Pearl of Csaba %	Madeleine angevine %	Madeleine royale º/o
Fruit set					
Diploid	35.3	38.2	39.6	31.5	40.1
Diploid $ imes$ tetraploid	30.6		25.5	17.6	30.3
Tetraploid $ imes$ diploid	40.8	46.3	50.0	26.5	46.2
Tetraploid open	31.7	48.4	33.4	18.7	29.7
Tetraploid bagged Not significant	32.3	—	30.3	45.0	21.4
Seed set					
Diploid	22.7	15.2	17.2	21.6	20.9
Diploid $ imes$ tetraploid	10.7	_	10.5	5.7	12.5
Tetraploid $ imes$ diploid	11.8	9.1	6.3	5.0	14.8
Tetraploid open	9.0	15.7	12.3	6.6	18.5
Tetraploid bagged	14.7	-	12.6	2.9	18.5
L.S.D. $(P < 5\%) = 0.59$					

Table 2 Fruit set and seed set

Fruit set in tetraploids tended to be generally lower than in the diploids, though there was no statistical significance. In the crosses, the set tended to be better when the tetraploid was used as the female parent than in the reverse cross.

Seed set was calculated on the basis of four ovules per berry as the full number. Except in Black Prince, the seed set in tetraploids was less than in the diploids. In the crosses, the seed set was generally less than in the parents. In the crosses of tetraploid \times diploid, while the fruit set tended to be more than in the diploids, the seed set was less. The same trend is reflected in the percentage of seedless fruits presented in Table 3.

	Bharat Early %	Black Prince •/0	Pearl of Csaba •/0	Madeleine angevine %	Madeleine royal "/f
Diploid	0.0	13.1	2.6	0.8	0.0
Diploid $ imes$ tetraploid	0.0		0.0	0.0	0.0
Tetraploid $ imes$ diploid	18.2	38.0	0.0	22.0	0.0
Tetraploid open	5.3	20.7	1.2	11.8	12.2
Tetraploid bagged	0.0		6.2	81.4	10.0
Not significant					

Table 3 Percentage of seedless fruits

The higher fruit set and seedlessness in the fruits of tretraploid \times diploid crosses in three varieties indicate the greater ability of the fruits to mature without seeds, i. e. a greater parthenocarpic tendency in the tetraploids of at least three of the varieties.

The percentage of floating seeds (empty seeds) is presented in Table 4. When the tetraploid is used as female parent, the seeds were practically all floaters, but the latter were considerably less when the diploid was used as female parent.

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Floating seeds							
	Bharat Early %	Black Prince %	Pearl of Csaba °/•	Madeleine angevine ®/0	Madeleine royale %		
Diploid	0.72	6.71	5.26	1.74	1.45		
Diploid $ imes$ tetraploid	41.40		30.00	20.00	0.00		
Tetraploid $ imes$ diploid	100.00	100.00	100.00	100.00	84.50		
Tetraploid open	100.00	100.00	100.00	96.00	100.00		
Tetraploid bagged	100.00	—	95.40	100.00	27.60		
L.S.D. $(P < 5\%) = 22.01$							

Even though the tetraploid seeds were practically all floaters, they weighed more than the sound seeds of the respective diploids in all varieties except Madeleine angevine, in which the tetraploid seeds were slightly lighter than diploid seeds.

The seeds of the crosses with tetraploid as female weighed nearly as much as those of the tetraploids and more than diploid seeds except again in Madeleine angevine.

Development of ovules

Periodical measurements of ovules in Madeleine angevine (vide Table 5) show that the percentage of normal ovules decreased progressively after anthesis in the tetraploid more than in the diploid. But even after 25 days there was a percentage of ovules which were normal. They also show aborted ovules of different lengths. Since an ovule stops growth when it aborts, the length of an aborted ovule indicates that it has aborted at a stage, at which the normal length of an ovule is equal to that of the aborted ovule.

		Diploid			Tetraploid	
Days from anthesis	No. of ovules examined	Average length in mm	Percentage of normal ovules	No. of ovules examined	Average length in mm	Percentage of normal ovules
0	20	0.639	100	19	0.702	95
				1	0.504	
2	16	0.900	80	14	0.972	70
	4	0.747		3	0.684	
				3	0.486	
4	13	1.161	65	10	1.089	50
	4	0.918		4	0.954	
	3	0.675		5	0.702	
				1	0.495	
8	14	1.539	70	8	1.575	40
	4	0.900		6	0.855	
	2	0.693		4	0.693	
				2	0.513	
15	13	6.066	65	5	5.994	25
	4	0.872		5	1.350	
	2	1.368		8	0.873	
25	8	6.750	67	1	6.390	25
	3	0.854		2	5.814	
	-			3	1.530	
				6	0.873	

Table 5

Measurement of ovules at different intervals after anthesis in Madeleine angevine

Note: The full number of ovules were not recovered from ovaries of 15 and 25 days age possibly due to their early degeneration.

Discussion

There are usually four ovules per ovary in the grape. In the non-parthenocarpic varieties, the fruits mature even if only one of the ovules develops into a seed. Hence fruit set was less affected in the tetraploids, though seed set was much reduced. Further, tetraploids and the tetraploid \times diploid crosses bore a few seedless fruits in the clusters indicating some parthenocarpic tendency in the tetraploids.

PRATT and EINSET (1961) characterized three stages of premeiotic abortion of grape ovules, as indicated by their length. Since arrest of growth immediately follows abortion, the length of an ovule is an indication of the normalcy or the stage at which abortion took place in it. The aborted ovules of the tetraploid grapes with shrivelled contents showed no other abnormalities such as are found in Thompson Seedless or Corinth. Abortion of ovules did not always occur during meiosis as suggested by STOUT (1936), but was manifested at different stages of growth. The final seed content of the fruits is the balance left after abortions at various stages.

Ovules that aborted earlier than eight days after anthesis, possibly due to lack of fertilization, are too small to be seen by the naked eye in the mature berries. Those aborted between eight and fifteen days after anthesis appear as 'aborted seeds' in the fruit. If all the ovules in any ovary abort, the fruit is recorded as a 'seedless' fruit. The ovules in which abortion takes place after they have grown for 25 days or more i.e., after they have attained almost full size and formed hard tissues, become the floating or empty seeds. $O_{\rm LMO}$ (1934) and STOUT (1936) were also of the opinion that empty seeds indicated the earlier formation of embryo and endosperm which later degenerated.

Compared to the diploid, the growth curves of the tetraploid berries show a shorter period II, an earlier onset of period III and a more rapid rate of growth during the second cycle (NARASIMHAM and MUKHERIEE 1969). The existence of these features in association with embryo abortion has a close parallel in the embryo abortion of early maturing cherries which also have a double sigmoid growth. TUKEY (1933 a) showed that the embryo abortion in these cherries as well as in early peaches (TUKEY 1933 b) was associated with earlier onset of period III and he also proved by embryo culture, that these embryos destined to abort were viable at period II.

Since some normal ovules were present even 25 days after anthesis in the tetraploid grapes, their abortion (leading to the formation of floating seeds) must have occurred later, about 30 days after anthesis. The onset of period III is also seen to occur in these varieties between 30—37 days from anthesis coinciding with the above period, the situation being strikingly similar to that of the cherries reported by TUKEY. BRINK and COOPER (1941) suggested that post-fertilization abortion occurred due to lack of adjustment between the embryo and endosperm on one hand and the maternal tissues on the other and named the phenomenon 'somatoplastic sterility'. They included the nucellus and the integuments as the competing maternal tissues starving the endosperm. In the case of these tetraploids, the rapidly growing ovary seems to have been the competing factor resulting in somatoplastic sterility.

This second cycle of growth is shown by COOMBE (1960) to be due to osmotic attraction of water consequent on the influx of sugars into the berries. The higher TSS (NARASIMHAM and MUKHERIEE 1969) recorded in the tetraploids as well as the larger cell size might have been the cause of higher rates of growth. This complex feature leading to the expression of a type of somatoplastic sterility, if it persists over the years, would be a new response of grape to autotetraploidy.

The empty seededness in the crosses is associated more with the maternal parent than with the pollen parent, being nearly 100 per cent when tetraploid was the mother and considerably less when the diploid was the mother. In the crosses involving varieties with (Pearl of Csaba) and without floating seeds, OLMO (1942) found that the maternal parent was responsible for their occurrence and not the pollen parent. The features observed in the present studies can be explained on the basis of the somatoplastic sterility proposed above. In the diploid \times tetraploid cross, the maternal tissues are diploid and normal, and the tetraploid endosperm is perhaps responsible for the maladjustment. Hence the percentage of floating seeds is smaller. In the reverse cross, both the pentaploid endosperm as well as the rapidly growing ovary contribute to maladjustment and hence the total empty seedness.

The occurrence of more floating seeds in the tetraploid \times diploid cross than in the reverse cross is contrary to that reported by ALLEY (1957). RANDALL (1936) found that both crosses gave some viable seeds. He was of the view that the functioning

aneuploid gametes from the tetraploid parent cause the floating seeds. Since more aneuploid eggs function than aneuploid pollen, the explanation fits with the present observation. But it appears improbable that 100 percent floating seeds are all due to this reason. The postulation of somatoplastic sterility, on the other hand, provides a mechanism for the occurrence of floating seeds and explains the various features studied.

The occurrence of nearly 100 per cent empty seeds in tetraploids poses a problem in tetraploid breeding since the seeds will not germinate. But no such difficulty has been reported by OLMO and KOYAMA (1962), or DERMEN (1964). The unusual phenomenon in these tetraploids requires to be examined further. Because none of the varieties is used for polyploidy elsewhere, no comparison can be made.

From the point of view of triploid production, the complete empty seededness in the tetraploid \times diploid cross precludes the use of this cross for this purpose. The reverse cross, fortunately, has yielded a fair percentage of filled seeds in the varieties studied. The higher seed weight in this cross is an indication that diploid pollen has participated in the fertilization. Diploid \times tetraploid crosses have been generally unsuccessful in annual plants for various reasons (STEBBINS 1958). On the other hand, in woody plants like pear and *Populus* the position was different. ZIELINSKI (1962) obtained more seeds in this cross than even the diploid in some pear varieties. Johnsson (1945) found that 46 percent of the progeny from this cross were triploid in *Populus tremula*. In grapes, the diploid \times tetraploid crosses should be attempted for triploid production, till the problem of empty seededness in the reverse cross is overcome.

Summary

The fruit and seed set as well as the occurrence of empty seeds in diploids, tetraploids and their crosses in five varieties of grapes were studied. Ovule lengths at certain intervals after anthesis in the diploid and tetraploid of one variety have been presented to indicate development of ovules. Late abortions of ovules resulting in empty seededness in tetraploids were found to be related to the shortening of period II of berry growth, earlier and greater accumulation of TSS, the earlier onset of period III and the higher rate of growth of the ovary. The consequent lack of adjustment between the pace of growth of endosperm and the ovary probably led to somatoplasitc sterility.

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