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Effect of some genetic and environmental factors on spontaneous polyembryony in grape (Vitis vinifera L.)

by

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Effet de quelques facteurs génétiques et du milieu sur la polyembryonie spontanée chez la vigne (Vitis vinifera L.)

Résumé. — La variabilité du caractére «polyembryonie spontanée» été étudiée sur 35 cépages de *V. vinifera* L. La fréquence observée des plantules jumelles parmi les pépins germés varie de 0 à 0,35 %, avec une moyenne de 0,054 %. Merlot est la variété la plus intéressante à cet égard.

La polyembryonie chez la vigne semble être une caractéristique variétale contrôlée génétiquement. Cependant, elle peut être influencée par certains facteurs du milieu: Un faible taux de nouaison, induit par les conditions climatiques ou tout autre facteur, est généralement corrélé avec une fréquence élevée de polyembryonie.

Les différences observées dans les cinétiques comparées de germination, et l'influence de la durée de stratification des pépins au froid, montrent que les pépins polyembryonnés sont moins aptes à germer que les pépins monoembryonnés. La température optimale de germination des pépins polyembryonnés est 23 °C; une température plus haute augmente la compétition entre les embryons multiples, et accroît la fréquence des «jumeaux cachés».

La faiblesse et la léthalité de nombreuses plantules issues des pépins polyembryonnés réduit fortement la probabilité d'obtenir des plantes haploïdes. Jusqu'à maintenant, seules des plantules diploïdes ont pu être obtenues. Cependant, l'instabilité apparente de l'état haploïde chez lo vigne laisse penser que des plantes diploïdes homozygotes, issues du développement parthénogénétique de cellules du sac embryonnaire, pourraient être produites par cette voie.

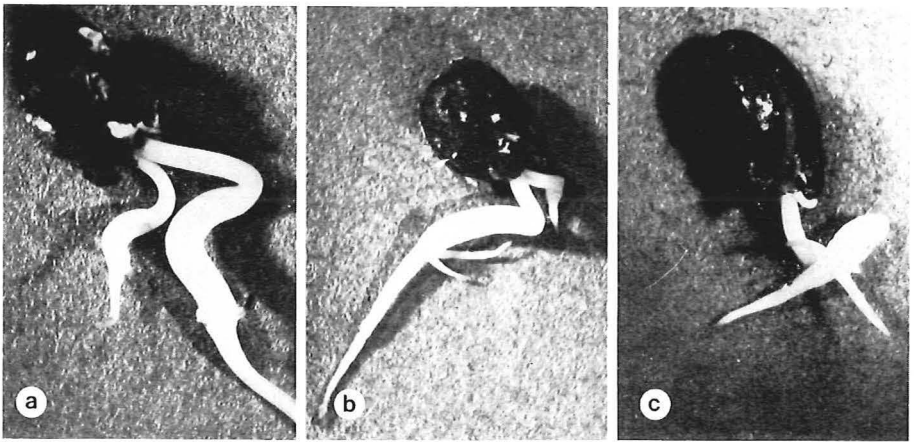
Introduction

Haploidy in grape would be of considerable interest from the cytological and genetical point of view. So far, haploid plants have not been found in *Vitis*. Polyembryonic seeds are a classical source of spontaneous haploids in Angiosperms and the subject has been reviewed by Webber (1940), Kappert (1950), Kimber and Riley (1963), Magoon and Khanna (1963), Maheshwari and Rangaswamy (1965), Lacadena (1974).

A first report on polyembryony in grape was made by Negrul (1934) who observed twin seedlings in the Russian cultivar Nimrang. However, Mullins and Srinivasan (1976) suggest that *V. vinifera* is a monoembryonic species. They based their opinion on the results of Stout (1936), who was unable to obtain any polyembryonic seeds using the same cultivar Nimrang in Geneva, N. Y. In fact, the most likely reason that Stout was unable to duplicate Negrul's research is due to the great difference in environmental factors between New York and Soviet Union.

In France, THEVENOT (1972) observed polyembryonic seeds in some cultivars of Alsace vineyards, but no report of haploids was made. In 1975, systematic screening for polyembryonic seeds and haploid plants in *V. vinifera* was initiated in Bordeaux. Preliminary and incomplete results were published in the proceedings of the 2nd International Symposium on grape breeding (BOUQUET 1978 a). Although the research for haploidy was unsuccessful, a lot of information was obtained on the variability of the polyembryony in grape.

Mutiple embryos are probably formed very early in the seeds, during or immediately after blooming period. So, environmental factors like climatic conditions and physiological state of the vine may have an effect on the frequency of polyembryony which is probably genetically determined, too. Direct observation of multiple embryos in the seeds is practically impossible in grape, so we can have only an estimate of this frequency by the observed proportion of seeds which give twin or multiple seedlings after germination. This observed frequency may be modified by some other environmental factors having an effect on the physiology of the seed like chilling or temperature of germination.



Germination of polyembryonic seeds from *V. vinifera* L. cv. Merlot: degrees of dissymetry between twin seedlings.

Germination de pépins polyembryonnés de *V. vinifera* L. cv. Merlot: degrés de dissymétrie à l'intérieur des couples de jumeaux.

Material and methods

Open pollinated seeds were harvested, extracted and cleaned with diluted commercial chlorox. They were chilled and conserved in moist sawdust at 5+ °C until utilization. After chilling, seeds were washed carefully, treated with fungicide, and placed on moistened paper towels in plastic dishes covered with plastic wraps. Sawdust was added in the dishes to prevent development of moulds.

They were then incubated at 27 °C because grape seeds germinate faster and vigorously at this temperature. But we shall see that optimal temperature of germination is not necessarily the same for monoembryonic and polyembryonic seeds. When radicle emerged and was 1—2 cm long (Fig.), seeds were screened for twin

Table 1

Genetical variability of spontaneous polyembryony in *V. vinifera* L. (seeds harvested in 1975; temperature of germination: 27 °C)

Variabilité génétique de la polyembryonie spontanée chez *V. vinifera* L. (pépins récoltés en 1975; température de germination: 27 °C)

Varieties	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
Aligote	11000	6125	55.7	2	0.033
Baroque	10700	6286	58.7	2	0.032
Cabernet franc	30500	14570	47.8	3	0.021
Cabernet Sauvignon	47100	21905	46.5	10	0.046
Carignan	18900	6369	33.7	5	0.079
Chardonnay	61100	26576	43.5	17	0.064
Chenin	24500	17702	72.3	0	0
Colombard	20000	9481	47.4	0	0
Cot	12000	7695	64.1	0	0
Fer Servadou	13000	9226	71.0	0	0
Folle blanche	33800	14228	42.1	6	0.042
Gamay noir	28400	15237	53.6	8	0.052
Grenache noir	30100	16194	53.8	4	0.025
Grolleau	27700	11891	42.9	28	0.235
Gros Manseng	8300	6936	83.2	0	0
Jurançon noir	28500	12371	43.4	5	0.040
Maccabeu	14800	4185	28.2	0	0
Mauzac	119800	91545	76.4	160	0.175
Melon	44000	21766	49.5	6	0.027
Merille	8700	5213	60.0	1	0.019
Merlot	108900	95068	87.3	331	0.348
Mourvèdre	8100	4572	56.2	5	0.109
Muscadelle	12300	4223	34.4	1	0.023
Négrette	9600	5015	52.2	1	0.019
Perle de Csaba	12600	4731	37.4	1	0.021
Petit Verdot	26000	8777	34.0	1	0.011
Petit Meunier	53620	23945	44.7	18	0.075
Pinot noir	40800	18686	45.8	22	0.117
Riesling	53100	28935	54.5	45	0.155
Sauvignon	14300	5294	37.0	3	0.056
Sémillon	15000	7171	47.8	1	0.014
Sylvaner	9800	4754	48.5	0	0
Tannat	9000	6558	72.9	1	0.015
Traminer	20200	3764	18.6	0	0
Ugni blanc	76500	61483	80.4	10	0.016
Total	1062720	608477	57.3	697	0.115

Table 2

Clonal variability of spontaneous polyembryony in *V. vinifera* L. cv. Merlot and influence of grafting (seeds harvested in 1976; temperature of germination: 27 °C)

Variabilité clonale de la polyembryonie spontanée chez *V. vinifera* L. cv. Merlot et influence du greffage (pépins récoltés en 1976; température de germination: 27 °C)

Clones		Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
Cl. 2527	ungrafted GF	12000	8716	73.6	40	0.458
	grafted/420 A GP	11000	7922	73.0	14	0.176
Cl. 2529	ungrafted GF	9000	6354	70.6	19	0.300
	grafted/420 A GP	9000	6876	76.4	13	0.189
Cl. 2530	ungrafted GF	9800	7648	78.0	27	0.353
	grafted/420 A GP	9200	6819	74.1	19	0.278
Cl. 3196	ungrafted GF	10700	8019	74.9	37	0.461
	grafted/420 A GP	10300	7417	72.0	24	0.324
Cl. 3197	ungrafted GF	9500	6809	71.6	27	0.396
	grafted/420 A GP	9800	7519	76.7	29	0.385
Cl. 3211	ungrafted GF	14000	9208	65.8	32	0.347
	grafted/420 A GP	6000	3994	66.5	7	0.175
Cl. 3224	ungrafted GF	7300	5294	72.5	39	0.737
	grafted/420 A GP	8000	6052	75.6	22	0.364
Cl. 3237	ungrafted GF	13300	7898	59.4	18	0.228
	grafted/420 A GP	5700	3948	69.6	10	0.253
Total	ungrafted GF	85600	59946	70.0	239	0.399 *
	grafted/420 A GP	69000	50547	73.3	140	0.277 *

GF = Domaine de la Grande Ferrade, Pont-de-la-Maye. GP = Domaine du Grand Parc, Latresne.
* = Significant at the 5 % level.

seedlings. Two possible sources of error must be taken into consideration when calculating the frequencies of twin seedlings. The first one is due to "pseudo-twins" misjudged because forking of the root meristem. But, when the basal portion of hypocotyl emerges from the seed, the confusion is not possible. The second and most important source of error is due to the occurrence of "cryptic twins" in which the smaller member can be hidden by the larger. In *V. vinifera,* where a majority of twin seedlings are very dissymetrical, the frequency of polyembryony is probably underestimated.

After screening, polyembryonic seeds were planted and grown in a greenhouse. Different media were used (peat, sand, perlite, sawdust, . . .) but none gave good results as for the survival of the smallest plants from dissymetrical twins which are extremely fragile in non-sterile conditions. So far, only vigorously growing plantlets could be screened cytologically for haploidy. Chromosome countings were made on root tips according to the classical procedure of Feulgen's stain after pretreatment in α-monobromonaphthalene and fixation in acetic acid-alcohol 1 : 3 (BOUQUET 1978 b).

Results

1. Intraspecific variability of polyembryony in grape (*V. vinifera* L.)

Frequency of polyembryony was estimated on 35 varieties of *V. vinifera.* Seeds were harvested in 1975. On the whole, screening was made on 1 062 720 seeds, among which 608 477 germinated (57.3 %). Results are given in Table 1.

Frequency of polyembryony among germinated seeds shows a large variation from 0 to 0.35 %, according to the varieties. Most interesting are Merlot, Grolleau, Mauzac, Riesling, Pinot noir and Mourvèdre for which the frequency of polyembryony is above 0.1 %. 24 varieties (80 %) show a frequency below 0.05 %. For some varieties like Chenin, Colombard, Cot, Fer Servadou, Sylvaner, Traminer, research was unsuccessful and frequency of polyembryony can be estimated below 0.02 %. Frequency of polyembryony observed on the whole is 0.115 %, but is not significant because variation in the number of seeds screened by variety is considerable.

Average frequency of polyembryony in *V. vinifera,* calculated with the same number of seeds per variety, can be estimated at 0.054 %.

Among the polyembryonic seeds observed in the variety Merlot, which shows the highest frequency of polyembryony, 88 % gave twin seedlings, 11.5 % triple seedlings, and 0.5 % quadruple seedlings.

There is no correlation between frequency of polyembryony and geographical origin of the varieties. But we can observe that the four varieties belonging to the family of Cots (LEVADOUX 1956), namely Cot, Tannat, Négrette and Merille show comparable frequencies of polyembryony, below 0.02 %. Likewise, Folle blanche and Jurançon noir, belonging to the same family of Folloides, show similar frequencies: 0.042 and 0.040 %. On the contrary, we can observe a considerable variation of polyembryony among the varieties belonging to the family of Noiriens, namely Pinot noir, Meunier, Chardonnay, Traminer, Gamay and Melon. Then again, among varieties belonging to the family of Carmenets, namely Petit Verdot, Fer Servadou, Cabernet franc, Cabernet Sauvignon and Merlot, we can observe that the frequency of polyembryony shows a variation apparently correlated with the degree of evolu-

Table 3

Influence of the age of the vines on spontaneous polyembryony in *V. vinifera* L. cv. Merlot (1977; temperature of germination: 23 °C)

Influence de l'âge des vignes sur la polyembryonie spontanée chez *V. vinifera* L. cv. Merlot (1977; température de germination: 23 °C)

Age	Clones	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
8 years	Cl. 2141 GF	9400	5571	59.3	19	0.341
	Cl. 3216 GF	19000	12110	63.7	25	0.206
	Total	28400	17681	62.3	44	0.249
20 years	Cl. 2141 GP	8600	6018	70.0	19	0.316
	Cl. 3216 GP	15000	10148	67.6	17	0.167
	Total	23600	16166	68.5	36	0.223

tion of the varieties. Petit Verdot and Fer Servadou, which show the lowest frequency of polyembryony are generally considered as archaïc varieties very closely related to the wild grape *(V. vinifera* L. ssp. *silvestris).* This can be related to the fact that no twin seedling was found in a screening made on 3 000 seeds collected in wild grapevines native of south-western Pyrenees.

2. Clonal variability of polyembryony in *V. vinifera* L. cv. Merlot

In 1977, frequency of polyembryony was estimated on 8 clones of *V. vinifera* L. cv. Merlot. Each clone was planted in two locations: on its own roots in a light gravelly soil and grafted on 420 A in a clay soil with moderate lime. Seeds were harvested in 1976. Results are given in Table 2.

Frequency of polyembryony shows a rather wide variation from 0.175 to 0.737 %, but two-ways analysis of variance after angular transformation (arc sin $\sqrt{x}$) shows that variation between clones is not significant. This result must be considered cautiously because there is no replication within each treatment. So residual error can include genotype × environment interactions which can minimize clonal effect.

We can conclude, however, that polyembryony in *V. vinifera* L. cv. Merlot is mainly a varietal characteristic, even if differences between clones can appear occasionnally.

3. Effect of environmental factors on formation of polyembryonic seeds

a) Soil and culture conditions

Frequency of polyembryony was estimated on 8 clones of *V. vinifera* cv. Merlot. Considerable differences of vigour and berry set were induced on each clone by combination of two factors: grafting and fertility of the soil. Clones of Merlot, 20 years old, grafted on 420 A and planted in a clay soil with good fertility, show moderate to high vigour with a good berry set. Clones of Merlot, 8 years old, planted on their own roots in a poor gravelly soil, show very low vigour with a bad berry set. Results are given in Table 2. Frequency of polyembryony in the low vigour and bad berry set conditions was 0.399 %, against 0.277 % in the high vigour and good berry set conditions. Statistical analysis show that difference is significant.

We must note that the frequency of polyembryony of the variety Merlot, given in Table 1, was estimated on seeds harvested in 1975 on the same vines, ungrafted, which have given in 1976 the highest level of polyembryony. Other varieties of Table 1 were grafted, so the difference between Merlot and other varieties with high level of polyembryony (Grolleau, Mauzac, Riesling, . . .) is probably overestimated.

Frequency of polyembryony was estimated on two other clones of Merlot. Vines were grafted on 420 A and planted in 1958 and 1970 in two different locations. So, influence of the age of the vines cannot be separated from the influence of the soil fertility. Seeds were harvested in 1977. Results are given in Table 3. Age of the vines induces a slight decrease in the frequency of polyembryony. Vigour of the oldest vines was superior to the vigour of the youngest ones, but differences in berry set were not evident. Though they are not statistically significant, these results are consistent with the former.

So, we can conclude that environmental factors conditionning growth and development of the vines can have a unnegligible influence on the frequency of polyembryony.

Table 4

Influence of climatic conditions on spontaneous polyembryony in *V. vinifera* L. cvs. Merlot and Mourvèdre (temperature of germination: 27 °C)

Influence des conditions climatiques sur la polyembryonie spontanée chez *V. vinifera* L. cvs. Merlot et Mourvèdre (température de germination: 27 °)

Variety	Year	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
Merlot GF ungrafted	1975	108900	95068	87.3	331	0.348
	1976 a	85600	59946	70.0	239	0.399
	1976 b	48200	38774	80.4	148	0.382
	1977	5400	2919	54.0	15	0.513
	1978	14100	10705	75.9	106	0.986
Mourvèdre GF grafted on 420 A	1975	8100	4572	56.2	5	0.109
	1976	45600	36431	79.9	26	0.071
	1978 c	7500	3310	44.1	2	0.060

a = Seeds collected from the 8 clones of Table 2.
b = Seeds collected from other clones.
c = Vines covered with plastic greenhouse.

Table 5

Influence of climatic conditions on spontaneous polyembryony in two clones of *V. vinifera* L. cv. Merlot (temperature of germination: 23 °C)

Influence des conditions climatiques sur la polyembryonie spontanée chez deux clones de *V. vinifera* L. cv. Merlot (température de germination: 23 °C)

Year	Clones	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
1976	Cl. 2141 GF	8150	7050	86.5	15	0.212
	Cl. 3216 GF	25500	20524	80.4	38	0.185
	Total	33650	27574	81.9	53	0.192
1977	Cl. 2141 GF	9400	5571	59.3	19	0.341
	Cl. 3216 GF	19000	12110	63.7	25	0.206
	Total	28400	17681	62.3	44	0.249

Table 6

Climatic data recorded in Pont-de-la-Maye during blooming period (years 1975 to 1978)
Données climatiques recueillies à Pont-de-la-Maye durant la période de floraison (années 1975 à 1978)

	June 1975	June 1976	June 1977	June 1978
Mean temperature (°C)	18.8	22.5	17.4	17.6
Extreme temperatures (°C)	13—24	15—30	12—22	12.8—22.4
Rainfall (mm)	12	7.3	81.4	81.8

b) Climatic conditions

Influence of climatic conditions on spontaneous polyembryony was studied on the varieties Merlot and Mourvèdre. Results are given in Tables 4 and 5. Table 6 gives climatic data recorded in Pont de la Maye during blooming periods from 1975 till 1978.

In 1975, climatic conditions were very favourable to berry set on Merlot. Percentage of germination of the seeds was good. In 1976, high temperatures and water stress caused some berry drop. We observed a slight increase in the frequency of polyembryony in Merlot GF ungrafted from 1975 to 1976. The difference observed between the percentages of germination of the lots a and b is probably due to the fact that the second lot was germinated 1 year later (see Table 7). In 1977 and 1978, berry set was strongly reduced by cold and rainy weather. Percentages of germination were reduced too but we observed simultaneously a considerable increase in the frequency of polyembryony. Increase in polyembryony from 1976 to 1977 is 34 % on Merlot ungrafted GF and 30 % on Merlot GF cl. 2141 and 3216. Increase in polyembryony from 1975 to 1978 is 183 % on Merlot ungrafted GF.

From this we can conclude that occurrence of polyembryony, considered as an abnormality during fecondation and early development of the embryo, is increased if climatic conditions during blooming period are unfavourable to berry set.

Observations made on the variety Mourvèdre are apparently inconsistent with those made on Merlot during the same years. This can be explained by the differences in the number of seeds screened in the 3 years, but also by the fact that Mourvèdre, a late-ripening variety native of Spain, is more adapted to hot and dry climates than Merlot. So, climatic conditions during blooming period were probably more favourable to berry set in 1976 and also 1978 (the vines were covered by a plastic greenhouse) than in 1975.

4. Effect of environmental factors on the expression of polyembryony

a) Length of chilling period

With 15 lots of seeds, a germinating experiment was set up at two different times: The first germination was made after a period of chilling varying for each lot from 3 to 12 months, the second germination was made 1 year later, i.e. after a period of chilling varying from 15 to 24 months. Results are given in Table 7.

On the whole, we can observe an increase of the percentage of germination associated with a slight, but not significant decrease of the frequency of polyembryony.

But variation between varieties is important. Increase in the percentage of germination with a longer period of chilling is currently observed in grape seeds. Only two varieties, Cabernet franc and Cabernet-Sauvignon, show a significant decrease of germination after a longer chilling. Causes are unknown.

Four varieties, Chardonnay, Pinot noir, Meunier and Riesling, show an increase in the percentage of germination associated with a considerable increase in the frequency of polyembryony. We can suppose that for these varieties, originating from northern vineyards, chilling requirements are higher in polyembryonic seeds than in monoembryonic seeds.

Table 7

Influence of the duration of chilling on the percentage of observed polyembryony (temperature of germination: 27 °C)

Influence de la durée de stratification au froid sur le pourcentage de polyembryonie observée (température de germination: 27 °C)

Varieties	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
	1st germination (chilling: 3—12 months)				
Cabernet franc	15500	8304	53.7	2	0.024
Cabernet Sauvignon	17600	10603	60.2	5	0.047
Chardonnay	31100	10887	35.0	3	0.028
Folle blanche	15000	4631	30.8	2	0.043
Gamay	13400	7020	52.4	3	0.043
Grolleau	15700	6609	42.0	17	0.257
Jurançon noir	14000	4340	31.0	2	0.046
Mauzac	25300	19538	77.2	32	0.164
Merlot 1976	129900	92571	71.2	334	0.361
Meunier	26000	10379	39.9	4	0.038
Mourvèdre 1976	13300	10446	78.5	10	0.095
Pinot noir	22500	9479	42.1	6	0.063
Riesling	14800	6762	46.0	6	0.089
Total	354100	201569	56.9	426	0.211
	2nd germination (chilling: 15—24 months)				
Cabernet franc	15000	6266	41.8	1	0.016
Cabernet Sauvignon	29500	11302	38.3	5	0.044
Chardonnay	30000	15689	52.3	14	0.089
Folle blanche	18800	9597	51.0	4	0.042
Gamay	15000	8217	54.8	5	0.061
Grolleau	12000	5282	44.0	11	0.208
Jurançon noir	14500	8031	55.4	3	0.037
Mauzac	94500	72007	76.2	128	0.178
Merlot 1976	83800	68389	81.6	242	0.353
Meunier	27620	13566	49.9	14	0.103
Mourvèdre 1976	32300	25985	80.4	16	0.061
Pinot noir	18300	9207	50.3	16	0.174
Riesling	38300	22173	57.1	39	0.176
Total	429600	275711	64.2	498	0.180

Table 8

Effect of the temperature of germination on the percentage of observed polyembryony in *V. vinifera* L. cv. Merlot (1976)

Effet de la température de germination sur le pourcentage de polyembryonie observée chez *V. vinifera* L. cv. Merlot (1976)

Temperature of germination (°C)	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
21	8000	6536	81.7	21	0.321
23	8000	6864	85.8	25	0.364
25	8000	6808	85.1	23	0.337
27	8000	6696	83.7	14	0.209

Table 9

Effect of the temperature of germination on the percentage of observed polyembryony in *V. vinifera* L. cv. Merlot (1976)

Effet de la température de germination sur le pourcentage de polyembryonie observée chez *V. vinifera* L. cv. Merlot (1976)

Temperature of germination (°C)	Clones	Number of seeds	Number of seeds germinated	Percentage of germination	Number of twins observed	Percentage of polyembryony
23	Cl. 2141 GF	8150	7050	86.5	15	0.212
	Cl. 3216 GF	25500	20524	80.4	38	0.185
	Total	33650	27574	81.9	53	0.192
27	Cl. 2142 GF	16000	13586	84.9	17	0.125
	Cl. 3216 GF	16500	12711	77.0	14	0.110
	Total	32500	26297	80.9	31	0.118

Table 10

Cumulative percentages of germination of monoembryonic and polyembryonic seeds in *V. vinifera* L. cv. Merlot (1976; temperature of germination: 27 °C)

Pourcentages cumulés de germination des pépins mono et polyembryonnés chez. *V. vinifera* L. cv. Merlot (1976; température de germination: 27 °C)

Duration of germination (d)	Number of seeds germinated	Cumulative percentage of germination (monoembryonic seeds)	Number of twins observed	Percentage of polyembryony	Cumulative percentage of germination (polyembryonic seeds)
5—6	56365	40.0	63	0.112	12.8
7—8	48699	74.6	178	0.365	48.9
9—10	27480	94.1	174	0.633	84.2
11—12	7053	99.1	58	0.822	95.9
13 and more	1320	100.0	20	1.520	100.0
Total	140917	100.0	493	0.350	100.0

Table 11

Cumulative percentages of germination of monoembryonic and polyembryonic seeds in *V. vinifera* L. cv. Merlot cl. 2141 and 3216 (1976 and 1977; temperature of germination: 23 °C)

Pourcentages cumulés de germination des pépins mono et polyembryonnés chez *V. vinifera* L. cv. Merlot cl. 2141 et 3216 (1976 et 1977; température de germination: 23 °C)

Duration of germination (d)	Number of seeds germinated	Cumulative percentage of germination (monoembryonic seeds)	Number of twins observed	Percentage of polyembryony	Cumulative percentage of germination (polyembryonic seeds)
5—6	23249	37.8	13	0.056	9.8
7—8	21159	72.3	49	0.232	46.6
9—10	12558	92.7	51	0.406	84.9
11—12	3433	98.3	15	0.437	97.8
13 and more	1022	100.0	5	0.489	100.0
Total	61421	100.0	133	0.210	100.0

b) Temperature of germination

Temperature currently used in grape for germination is 27 °C because seeds germinate faster and vigorously. But the observation of a high frequency of very dissymetrical twin seedlings let us think that optimal temperature for germination of polyembryonic seeds and expression of the character may be not the same than for monoembryonic seeds.

Four temperature regimes of germination were tested in 1978 with a lot of seeds harvested in 1976 (cv. Merlot, cl. 2529, 2530 and 3211 GP). Results are given in Table 8 and show clearly that optimal temperature for the expression of polyembryony in the variety Merlot is 23 °C. Lowest frequency of polyembryony is observed at 27 °C and increase in polyembryony from 27 to 23 °C is 74.1 %.

Germination at 23 °C was also tested in 1978 with two other clones of Merlot (cl. 2141 and 3216) and compared to germination made at 27 °C in 1977 with the same lot of seeds harvested in 1976. So seeds germinated at 23 °C were chilled 1 year longer than seeds germinated at 27 °C. Results are given in Table 9: Increase in polyembryony from 27 to 23 °C is 69 %. These findings are very consistent with the former if we consider that a longer chilling period causes no significant decrease in the frequency of polyembryony in the variety Merlot (Table 7).

We can suppose that high temperature of germination, above 25 °C, increases the frequency of "cryptic twins", because the development of the smaller member is inhibited by the vigorous growth of the larger.

5. Kinetics of germination of monoembryonic and polyembryonic seeds

Kinetics of germination were measured on a lot of 166 000 seeds of the variety Merlot of which 140 917 germinated. Seeds were harvested in 1976. Germination temperature was 27 °C. Results are given in Table 10.

Results show clearly that polyembryonic seeds germinate more slowly than monoembryonic seeds. But delay of germination is not more than 2 d.

Kinetics of germination were measured too on 85 650 seeds of the variety Merlot (cl. 2141 and 3216) germinated at 23 °C. Results are given in Table 11 and are not significantly different from those obtained with a temperature of 27 °C.

Discussion

On the basis of our results, the average frequency of polyembryony in grape (*V. vinifera* L.) can be estimated at 0.054 %. This value is apparently consistent with the results of Thevenot (1972) who observed 220 twin seedlings out of 470 800 germinated seeds (0.046 %) from 9 varieties of Alsace vineyards.

Frequencies of observed polyembryony are 0.22 % on *Asparagus* (Thevenin 1968), 0.24 % on *Capsicum* (Pochard 1969), 0.10 % on maize (Sarkar and Coe 1966) and 4 % on soybean (Kenworthy *et al.* 1973). So, as compared with other plants, frequency of observed polyembryony in grape is relatively low.

Frequencies of polyembryony observed in Bordeaux on Pinot noir, Riesling, Traminer and Sylvaner are not very different from those found in the same varieties in Colmar by Thevenot. We stated similar frequencies of polyembryony in closely related varieties. Thevenot, too, observed similar frequencies in the varieties Pinot

noir and Pinot gris. Thus, we can conclude that polyembryony in grape is mainly under genetical control and is a varietal characteristic.

Differences of polyembryony observed between clones of the variety Merlot are not statistically significant. But Merlot is a variety which appeared rather lately, so we cannot generalize this result to older varieties, like Pinot or Grenache, which have probably a much wider clonal variability.

Polyembryony can be greatly influenced by environmental factors. THEVENOT observed wide differences of polyembryony in lots of seeds of the varieties Riesling and Auxerrois, harvested in 1970 and 1971. He concluded that polyembryony, considered as an abnormal phenomenon, was favoured when the fecondation was disturbed by climatic conditions. Our results on the variety Merlot corroborate this hypothesis. We observed that a low berry set, induced by climatic conditions or other factors, is generally correlated with a high frequency of polyembryony.

THEVENOT found that a lot of seeds of the variety Riesling, harvested in 1970 and germinated in 1972, after a long period of chilling, gave a frequency of polyembryony practically twice as high as the frequency observed on the same lot germinated in 1971. We observed similar differences in Bordeaux on Riesling, but also on the other northern varieties Pinot noir, Meunier and Chardonnay. These results let us think that polyembryonic seeds are less fit to germinate than monoembryonic seeds, which is corroborated by the differences we observed in the kinetics of germination, and is in harmony with the observations of THEVENIN (1968) on *Asparagus*.

Some polyembryonic seeds give twin seedlings absolutely symmetrical, which is probably due to segmentation of the zygote in the first stages of embryogenesis. Yet, the majority of polyembryonic seeds give dissymetrical seedlings, particularly in the variety Merlot, where the dissymetry is considerable and the frequency of "cryptic twins" probably high. This frequency of "cryptic twins" can be reduced by lowering the temperature of germination from 27 to 23 °C. A temperature of 23 °C is apparently optimal for germination of polyembryonic seeds; higher temperatures increase the competition between the polyembryos to the prejudice of the smallest ones. On the other hand, lower temperatures reduce the speed of germination, and make the small embryos more susceptible to development of moulds and bacteria.

The culture of twin seedlings under greenhouse conditions gives very bad results as for the survival of the smallest plants which are extremely fragile in non-sterile conditions. So, only vigorously growing plantlets could be checked cytologically and revealed typical diploid metaphases. Nevertheless, mixoploid structures with typical haploid, diploid and aneuploid cells were observed on very weak seedlings obtained from polyembryonic seeds of the variety Merlot and fixed as they germinated (BOUQUET 1978 a). This cytological situation may be due to different types of mitotic irregularities during chromatid segregation and chromosomal distribution at the anaphase. The degree of irregularity and the importance of the loss of chromosomes can be related to the non-viability of numerous seedlings. However, if chromosomal doublings occur sufficiently early during the embryogenesis, diploid homozygote seedlings can be produced from parthenogenetic development of haploid cells of the embryo sac. Grapevine is known to contain a heavy load of deleterious recessive genes and to show considerable inbreeding effect when selfing. Merlot is one of the most susceptible varieties to inbreeding depression. So the lethality of some plantlets obtained from polyembryonic seeds could be ascribed to complete homozygosis. Unfortunately, lack of genetic markers makes the identification of homozygote genotypes in grape very difficult.

Apparent instability of the haploid condition in grape is corroborated by the observations of GRESSHOF and DOY (1974) on the polyploidization of haploid cells *in vitro* and the results of RAJASEKARAN and MULLINS (1979) who, by anther culture, obtained diploid and haploid calluses, but only diploid plantlets.

Summary

Variability of polyembryony was studied in 35 varieties of *Vitis vinifera* L. The observed frequency of twin seedlings is varying from 0 to 0.35 % with a mean of 0.054 %. Merlot is the most interesting variety.

Polyembryony in grape is mainly under genetical control and is a varietal characteristic. However, polyembryony is influenced by environmental factors: A low berry set, induced by climatic conditions or other factors, is generally correlated with a high frequency of twin seedlings.

Differences observed in the kinetics of germination and influence of the length of chilling show that polyembryonic seeds are less fit to germinate than monoembryonic seeds. Optimal temperature for germination of polyembryonic seeds is 23 °C; higher temperatures increase the competition between polyembryos and the occurrence of "cryptic twins".

Weakness and lethality of numerous plantlets obtained from polyembryonic seeds reduce strongly the probability of obtaining haploid plants. So far, only diploid seedlings were obtained, but apparent instability of the haploid condition in grape let us think that diploid homozygote seedlings originating from parthenogenetic development of cells of the embryo sac, could be produced by this way.

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