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Investigations on anomalies of ovule development and on pollination in mutated grapevines, cv. Barbera[1])

by

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Etudes sur les anomalies du développement de l'ovule et la pollinisation dans deux vignes mutées cv. Barbera

Résumé: Le développement de l'ovule et la pollinisation d'une plante tétraploïde et d'une chimère péricline 2n-4n ont été étudiés afin de rechercher les causes de la fertilité réduite remarquée dans ces plantes.

Des anomalies dans la formation du sac embryonnaire et l'absence de fécondation, surtout dues à des difficultés pendant la pollinisation, ont été observées et rapportées à la fertilité.

Key words: flower biology, fecundation, histology.

Introduction

Investigations on the embryo sac development and fertilization in grapevine were carried out by several authors.

The studies of KASSEMEYER and STAUDT (1981) are among the last and more detailed ones. Besides, the same authors (1982) and STAUDT and KASSRAWI (1973) describe some causes of shedding and berry dropping for diploid and tetraploid grapevines.

Anomalies of ovule development are reported by BARRITT (1970) in seedless grapes, by PRATT and EINSET (1961) in small-clustered Concord grapes, and by CARRARO *et al.* (1979) in Picolit giallo with low productivity. The latter authors (LOMBARDO *et al.* 1983) describe in the same cultivar some problems of pollination as related to calyptra fall.

In this paper, we investigate the causes of low fertility of two mutated plants of *Vitis vinifera* cv. Barbera (a tetraploid and a periclinal chimera 2n-4n) already described in previous works (SACERDOTE *et al.* 1981; VALLANIA *et al.* 1982; ME *et al.* 1984).

These plants show flowers shedding after bloom and reduced number of seeds/berry. Since anomalies in microsporogenesis do not seem to be sufficient to explain these phenomena, we studied ovule development and pollination problems related to morphological and caryological aspects.

Materials and methods

The grapevines studied were a tetraploid and a 2n-4n periclinal chimera plant, cv. Barbera, besides a diploid plant of the same cultivar used as control. They were grafted on 420 A and grown in a vineyard on the outskirts of Torino (north Italy).

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Samples of flowers and berries were collected in 1984 and 1985, every two days, from the middle of May to the end of June. This period included about 20 d before and 20 d after flowering. Moreover, shedding flowers were picked up in the week following full bloom.

Samples were fixed in Navashin solution and embedded in paraffin; sections 10—15 μm thick, were stained with Feulgen and light green.

Data about seed number, counted on 100 ripe berries each year, were elaborated with Duncan's significance test, while figures regarding percentage of germinable pollen were drawn from data used for a previous work (ME *et al.* 1984).

In order to observe pollination, sections were stained with aniline blue 0.1 % in K_3PO_4 0.1 N aqueous solution (MARTIN 1959) and examined in UV light to verify pollen tubes development.

Average number of seeds/berry at ripeness

Nombre moyen de pépins/baie à la maturité

	1984	1985
Chimera	1.14 A	1.15 A
Tetraploid	1.12 A	1.23 A
Control	2.04 B	2.00 B

Duncan's significance test: Mean values within rows followed by the same letter are not significantly different for P = 0.01.

Results

The average numbers of seeds/berry at ripeness were significantly different for control and mutated plants (Table). The observations on ovules pointed out some anomalies in their growth which could be a cause of this difference.

In normal ovule development, functional megaspore was evident 7—15 d before anthesis (Fig. 1). In the following period (7—10 d), embryo sac formation occurred: 8 polar nuclei were originated by the megaspore after 3 mitosises; 3 antipodal nuclei degenerated before bloom, 3 micropylar nuclei constituted the 2 synergids and the egg cell, while the 2 remaining nuclei fused into the endosperm nucleus.

Fig. 1: Functional megaspore 7 d before full bloom. Control. × 450.

Fig. 2: Normal developed embryo sac at calyptra fall. Control. × 450.

Fig. 3: Nucellus without embryo sac 2 d after bloom. Chimera. × 120.

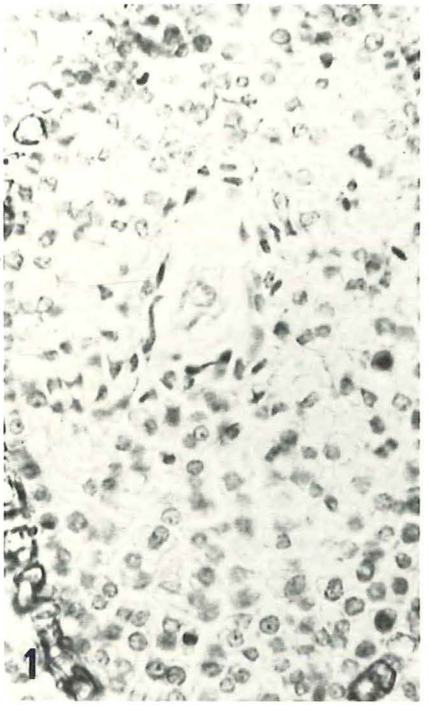
Fig. 4: Non-fused polar nuclei near the egg apparatus at bloom. Chimera. × 400.

Fig. 1: Mégaspore fonctionnelle 7 d avant la pleine floraison. Témoin. × 450.

Fig. 2: Sac embryonnaire normal à la floraison. Témoin. × 450.

Fig. 3: Nucelle sans sac embryonnaire 2 d après la floraison. Chimère. × 120.

Fig. 4: Noyaux polaires non-fusionnés près de l'oosphère et les synergides à l'anthèse. Chimère. × 400.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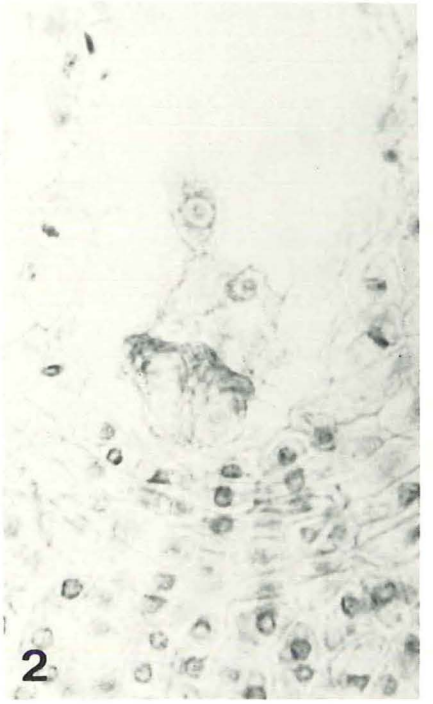
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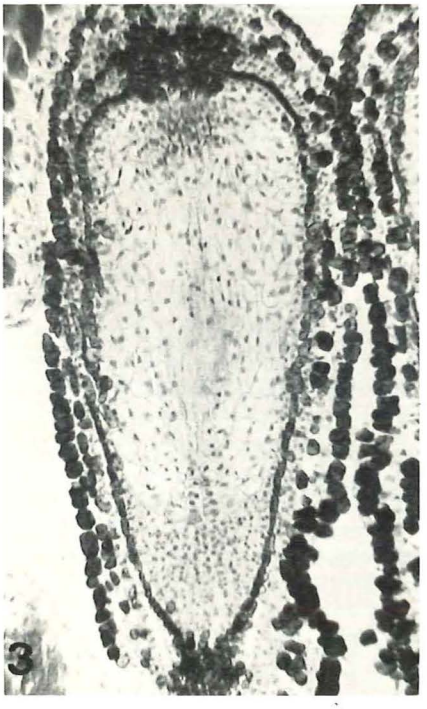
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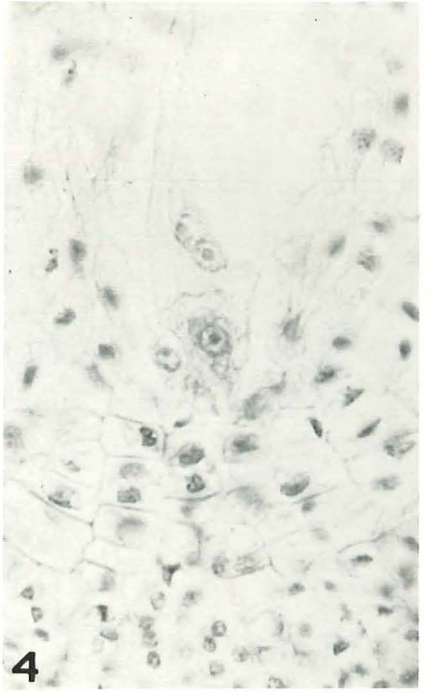
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This fusion occurred in the center of the embryo sac, but the endosperm nucleus migrated to the synergids a little before bloom.

At calyptra fall, the egg apparatus was constituted by the 2 synergids and the egg cell inserted on the micropylar cells of the nucellus, with the endosperm nucleus close to them (Fig. 2).

After double fertilization, the endosperm nucleus migrated towards the center of the embryo sac and the synergids began to degenerate.

In the following days, the endosperm divided several times while no mitosis of the zygote was noticed in the considered period.

Besides normal ovule development, some anomalies were observed:

7—15 d before anthesis, nucelli without functional megaspore were found both in control and mutated plants (Fig. 3). Nevertheless, degeneration of all megaspores was not observed except in some nucelli of the chimera plant. At bloom, these ovules were similar as for size and shape to normal ones, but they did not have an embryo sac which was replaced by nucellar tissue. About 1 week after anthesis the central core of the nucellus began to degenerate; the nuclei contracted, the cytoplasm coagulated and the cells collapsed.

In mutated plants, difficulties in fusion of polar nuclei to form the secondary embryo sac nucleus were also observed.

This anomaly was evident at bloom (Fig. 4) and was often associated to non-migration of the endosperm nucleus to the synergids before fertilization (Fig. 5).

However, well fused non-migrated nuclei were found in non-pollinated flowers even 2 d after bloom.

The non-migrated endosperm nucleus began to degenerate 6 d after anthesis, while, in the normal non-pollinated embryo sac, degeneration of egg cell and endosperm started usually after 2 d.

In addition, two big nucleoli were frequently observed in fertilized endosperm nuclei after the first two mitosises.

Problems in nuclei distribution for embryo sac formation occurred in the chimera plant as testified by Fig. 6, where 5 nuclei (1 not visible) were still crowded together in the center of the nucellus 5 d after bloom, while the synergids and the egg cell had already degenerated.

Pollinated ovules were found in degeneration 3—13 d after full bloom, both in control and mutated plants. In these ovules the degeneration usually began from the zygote, while the endosperm nucleus normally migrated after fertilization and sometimes one mitosis or more occurred (Fig. 7).

In two years of observations, mutated plants, particularly the chimera, showed difficulties in calyptra fall of flowers at bloom; the stamens compared with pistil were shorter than in control (Fig. 8) and the percentage of germinable pollen was lower: tetraploid 34.7 % B; chimera 51.9 % B; control 80.7 % A (values followed by the same letter are not significantly different for $P = 0.01$).

Observations on dropped flowers of these plants demonstrated that 75 % of their ovules were not pollinated.

Non-fertilized ovules showed degeneration of the egg apparatus and the secondary embryo sac nucleus, which was still close to the synergids, 2 d after bloom (Fig. 9).

Generally, all the degenerations described developed with a gradual detachment of the nucellus from the inner integument and/or the inner integument from the outer one.

Contracted nucelli were found 8 d after anthesis (Fig. 10).

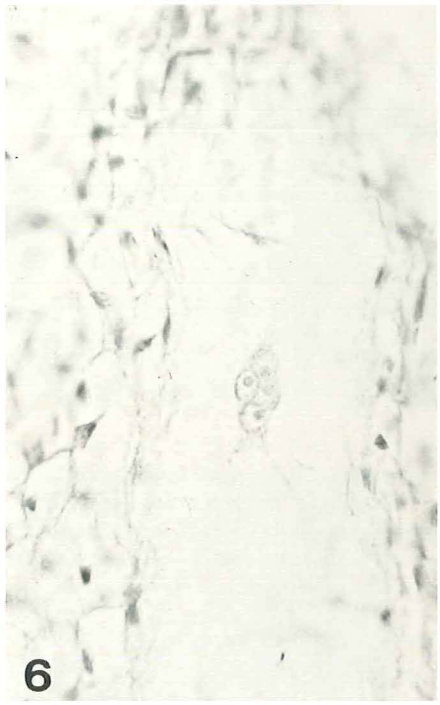


Fig. 5: Poorly fused and non-migrated polar nuclei 2 d after bloom. Tetraploid. × 300.

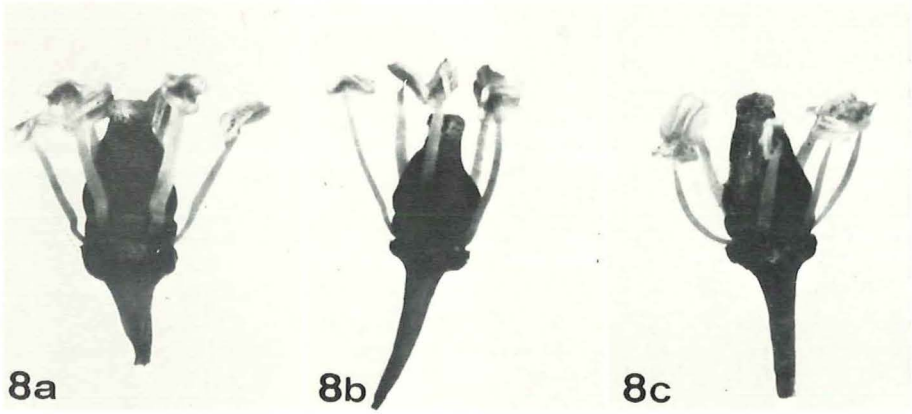
Fig. 6: Anomalous embryo sac formation: nuclei crowded together 5 d after full bloom (shed flower). Chimera. × 400.

Fig. 7: Degenerating nucellus 13 d after full bloom. Two endosperm nuclei are visible. Chimera. × 200.

Fig. 5: Noyaux polaires mal fusionnés et non-migrés 2 d après l'anthèse. Tétraploïde. × 300.

Fig. 6: Formation anormale du sac embryonnaire: noyaux groupés 5 d après l'anthèse (fleur coulée). Chimère. × 400.

Fig. 7: Dégénération du nucelle 13 d après la floraison. On peut voir deux noyaux de l'endosperme. Chimère. × 200.



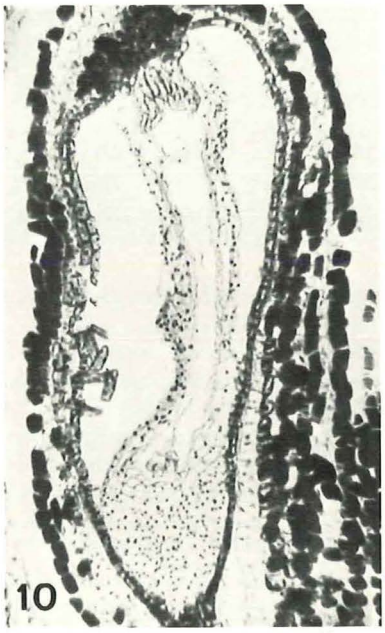


Fig. 8: In mutated plants stamens compared with pistil are shorter than in control: a) Flower of the tetraploid, b) flower of the control, c) flower of the chimera. × 7.

Fig. 9: Endosperm nucleus degeneration in non-fertilized ovule 2 d after bloom. Tetraploid. × 350.

Fig. 10: Contracted nucellus detached from integument 8 d after full bloom. Tetraploid. × 120.

Fig. 8: Dans les plantes mutées les étamines comparées au pistil, sont plus courtes que dans le témoin: a) Fleur du tétraploïde, b) fleur du témoin, c) fleur de la chimère. × 7.

Fig. 9: Dégénération du noyau de l'endosperme dans un ovule non fécondé 2 d après l'anthèse. Tétraploïde. × 350.

Fig. 10: Nucelle contracté détaché du tégument 8 d après l'anthèse. Tétraploïde. × 120.

Discussion

The abscence of the embryo sac in ovules leads to two anomalies; the first already described by KASSEMEYER and STAUDT (1982) is probably due to an irregular differentiation or degeneration of the megaspore mother cell; the second, noticed only in the chimera plants and consisting in degeneration of all megaspores, was presumably caused by meiotic irregularity. This hypothesis would be confirmed by the anomalies observed in meiosis of pollen mother cells of these plants (ME *et al.* 1984), but it is not clear why no functional megaspore degeneration was found in the tetraploid.

The difficulties in fusion of polar nuclei and the non-migration of secondary embryo sac nucleus just before anthesis were noticed only in the mutated plants and appear to be related to tetraploidy.

Irregular embryo sac formation shows also with the disorganization in nuclei distribution, so that the 3 antipodal nuclei and the 2 polar nuclei crowd together instead of degenerating and forming the endosperm, respectively.

As degeneration starts from the egg apparatus in both pollinated and non-pollinated ovules, it appears that regular formation and fertilization of the secondary embryo sac nucleus and endosperm development are required for zygote survival.

On the other hand, apparently fertilized ovules can abort even after a few divisions of endosperm. In these cases, degeneration also starts from the zygote which could mean either an abnormal fertilization of the egg cell or other causes, which may be physiological or nutritional, to be further investigated.

Another reason for ovule degeneration is non-pollination, which resulted from the concurrence of several factors, firstly the high incidence of flowers without calyptra fall at bloom, which reduces the possibility of cross-pollination.

Cleistogamy, on the other hand, is obstructed by reduced length of stamens which are shorter than in the control and do not protrude over the stigma; reduced percentage of vital and germinable pollen also contributes to make pollination difficult. It was not investigated to see if spermatic nuclei formed and if normal double fecondation occurred in pollinated ovules.

In conclusion, shedding of the flowers seems to be mainly related to non-pollination, even if ovule anomalies have been noticed, and physiological or nutritional causes cannot be excluded.

The reduced number of seeds in the berries of mutated plants at ripeness can also be explained by these factors.

Summary

In order to point out the reasons for reduced fertility in tetraploid and 2n-4n periclinal chimera grapevines, ovule development and pollination shown by these plants were investigated.

Anomalies in embryo sac formation and non-fertilization, mainly due to difficulties in pollination, were observed and related to fertility.

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