

Pollen Morphology of some Cultivated Varieties of Strawberry

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دراسة مورفولوجية حبوب لقاح نبات الفراولة كوسيلة للتعرف على الأصناف المختلفة

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يتناول البحث دراسة حبوب لقاح اثني عشر تركيب وراثي (صنف) من نبات الفراولة المزروعة في مصر وذلك باستخدام المجهر الضوئي العادي علاوة على المجهر الإلكتروني الماسح حيث تهدف الدراسة إلى التعرف على أوجه الشبه والاختلاف في الأشكال والزخرفة والتوزيع الحجمي لحبوب لقاح الفراولة. تم اختيار إحدى عشر صنفاً تنتمي إلى النوع *Fragaria x ananassa* وهي "كابيتولا، سيلفا، دو جلاس، سيسكيب، دوريت، شاندر، باركر، فرن، سيجويا، أوزوجراندي وباخارو" كما أضيف صنف ينتمي إلى النوع *Fragaria vesca*.

أوضحت نتائج الدراسة أن هناك اختلافاً واضحاً بين الأصناف في حجم وشكل حبوب اللقاح علاوة على الحزوز أو التجايف على طبقة الجدار الخارجي لحبة اللقاح وهذه خصائص مفيدة كوسيلة للتعرف على الصنف وتمييزه ومن الجدير بالذكر أن الأصناف قيد الاختبار أمكن تقسيمها إلى سبع مجاميع يمكن تمييز إحداها عن الباقي. هذا وقد أظهرت القياسات الكمية بنظام تحليل الصور لأقطار حبوب اللقاح عن وجود اختلافاً كبيراً بين الأصناف منسوباً لأقطار حبوب لقاحها مما يمكن من الاستدلال والتعرف على كل صنف على حدة.

ويمكن القول عموماً بأن حبوب لقاح نبات الفراولة كروية إلى هليلجية إلى مثلثية الشكل وتشارك بأن لها فتحة إنبات ذات ثلاث أحاديث مثقوبة *tricolporate* وطويلة كما أن زركشة الجدار الخارجي تأخذ شكل الحزوز الطويلة المتوازية وفي بعض الأصناف متفرعة.

وأوضح تقدير حيوية حبوب اللقاح أن التراكيب الوراثية المختلفة قيد الدراسة أظهرت تبايناً واضحاً حيث أظهر الصنف "كابيتولا" حيوية حبوب اللقاح وصلت إلى ٢٣، ٩٤٪ بينما انخفضت في الصنف "أوزوجلاندي" إلى ٢٤، ٤٠٪.

وتوصي الدراسة الحالية باستخدام هذا الفحص بصورة روتينية للتعرف على التراكيب الوراثية لنباتات الفراولة خاصة عندما يكون الهدف هو جودة المنتج وهي تساعد في التعرف على الهجن في برامج زراعة الفاكهة.

Key Words : Morphology, Varieties of Strawberry

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Abstract

Pollen grains of twelve cultivated varieties of strawberries grown in Egypt were morphologically studied using both light microscopy and SEM observations. These varieties include eleven cultivars of *Fragaria x ananassa* (c.v: Capitola, Douglas, Chandler, Selva, Fern, Seascape, Dorite, Pajaro, Parker, Sequioa, Oso Grande) and one cultivar of *Fragaria vesca*. Pollen grains of *Fragaria* are spheroidal, subprolate ellipsoidal to triangular in outline. Tricolporate with long colpae tapering to pointed ends. Exine sculpture is always striate.

These studied cultivars are discrepant mainly in pollen size, shape and striation pattern, which may be useful for identification of the cultivars and can be merged into seven morphological pollen groups. The differences in pollen morphology among *Fragaria* species and cultivars apparently corresponds with ploidy levels of the species. The quantitative measurements of pollen grains diameter using image analysis showed that there is a great variation in and between different strawberry cultivars studied. Generally, pollen grains of *Fragaria* are spheroidal, ellipsoidal to triangular in outline when dry, but readily take up moisture and expand, becoming oblately flattened and angular in outline.

INTRODUCTION

Recent Scanning Electron Microscope (SEM) studies on pollen morphology and ultrastructure of the exine have shown a tremendous results to distinguish between tree fruit species. Of these, are the work of Fogle [1, 2]; Mass [3]; Westwood and Challice [4]; Martens and Fretz [5]; Simpson & Skvarla [6]; Constance & Chuang [7]; and Marcucci et al. [8].

Until recently the genus *Fragaria* (Strawberry) had not been studied enough to describe its species adequately. Previous studies on pollen morphology of *Fragaria* illustrated that pollen grain diameter in Strawberry is closely related to ploidy with greater variation in size of pollen among pentaploid clones than among diploid and tetraploid clones.

Several light microscopy studies on pollen of *Fragaria* species have been published [9, 10], but few attempts has been made to study the pollen morphology by means of Light microscopy and Scanning Electron Microscope (SEM) of the *Fragaria* species. Maas [3] examined pollen morphology of Strawberry and other small-fruit crops by SEM. Wodehouse [11] and Thakur & Thakur [12] suggested that pollen morphology, including size, shape and exine pattern, may be useful for identification of species groups.

The purpose of the present study is to examine the usefulness of pollen morphology for identification of cultivars of Strawberry *Fragaria x ananassa* and *Fragaria vesca*.

Material and Methods

In the present study, pollen grains of twelve cultivated varieties covered the most important cultivars of strawberries grown in Egypt, were morphologically studied using both light microscopy and SEM.

Anthers from open flowers were collected and stored in a solution of ethyl alcohol 70% at room temperature. Anthers were dehydrated in serial solutions of ethyl alcohol started from 80, 90 and 100% for fifteen minutes each. The anthers and loose pollen for each cultivars were then dried for an additional six hour.

Double-sided adhesive tape were applied to the top of the SEM stubs. A coverslip was placed on each stub to give natural and smooth background. Some of the dried anthers of each cultivar were gently pressed and placed on the coverslip.

The specimens were then coated with gold using JEOL JFC-1100E Sputter coater alloy about 40 - 60 nm thickness and examined and photographed with a JEOL JSM - 5300 Scanning Electron Microscope at 25 Kv.

Pollen grains were mounted in gelatin and placed on a glass slide. For each cultivars, the grain size of at least 50 random pollen grains were measured.

Results and discussion

In the present investigation eleven cultivars of *Fragaria x ananassa* and one cultivar of *Fragaria vesca* have been morphologically studied. The total number, percentage of viable (stainable) and aborted pollen grains in random

samples of some cultivated strawberry varieties (*Fragaria x ananassa*) are given in table (1), while the quantitative measurements of pollen grains diameter using image analysis (table 2) showed that there is a great variation in and between different strawberry cultivars studied.

The percentage of the different pollen grain diameter in most of the cultivars studied is shown in Figure 1. Description of the studied species and cultivars of *Fragaria* is presented in the next paragraphs.

Variety (Cultivar)	Total No. of pollen examined	Viable pollen		Aborted pollen	
		No.	%	No.	%
Capitola	4340	4090	94.23	250	5.77
Douglas	4725	4440	93.96	285	6.04
Chandler	7270	6770	93.12	500	6.88
Selva	7710	6880	89.23	830	10.77
Fern	6050	4940	81.65	1110	18.35
Seascape	8330	6720	78.78	1810	21.22
Dorite	7060	5350	75.78	1710	24.22
Pajaro	8900	6600	74.16	2300	25.84
Parker	4875	4650	69.66	1474	30.34
Sequoia	6200	2800	45.16	3400	54.84
Oso Grande	5965	2400	40.24	3565	59.76

Table (1): Total number and percentage of viable (stainable) and aborted pollen grains in random samples of some cultivated strawberry varieties (*Fragaria x ananassa*).

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Strawberry <i>Fragaria x ananassa</i> cultivars	Measurements (μ)		Measurements (μ^3)	
	Range Diameter	Average Diameter	Range	Average
Capitola	18-27	22.5	3055-10310	6682.5
Selva	15-22	18.5	1768-5578	3673
Douglas	18-25	21.5	3055-8185	5620
Seascape	15-25	20	1768-8185	4976.5
Dorite	15-22	18.5	1768-5578	3673
Chandler	16-23	19.5	2146-6373	4259.5
Parker	15-24	19.5	1768-7241	4504.5
Fern	17-25	21	2573-8185	5379
Sequoia	15-23	19	1768-6373	4070.5
Pajaro	17-22	19.5	2573-5578	4075.5

Table 2 : Quantitative measurements using image analysis of pollen of some strawberry *Fragaria x ananassa* cultivars .

The percentage of the different pollen grain diameter in most of the cultivars studied is shown in Figure 1.

Description of the studied species and cultivars of *Fragaria* is presented in the next paragraphs.

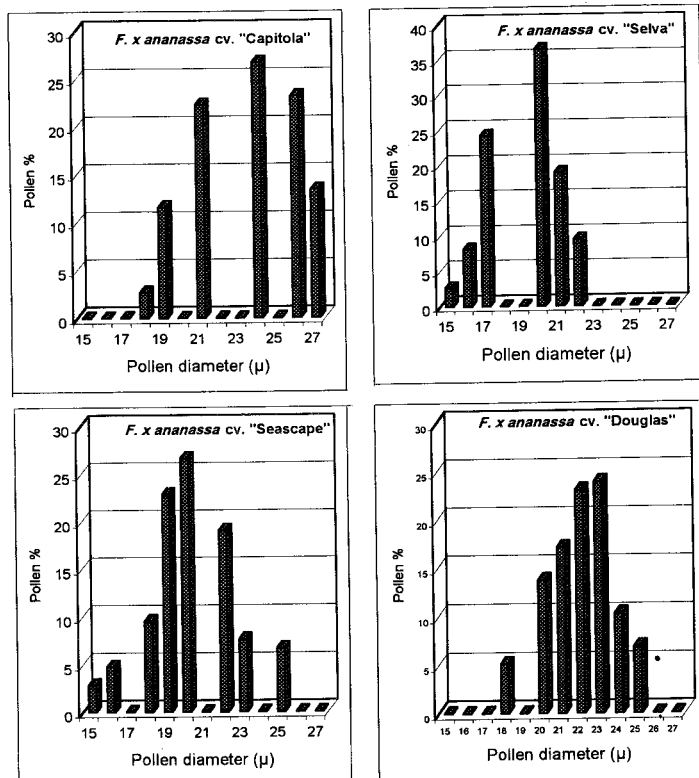
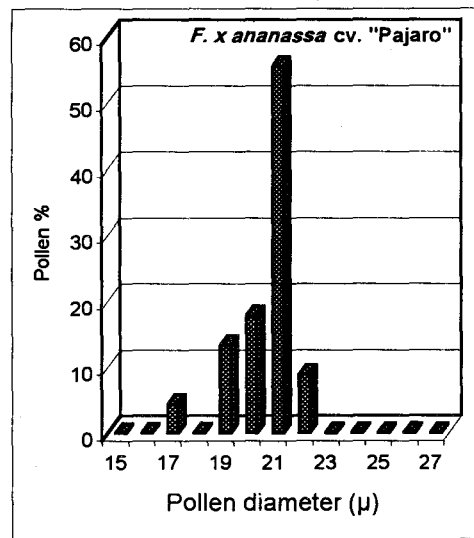
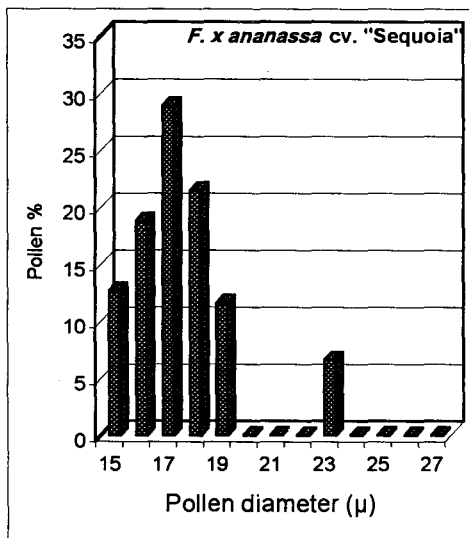
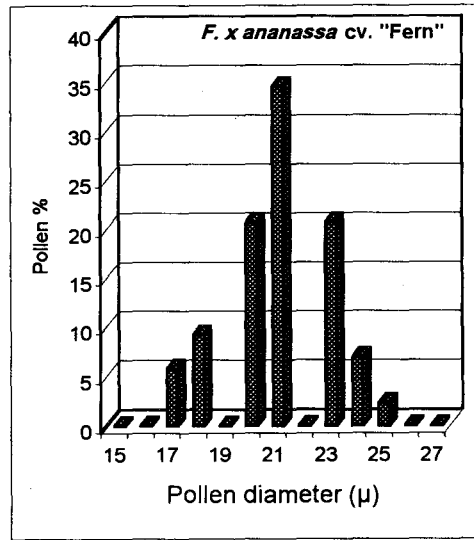
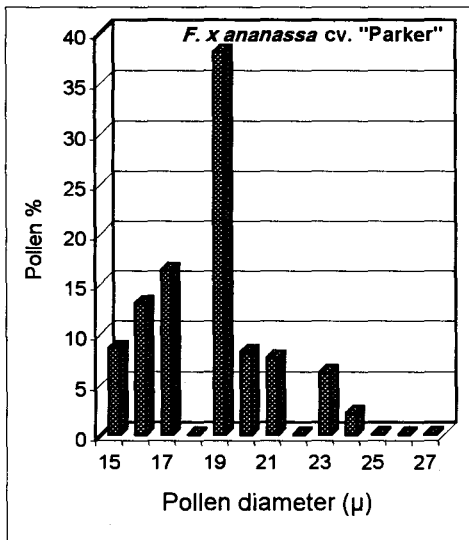
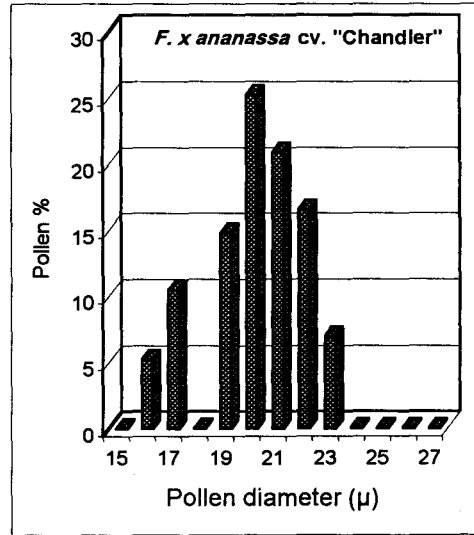
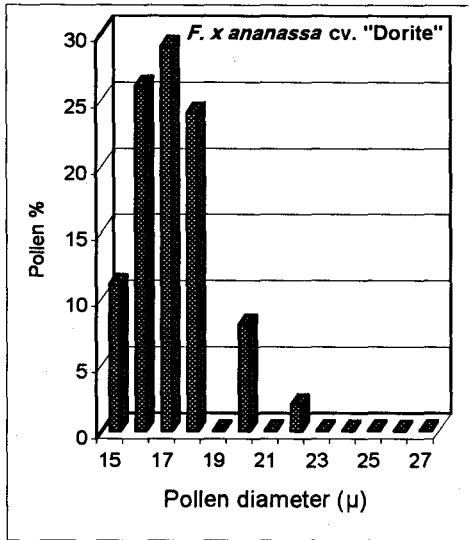


Figure (1) : Percentage of the pollen grain diameters of most of the studied cultivars.

Figure (1) : Continued



Genus : *Fragaria* Duchesne, 1766.

Species : *Fragaria x ananassa* Duch (octaploid, $2n = 8x = 56$).

Description : Pollen grains of *Fragaria x ananassa* are generally spherical, ellipsoidal to triangular in outline when dry, but readily take up moisture and expand, becoming oblately flattened and angular in outline. Tricolporate with long colpae tapering to pointed ends. Pollen exine sculpture is always striate.

The present SEM study showed that pollen grains of *Fragaria x ananassa* and *Fragaria vesca* cultivars are discrepant mainly in pollen grain diameter, shape and striation pattern, which may be useful for identification of the eleven cultivars and can be categorized into seven morphological pollen groups at which the pollen groups 1 to 5 are spherical to sub-spherical in shape, while groups 6 and 7 are typical triangular and elliptical in outlines respectively.

Pollen Group 1 (plate 1, figs. 1-8; plate 2, figs. 1-4):

Includes pollen grain of the cultivars "Capitola", "Selva" and "Pajaro".

Diagnosis: Spherical pollen grain, tricolporate, colpae widening in the equatorial region (mean 5 μm wide), and pointed toward the pole. Exine striate, running nearly parallel to the colpae, always disconnected and sometimes bifurcating, about 0.3 - 0.5 μm thick and 0.1- 0.3 μm apart.

Grain diameter: CV. "Capitola" : 18-27 μm

CV. "Selva": 15-22 μm

CV. "Pajaro": 17-22 μm

Pollen Group 2 (plate 2, figs. 5-7; plate 3, figs. 1-7):

Includes pollen grains of the cultivars "Douglas", "Seascape" and "Praker."

Diagnosis: This pollen group is very similar to pollen group 1, but differs in possessing more thick striations (0.5 - 0.8 μm) and being discontinuous.

Grain diameter: CV. "Douglas": 18-25 μm

CV. "Seascape": 15-25 μm

CV. "Parker": 15-24 μm

Remarks: The thicker striations characterizing this group are clearly observed under light microscope (plate 3, figs. 4 and 5). Wet pollen samples were used in this study for light microscope illuminating inconsistencies resulting from equatorial swelling (i.e., plate 3, fig. 4). Such swelling is also present in samples prepared for SEM examination that have been prepared from wet pollen.

Pollen Group 3 (plate 4, figs. 1-4):

Represented by pollen of cultivar "Chandler".

Diagnosis: Pollen of this group is characterized by exine surface covered with ridges rather than striations. Ridges are irregular, varied in thickness. Anastomosing ridges were present between adjacent ridges. Narrow longitudinal holes or furrows found between ridges.

Grain diameter: 16-23 μm

Pollen Group 4 (plate 4, figs. 5 and 6):

Represented by pollen of the cultivar "Oso Grande".

Diagnosis: Spherical pollen grains, differs from the preceding groups by possessing less prominent to indistinct striations which running parallel to the colpae. Smooth exine is found between the faint striations.

Grain diameter: 15-18 μm

Pollen Group 5 (plate 4, figs. 7-9):

Exemplified by pollen of the cultivar "Dorite".

Diagnosis: Subspheroidal (subprolate to prolate spheroidal) pollen grains, tricolporate. Exine sculpture consists of disconnected ridges or striations.

Grain diameter: 15 -22 μm .

Pollen Group 6 (plate 5, figs. 1-6):

Represented by pollen of the cultivars "Fern" and "Sequoia".

Diagnosis: The present group is characterized by its distinct morphological shape. Pollen grains are tricolporate, oblate spheroidal, triangular outline in polar view. Colpae are broad in the equatorial region (about 7 μm). Surface

sculpture is distinguished by prominent and distinct striations parallel to the colpae and sometimes disconnected. Striations are clearly visible under light microscope (plate 5, figs. 2 and 5).

Grain diameter: CV. "Fern": 17-25 μm
CV. "Sequoia": 15-23 μm

Pollen Group 7 (plate 5, figs. 7 and 8) :

Represented by one species, i.e., *Fragaria vesca* (Linnaeus, 1753). A diploid species, $2n = 2x 14$.

Diagnosis: Subprolate pollen grains, tricolporate, colpae are long, wide in the middle, pointed ends at the poles. Exine surface wrapped with very faint ridges. Ridges are branching, widely spaced, extending longitudinally and frequently anastomosing with adjacent ridges.

Grain diameter: average $P \times E = 20 \times 13 \mu\text{m}$ (P/E ratio = 1.53).

Conclusion and related genera

Variations in pollen morphology of the different Strawberry cultivars helped in the identification of the cultivars and varieties. The closest pollen allies to *Fragaria* in the Rose family are *Duchesnea* and *Potentilla*. Pollen from field-grown plants of *D. indica* and *P. recta* were examined by Maas [3]. Similarities among the pollen of *D. indica*, *P. recta* and *Fragaria x ananassa* pollen are their general size, form and ridging. However, one major difference is the presence of exine perforations on *P. recta* pollen.

In conclusion, differences in pollen morphology among *Fragaria* species and cultivars apparently corresponds with ploidy levels of the species.

References

- [1] Fogle, H.W., 1977a. Identification of Clones within four tree fruit species by pollen exine patterns. J. Amer. Soc. Hort. Sci., 102(5): 552-560.
- [2] Fogle, H.W., 1977b. Identification of tree fruit species by pollen ultrastructure. J. Amer. Soc. Hort. Sci., 102(5): 548-551.
- [3] Maas, J.L., 1977. Pollen ultrastructure of Strawberry and other small-fruit crops. J. Amer. Soc. Hort. Sci., 102(5): 561-571.
- [4] Westwood, M.N. and Challice, J.S., 1978. Morphology and surface topography of pollen and anthers of Pyrus species. J. Amer. Soc. Hort. Sci., 103(1): 28-37.
- [5] Martens, J. and Fretz, T.A., 1980. Identification of eight crabapples by pollen surface sculpture. J. Amer. Soc. Hort. Sci., 105(2): 257-263.
- [6] Simpson, B. B. and Skvarla, J. J., 1981. Pollen morphology and ultrastructure of *Krameria* (krameriaceae): Utility in questuins of intrafamilial and interfamilial classification. Amer. J. Bot., 68(2): 277-294.
- [7] Constance, L. and Chuang, T.I., 1982. SEM survey of pollen morphology and classification in hydrophyllaceae (waterleaf family). Amer. J. Bot., 69(1): 40-53.
- [8] Marcucci, M.C., Sansavini, S., Ciampolini, F. and Cresti, M., 1984. Distinguishing apple clones and cultivars by surface morphology and pollen physiology. J. Amer. Soc. Hort. Sci., 109(1): 10-19.
- [9] Heusser, C.J., 1971. Pollen and spores of Chile. Univ. of Arizona Press, Tucson.
- [10] Huang, T.C., 1972. Pollen flora of Taiwan. National Taiwan Univ. Bot. Dept. Press.
- [11] Wodehouse, R. P., 1935. Pollen grains: Their structure, identification and significance in science and medicine. McGraw-Hill, New York.
- [12] Thakur, D. R. and Thakur, S. S., 1970. Pollen morphology and germination in some temperate drupe plants. J. Palynology, 6: 96-100.

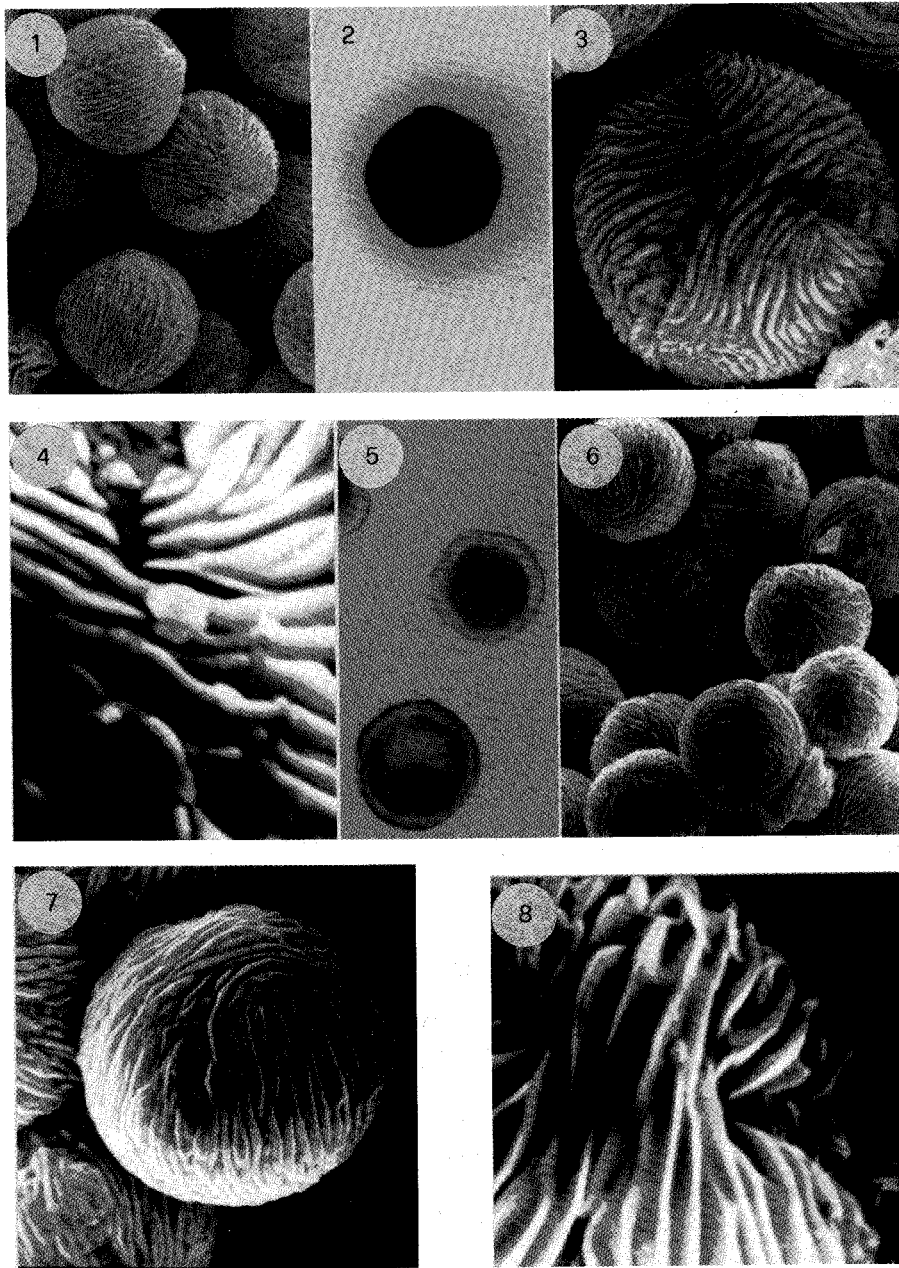


Plate 1

Pollen group 1:

1 - 4: *Fragaria x ananassa* CV. "Capitola".

1. SEM photograph showing spheroidal pollen in different views, x 1,500.

2. Light photograph of stainable grain, x 1,500.

3 & 4. SEM photographs of enlarged grain showing the disconnected and bifurcating striations, 3: x 3,500; 4: x 10,000.

5 - 8: *Fragaria x ananassa* CV. "Selva".

5. Light photograph showing thick outer exine, x 1,500.

6. SEM photograph showing spheroidal grains with fine striations, x 1,500.

7. SEM photograph of enlarged grain, x 3,500.

8. SEM photograph showing the bifurcating striations, x 10,000.

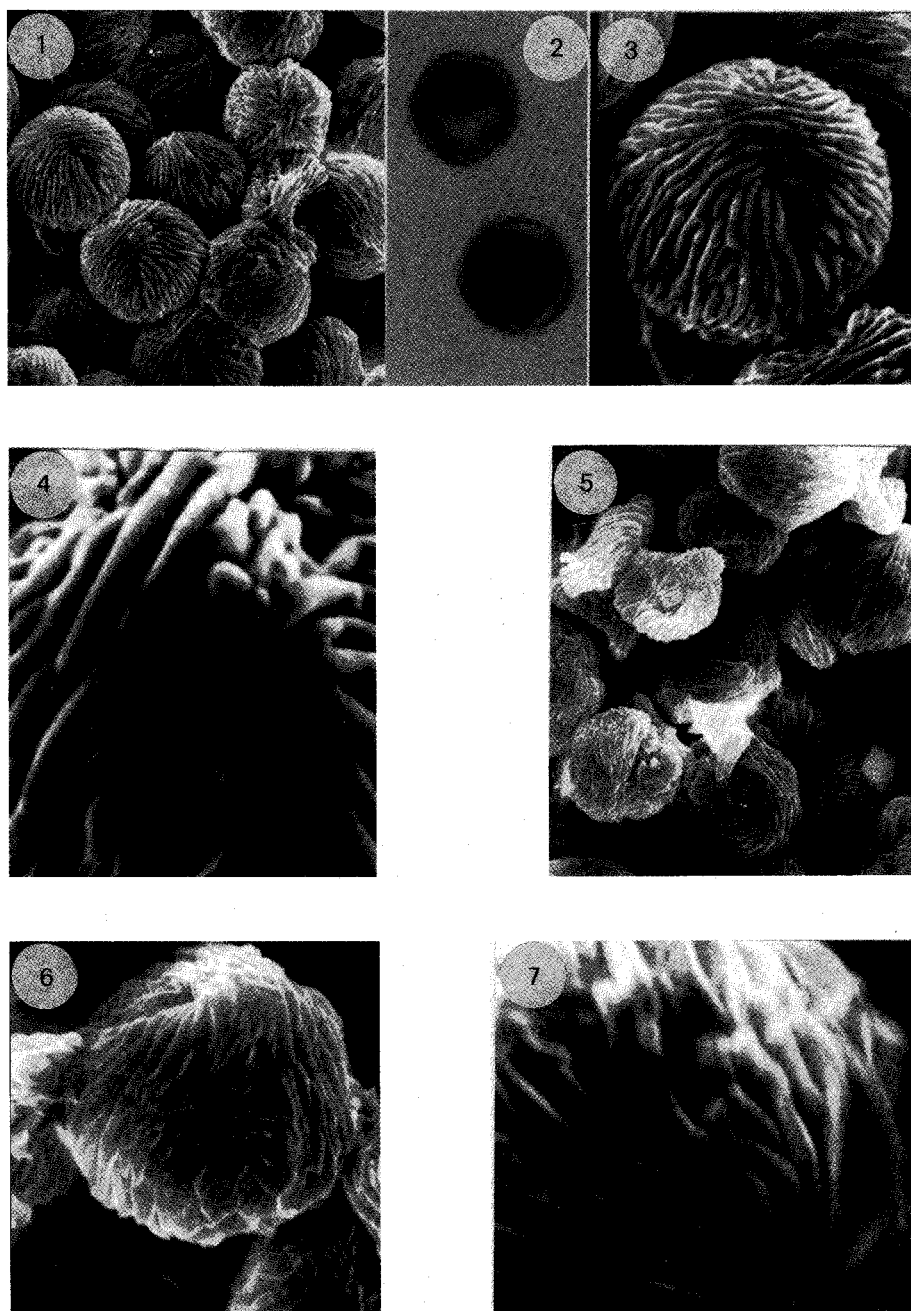


Plate 2

Pollen group 1:

1 - 4: *Fragaria x ananassa* CV. "Pajaro".

1. SEM photograph showing tricolporate pollen, x 1,500.
2. Light photograph x 1,500.
3. SEM photograph showing colpae that pointed toward the polar region, x 3,500.
4. SEM photograph of enlarged outer surface, x 10,000.

Pollen group 2:

5 - 7: *Fragaria x ananassa* CV. "Douglas".

5. SEM photograph x 1,500.
- 6 & 7 SEM photograph of enlarged grain showing thick striations and wide colpae, 6: x 3,500; 7: x 10,000.

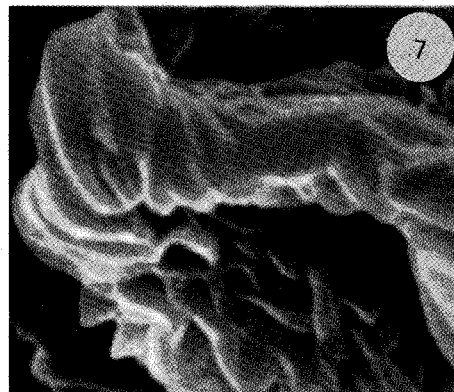
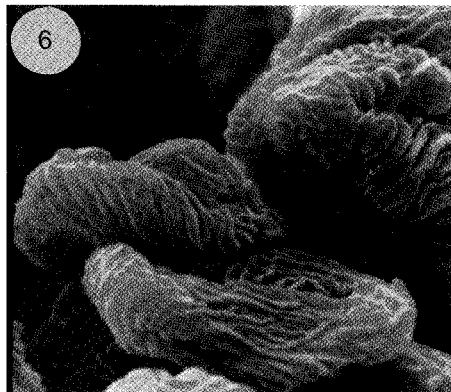
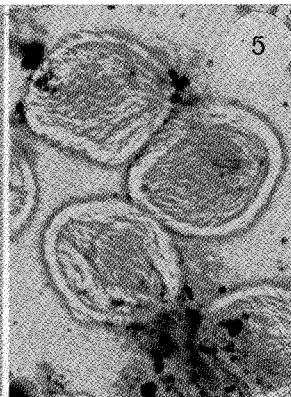
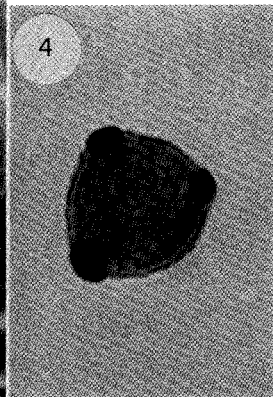
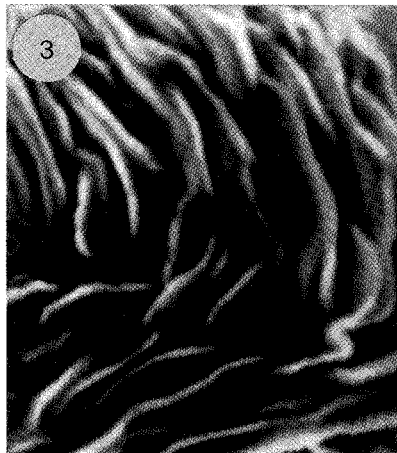
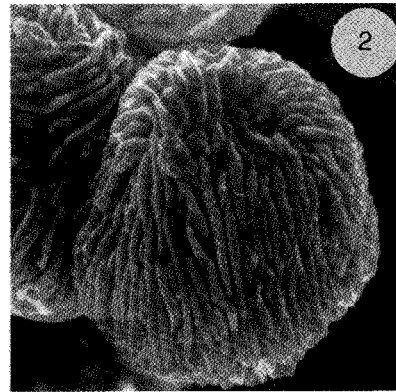
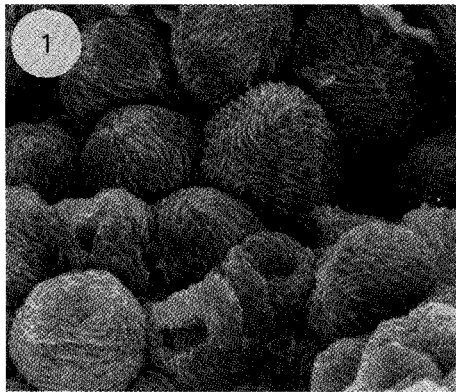


Plate 3

Pollen group 2:

1 - 4: *Fragaria x ananassa* CV. "Seascape"

1. SEM photograph showing generally spheroidal grains, x 1,500.
2. SEM photograph showing thick striations, x 3,500.
3. SEM photograph showing enlarged sculpture, x 10,000.
4. Light photograph of polar view showing equatorial swelling resulting from wet pollen, x 1,500.

5 - 7: *Fragaria x ananassa* CV. "Parker"

5. Light photograph showing well developed striations, x 1,500.
- 6 & 7 SEM photographs processing thick striations. 6: x 3,500; 7: x 10,000.

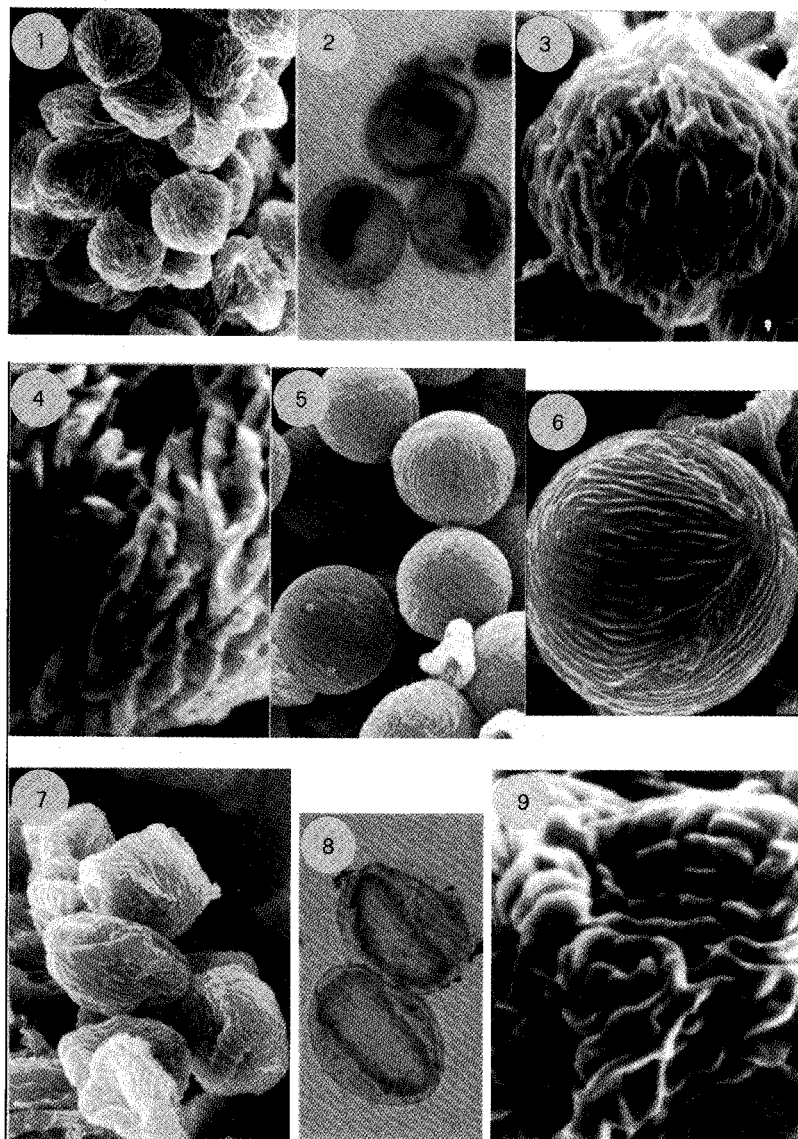


Plate 4

Pollen group 3:

1 - 4: *Fragaria x ananassa* CV. "Chandler"

1. SEM photograph showing spheroidal pollen with fine ridges, x 1,500.
2. Light photograph, x 1,500.
- 3 & 4 SEM photographs possessing irregular and anastomosing ridges separated by narrow longitudinal holes or furrows. 3: x 3,500; 4: x 10,000.

Pollen group 4:

5, 6: *Fragaria x ananassa* CV. "Oso Grande"

5. SEM photograph showing spheroidal grains covered with indistinct striations, x 1,500.
6. SEM photograph of enlarged grain possessing faint striations and long colpae reaching to the poles, x 3,500.

Pollen group 5:

7 - 9: *Fragaria x ananassa* CV. "Dorite"

7. SEM photograph showing subspheroidal grains, x 1,500
8. Light photograph showing subspheroidal to subprolate grains with long colpi, x 1,500
9. SEM photograph showing disconnected ridges at right angle to the colpi, x 3,500

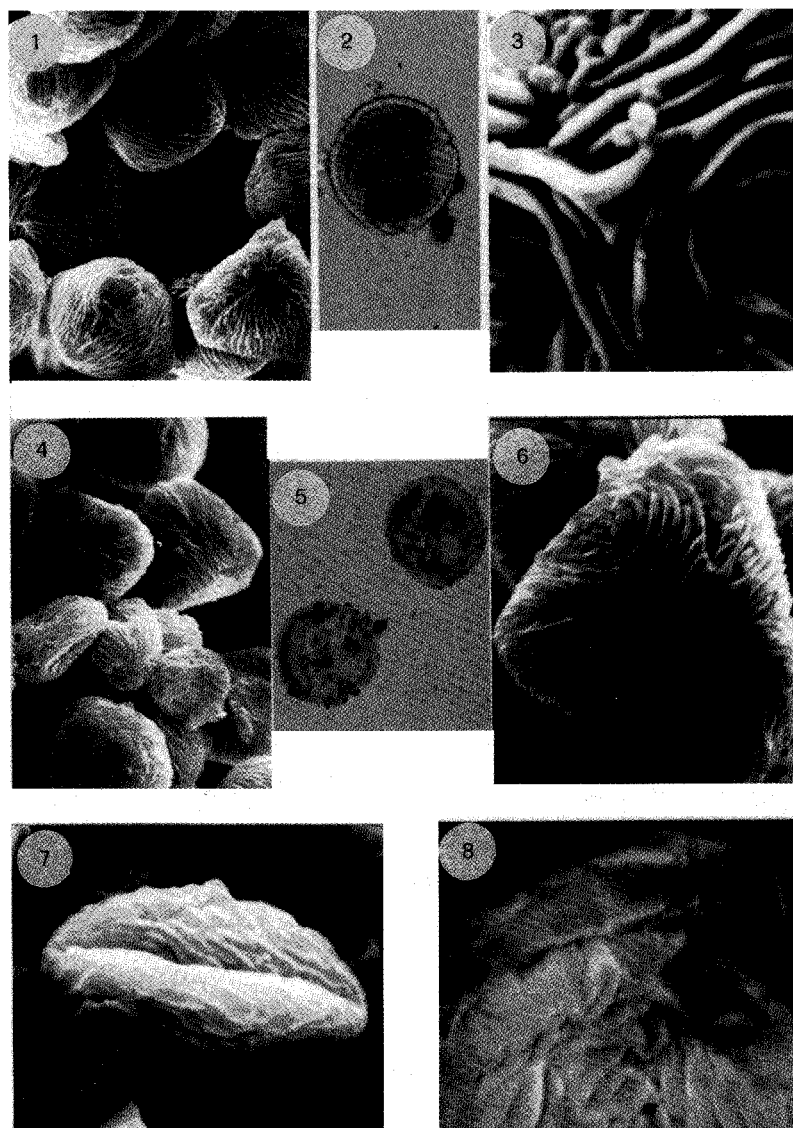


Plate 5

Pollen group 6:

1 - 3: *Fragaria x ananassa* CV. "Fern"

1. SEM photograph showing pollen grains with oblate spheroidal to triangular shape, x 1,500
2. Light photograph of polar view possessing clearly visible striations and colpae reaching to the poles, x 1,500
3. SEM photograph of enlarged grains with distinct striations, x 10,500

4 - 6: *Fragaria x ananassa* CV. "Sequoia"

4. SEM photograph showing pollen outlines from different views, x 1,500
5. Light photograph of oblate spheroidal grains with striations, x 1,500
6. SEM photograph showing polar view of triangular grain where colpae occupied the corners, x 3,500

Pollen group 7

7, 8: *Fragaria vesca*

7. SEM photograph showing subprolate pollen grains, tricolporate, colpi are long, pointed ends at the poles, x 3,500
8. SEM photograph showing part of grain with colpus, exine surface wrapped with very faint branching ridges, x 10,000.