Cytologia **44**: 391–396, 1979

Nuclear Behaviour in the Sterile Pollen of *Vanilla planifolia* (Andrews)[1]

P. N. Ravindran[2]

Central Plantation Grops Research Institute, Regional Station,
Vittal 574 243, Karnataka State, India

Received September 17, 1977

Vanilla planifolia (Andrews) (Syn. *V. fragrans* (Salisb.) Ames.) is the source of commercial vanillin, one of the important spices in use. Inspite of its economic importance very little is known about this plant. During the course of a study of pollen biology and cytology some interesting observations were made on the nuclear behaviour in the ungerminated-sterile-pollen grains of *Vanilla*. The present paper deals with this nuclear division and chromosome behaviour.

Materials and methods

Pollen was collected at 8.00 AM and stored in a desicator till used. The pollinia from the flowers were cut into small pieces and kept in a nutritive medium consisting of 10% sucrose, 100 ppm boric acid and 100 ppm calcium nitrate. Simultaneously pollen grains were placed on bits of non-waterproof cellophane bits floated on the nutritive medium. These were kept incubated for a period of 5–24 hours. These bits and pollinia were then taken out, and stained with 1% lactopropionic orcein.

Results and discussion

In *Vanilla* the pollen grains started germination in 30–40 minutes after incubation. The germination count was taken after five hours and was found to be 45%. Those pollen grains that did not germinate even after 5 hours were considered sterile. These sterile pollen grains were found to exhibit some unusual nuclear behaviour. Some of the pollen grains were shrivelled and did not take up any stain, and they were evidently sterile. Others took up stain and in them one, or two nuclei were visible. While some others were undergoing a nuclear division (Table 1). The chromosomes were well stained and the number most usually encountered was 14. In a few cases 16 chromosomes were also counted which is the gametic number of this species (Darlington and Wylie 1955). The behaviour of these chromosomes were normal in some grains, while in others various abnormalities were noted (Table 2). The commonest among them was fusion

[1] Contribution No. 147 from the Central Plantation Crops Research Institute, Regional Station, Vittal 574 243, India.

[2] Present address: CPCRI Regional Station, Calicut 673 011, Kerala State, India.

of chromosomes. Hence it was difficult in many cases to have an exact count of the chromosome number. Fusions similar to multiple associations were found in some pollen grains. Clumping of chromosomes were also prevalent. In some pollen grains the chromatin material appeared as pinched-off bits. An apparent reduction in chromatin content was noted in a few cases. Some of these abnormalities are shown in Plates 1 and 2. Abnormalities varied in different flower buds, ranging from 17.0% to 38.2% of the dividing pollens, as estimated from meta-, ana- and telophases.

Table 1. Ferquency of normal germinating, shrivelled and dividing pollen grains in *Vanilla planifolia*

Flower no.	No. of pollen grains observed	Normal germinating	Shrivelled	No. of pollen grains with			
				1 nucleus	2 nuclei	in division	% of dividing pollen
1	900	402	210	92	114	82	8.0
2	710	341	141	78	82	68	9.5
3	1190	495	194	132	181	218	18.5
4	775	365	183	68	92	67	8.6
5	470	232	84	48	56	53	11.3
6	1330	582	140	163	243	212	15.9

Table 2. Frequency of different division stages and abnormalities

Flower no.	Dividing cells	Prophase	Metaphase	Anaphase	Telophase
1	82	23	26	19	17
2	68	16	22	18	12
3	218	61	76	42	39
4	67	14	23	21	9
5	53	21	14	11	7
6	212	58	69	47	38

Flower no.	Abnormal metaphases	Abnormal anaphases	Abnormal telophases	% abnormalities
1	16	9	8	20.1
2	13	8	5	38.2
3	34	18	17	31.6
4	7	6	3	23.8
5	3	4	2	17.0
6	31	19	16	32.6

The pollen grains collected 24, 48, and 72 hours prior to flower opening were also studied. A good percentage of pollen grains at these stages carried either one nucleus or two nuclei. In the binucleate pollen grains one of the nuclei was deeply stained, and the other diffuse and lightly stained. The deeply stained one was the generative nucleus, and the other the vegetative nucleus. In some of the pollen grains various stages of a division were visible. This division showed abnormalities similar to those described above. These abnormalities were mainly

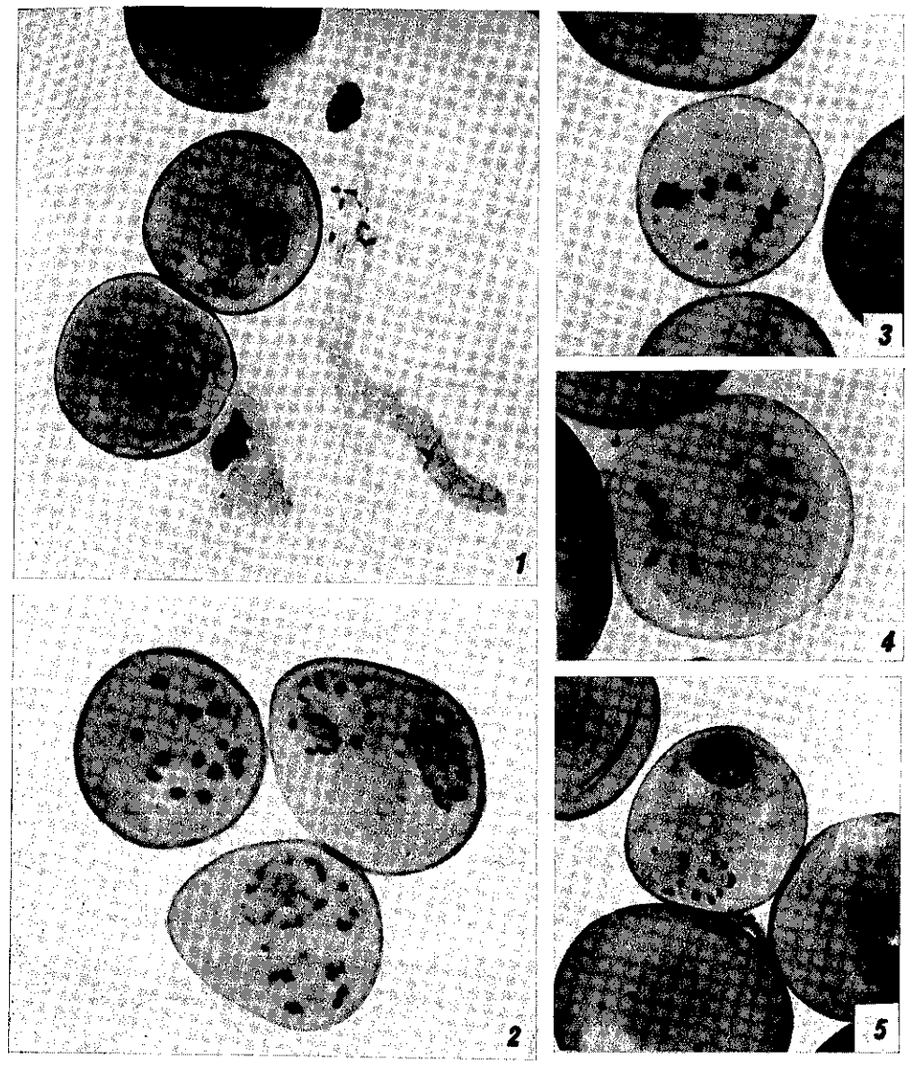
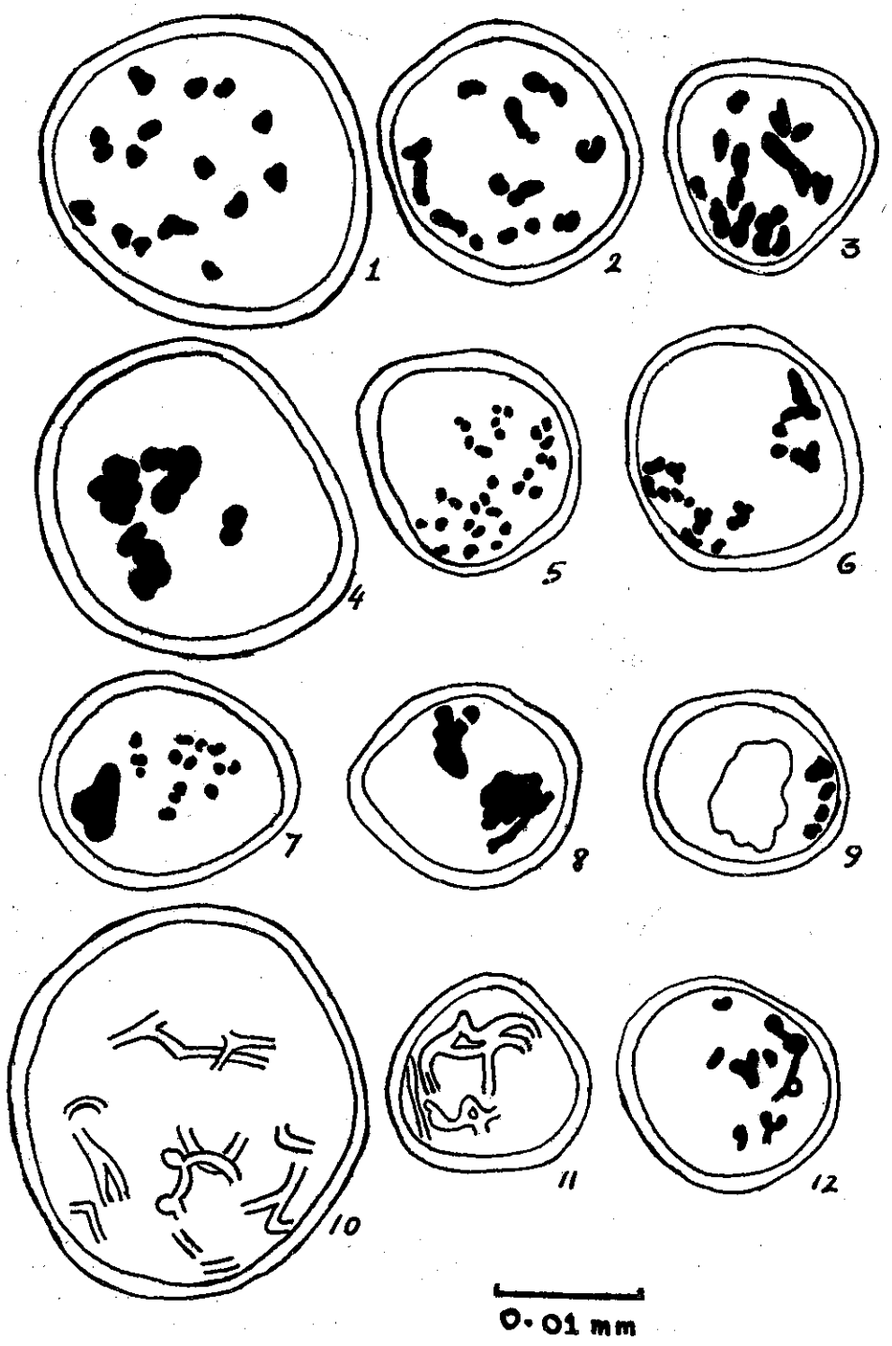


Plate 1. Figs. 1–5. (x1500) Photomicrographs of the nuclear behaviour in sterile pollen of *Vanilla planifolia*. 1, three pollen grains, two germinated and the middle one sterile. In the top pollen both the nuclei entered the tube, in the bottom one, one nucleus has entered the tube while the other still inside the pollen. Note the nuclear material in the sterile pollen. 2, three sterile pollens showing different stages of nuclear division. In one, the division has already advanced to metaphase and 14 chromosomes are visible. In others note the two groups of nuclear material. 3, a sterile pollen in metaphase. The chromosome number is less than normal gametic. Note the stickiness of the chromosomes. 4, a pollen in division showing two groups of chromosomes. From size difference these could be inferred as anaphase chromosomes. The number of chromosomes in the two groups differ. 5, a pollen showing one nucleus and a group of 13 chromosomes. The smaller size of the chromosomes indicates that these are anaphase chromosomes, but has not yet passed on to nuclear organization.



due to clumping and fusion of chromosomes. The maximum count was 16, while in many others only 14 chromosomes could be distinguished because of fusion of a few chromosomes.

Observations on the germinating pollen revealed that these contained two nuclei, a deeply stained generative nucleus and a diffuse slightly stained vegetative nucleus. The latter was found to be in different stages of degeneration. In all most all angiosperm species, the generative nucleus divides soon after pollen germinates to give rise to sperm nuclei (Steffen 1963). But in the present case no chromosome division was observed in the pollen tubes even after incubation for 96 hours. It may be because the division takes place only at a later stage, probably only after the pollen tube entered the ovule, as in the case of some plants (D' Amato 1947).

The nuclear division in the sterile pollen may either be the delayed first division which should have taken place soon after the organization of the pollen grains, or it may be a supernumerary division of the microspore nucleus. Such supernumenrary divisions were reported in other plants also (Koul 1970, Bhavanandran 1971, Abdel Hameed 1973). But absence of multinucleate pollen grains tend to show that these were not supernumerary divisions. A third possibility was that the division represented a premature division of the generative nucleus. Such premature divisions of generative nucleus were reported in *Ornithogalum nutans* and *Paris quadrifolia* (Geitler 1942). What initiated such premature divisions were not clearly understood, though higher relative humidity, and the use of aqueous medium were implicated as causes (Geitler 1942, Poddubnaya-Arnoldi 1936). Evidently a breakdown of the normal functioning might have taken place leading to such asynchrony.

Such abnormalities in the pollen grain mitosis combined with the comparatively high pollen sterility (fertility as assessed by pollen germination was only 45%) and the occasional chromosome associations met with in the dividing pollen grains led to conclude that *Vanilla planifolia* originated as a hybrid. The presence of higher associations in meiotic divisions and also multivalent like organisation

Plate 2. Figs. 1–12. Camera lucida drawing of the chromosome behaviour in sterile pollen grains of *Vanilla planifolia*. 1–3. Metaphase configurations in three pollen grains. 1, with 16 chromosomes. 2 and 3, the number could not be determined definitely due to fusion of some of the chromosomes. 4, deeply stained clumps of chromosomes were frequently encountered in these dividing nuclei of sterile pollen. This one is probably a metephase but chromosomes appeared in stained masses. 5–9. Different anaphase configurations noted in the sterile pollens. 5, two anaphase groups could be seen, each with 16 chromosomes. 6, anaphase groups with unequal number of chromosomes and chromosome clumping. 7, one deeply stained nucleus and chromosome clumping. 7, one stained nucleus and a group of 16 chromosomes which may have failed to organise into nucleus. 8, two nuclei in telophase. 9, one nucleus and four masses of chromatin which has failed to organize into nucleus. 10–12. Three configurations met within pollen collected 48 hrs before anthesis. 10, note typical diplotene like chromosomes some of which have undergone fusion similar to interchanges, thus giving rise to forked and branched chromosomes. 11, extreme interchange-like fusion of chromosomes leading to complex chromosome configurations. Evidently some type of pairing had taken place in both these cells. Such cells were not very common, but was not infrequent. 12, possibly a metaphase showing chromosome fusion and reduced chromosome number.

during pollen grain mitosis support this view (unpublished data). The low fertility of the hybrid was never a handicap because of the vegetative means of propagation. The asynchrony in the pollen grain mitosis might have resulted from this hybridity.

Abstract

Vanilla pollen was cultured on a nutritive medium and germination count after five hours was found to be 45%. In about 12% of the pollen grains a nuclear division was present. This division was similar to the first pollen mitosis. Various abnormalities in chromosome behaviour was recorded. The percentage abnormalities varied from 17.0 to 38.2 in different flowers. Similar nuclear divisions and chromosome abnormalities were also found in flower buds collected at 24, 48, and 72 hours prior to anthesis. This unusual behaviour might either be the result of delayed first pollen mitosis which should have taken place soon after the organisation of pollen grains or they represented a premature division of the generative nucleus.

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