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Pollen Ultrastructure of Peach Trees Regenerated from Tissue Culture

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ABSTRACT. Scanning electron microscopic and light microscopic studies were made on pollen grains of 26 peach trees regenerated from tissue culture. Characters that showed morphological variation included pollen grain length, width and length/width ratio. Pollen grains from all peach regenerated trees were elliptical and triaperturate with the germinal furrows extending almost to the full length of the grain. In all cases, the exine pattern was recticulate with irregular and semicircular shape pores. Pollen grains variability showed highly significant differences among the 26 peach regenerated trees. Pollen tube length of the 26 peach regenerated trees was not significantly different. Therefore these parameters could be used for distinguishing between those peach regenerated trees.

Introduction

One of the problems facing peach breeders and tissue culturists is the difficulty in distinguishing between the various peach cultivars during vegetative growth and fruit stages. Morphological characters of pollen grains proved to offer a reliable way for distinguishing some species of fruit trees and other tree species. Thakur and Thakur (1970) found that pollen grain size was not useful but pollen morphology afforded a new tool for the identification of stone fruit species. Fogle (1977a, b) reported that differences in size and exine surface of pollen grains were useful for distinguishing peaches, nectarines, apples, sweet cheery and European plums. Mass (1977) found that form, size, exine characteristics and pore structure were of taxonomic value in small fruit tree species. Martens and Fretz (1980) characterized 8 crab apples by observing pollen grain exine morphology. Shaheen (1983) and Shaheen *et al.*, (1986) indicated that pollen

grain morphology could be used as taxonomic tool for the identification of date palm males. Viability of pollen grains was tested in many fruit tree species (Brink, 1924; Brawbaker and Kwack, 1963 and Zielinski and Olenz, 1963). Multiple regenerated trees cultured from the same embryo were not easily distinguished from each other by comparing morphological characteristics of the tree or fruit.

The present investigation was undertaken to evaluate the surface morphology of pollen grains and their physiology for possible identification of 26 peach trees regenerated from embryo culture.

Materials and Methods

Pollen grains were collected from flowers of 26 peach trees derived from undifferentiated colli of embryonic source that were regenerated from embryo culture grown at the Horticultural Research Institute of Ontario (HRIO) in Vineland Station, Ontario, Canada (Table 1). Pollen grains collected from the mature anthers of the flowers of each of the regenerate tree were kept in desiccators until ready for use. Air-dried pollen grains from each regenerated tree were affixed to metal stubs using double-faced tape and then sputter-coated with a 60:40 gold palladium alloy 200A° thick and examined with a Hitachi S-570 SEM at an operating voltage of 15 Kv. Five samples from each regenerate tree were viewed before selecting representative pollen grains. Representative pollen grains were photographed at 2,000 × and 10,000 × to compare exine patterns of all regenerated trees.

TABLE 1. Number and name fo 26 peach trees regenerated from embryo culture.

Regenerated tree number	Regenerated tree name		Parentage	
1	$HB50_6$	25/7	Harbrite	⊗
2	$HB50_{10}$	23/7	Harbrite	⊗
3	$HB50_2$	25/10	Harbrite	⊗
4	$HB50_7$	23/7	Harbrite	⊗
5	$HB50_4$	27/7	Harbrite	⊗
6	HD53	7/8	Harrow Diamond	⊗
7	$HB50_2$	25/2	Harbrite	⊗
8	$HB53_7$	7/8	Harbrite	⊗
9	HD48	1/2	Harrow Diamond	⊗
10	FRH	2/23	Red Haven	⊗
11	$HB50_{11}$	23/24	Harbrite	⊗
12	HB53	7/8	Harbrite	⊗
13	$HD53_{13}$	7/18	Harrow Diamond	⊗
14	HD48	1/8	Harrow Diamond	⊗
15	FRH	1/14	Red Haven	⊗
16	HB50	16/9	Harbrite	⊗
17	FRH	1/4	Red Haven	⊗
18	HB50	25/2	Harbrite	⊗
19	$HD50_8$	23/4	Harbrite	⊗
20	$HB50_2$	25/4	Harbrite	⊗
21	$HD53_6$	7/8	Harrow Diamond	⊗

TABLE 1. Contd.

Regenerated tree number	Regenerated tree name		Parentage	
22	$HB50_2$	5/10	Harbrite	⊗
23	$HB50_{16}$	23/4	Harbrite	⊗
24	$HB50_{17}$	23/4	Harbrite	⊗
25	$HB50_9$	23/4	Harbrite	⊗
26	$HB50_6$	25/7	Harbrite	⊗

The measurements of length and width of 10 pollen grains were carried out by a Carl Zeiss light microscope. The germination test was done on a solid medium (prepared by making up 15% sucrose solution in 2% agar-agar, which poured into petri dishes (5 ml) and sterilized) as described by Janick and Moore (1975). After 24 hours of incubation at 26°C, 100 pollen grains were examined for germination from each petri dish. An initiation of a pollen tube was considered as evidence of germination, *i.e.*, viability. Statistical analyses were carried out using SAS (1987).

Results and Discussion

The pollen grains of all peach regenerated trees were similar in surface morphology (Fig. 1 and 2). Pollen grains from all regenerated trees were elliptical and triaperturate with the germinal furrows extending almost to the full length of the grain. In all cases, the exine pattern was recticulate with irregular and semicircular shaped pores. This pattern was similar to the pattern in the photograph of peach pollen presented by Fogle (1977a, b). Each regenerate tree consistently displayed a longitudinal and shallow exine type, which consisted of an intact tectum interrupted by round to oval perforations. All pollens had a distinct exine ridge, which ran roughly parallel along the grain. Typically, such ridges were narrow, closely spaced, frequently branched, and occasionally formed whorl patterns.

Statistical analysis for the morphological characteristics (length, width and length/width ratio) of the pollen grains indicated highly significant differences among the 26 peach regenerated trees (Table 2 & 3). These results are in line with those results obtained by Mass, 1977; Fogle, 1977a, b, and Westwood and Challica, 1978). Length of the pollen grains varied from 45.00 to 71.22 μm. The width of the pollen grains ranged between 15.00 to 37.50 μm. The length and width ratio which was used as an index of pollen grains shape ranged from 1.33 to 3.50.

TABLE 2. Least squares from analysis of variance of length, width and length/width pollen grains from 26 peach regenerated trees.

Source variation	d.f.	Mean square		
		Length	Width	Length/width
Replication	9	8.74	4.73	0.034
Regenerated tree	25	57.32**	53.50**	0.435**
Error	225	15.35	11.20	0.081

**P < 0.01

TABLE 3. Means of size and shape of pollen grains from 26 peach regenerated trees.

Regenerated tree number	Mean		
	Length (μm)	Width (μm)	Length / width
1	55.88	28.13	2.00
2	55.13	25.50	2.18
3	55.68	27.75	2.04
4	64.50	31.50	2.07
5	57.38	30.00	1.92
6	56.63	27.75	2.06
7	57.38	20.63	2.90
8	61.50	30.75	2.02
9	60.75	24.38	2.51
10	57.00	29.63	1.97
11	52.50	27.75	1.91
12	57.75	27.00	2.17
13	55.50	26.26	2.13
14	61.80	30.00	2.09
15	57.00	30.00	1.96
16	58.50	27.38	2.17
17	56.63	28.50	2.02
18	58.88	27.75	2.16
19	57.75	26.63	2.21
20	57.38	28.13	2.05
21	58.50	28.45	2.09
22	58.50	31.13	1.89
23	56.63	29.63	1.92
24	59.63	30.00	1.99
25	57.38	29.63	1.94
26	57.75	28.50	2.04
S.E	1.239	1.058	0.090
L.S.D. (0.05)	3.4523	2.9494	0.2513
L.S.D. (0.01)	4.5513	3.8883	0.3312

Nine regenerated trees had a ratio between 1.89 and 2.00 μm whereas the other regenerated trees (17 trees) had a ratio more than 2.00 μm (Table 3). These results showed that all regenerated trees had an elliptical and triaperturate pollen grains shape. The conformations are in line with those results obtained by Fogle (1977a).

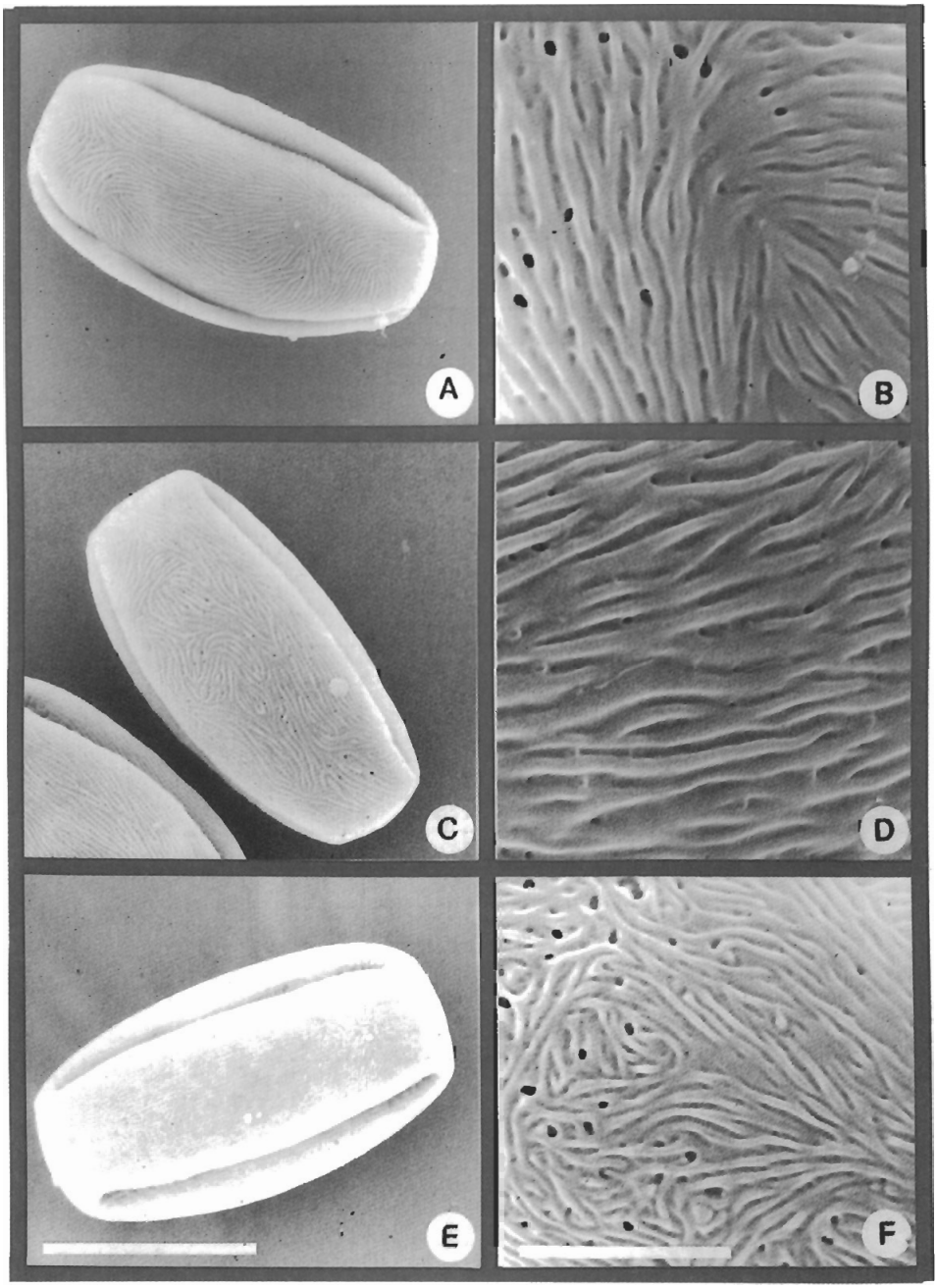

FIG. 1. Typical pollen grain and section of exine of peach regenerate trees of HB_{50} 16/9 (A and B), HD_{53} 7/18 (C and D), and FRH 1/4 (E and F). Bar represents 30 μm in A, C, E, and 6 μm in B, D, F.

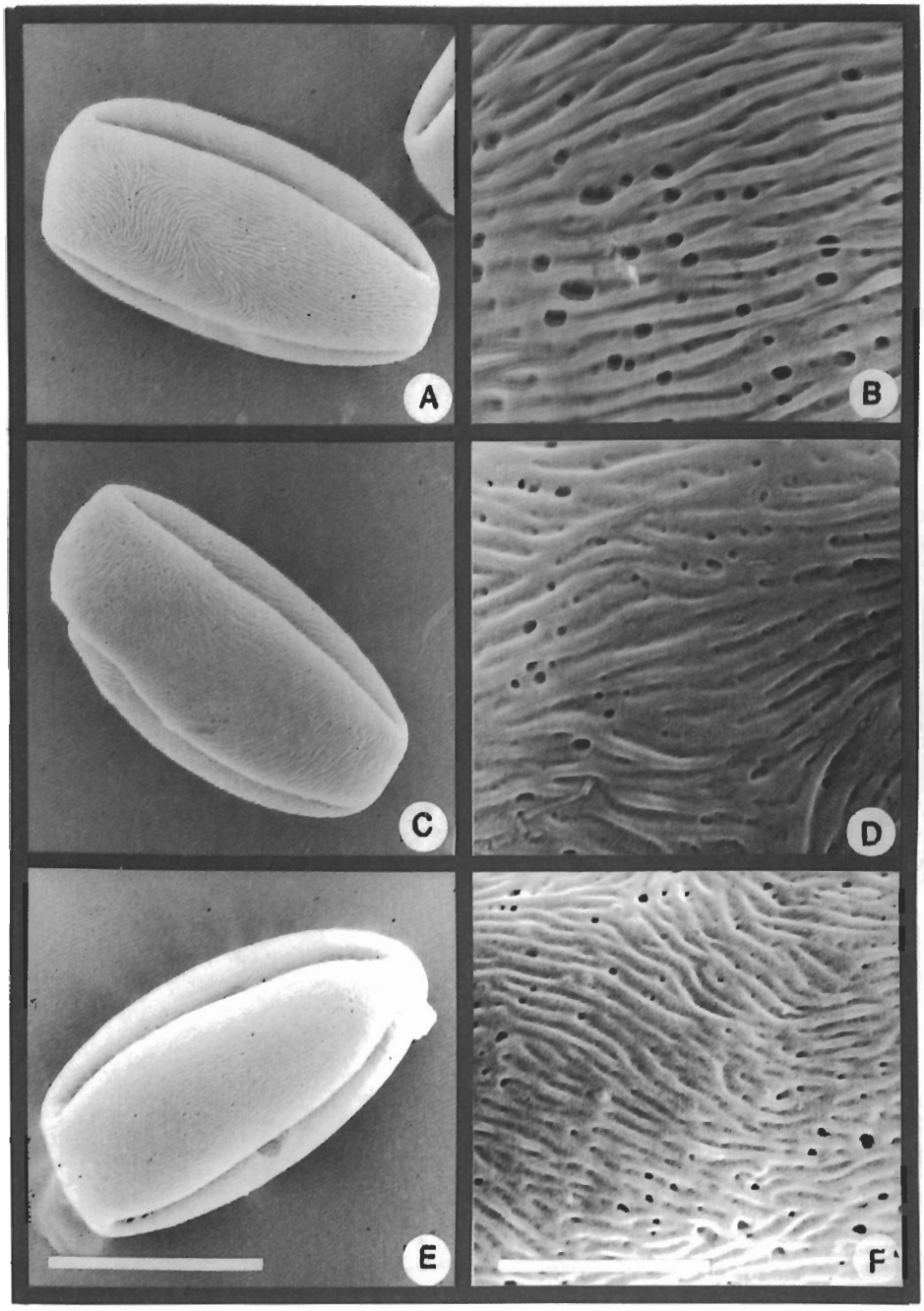

FIG. 2. Typical pollen grain and section of exine of peach regenerate trees of HB_{50} 25/2 (A and B), HD_{48} 1/2 (C and D), and FRH 1/14 (E and F). Bar represents 30 μm in A, C, E, and 6 μm in B, D, F.

The data concerning viability of the pollen grains from the 26 peach regenerated trees are shown in Table 4. It indicates highly significant difference among the 26 regenerated trees. Pollen grains of the regenerated trees could be classified according to their viability into four classes, the first class had viability ≤ 64% (3 regenerated trees), the second class had a viability between 65 to 69% (3 regenerated trees), the third class had a viability between 70 to 74% (8 regenerated trees) and fourth class had a viability ≤ 75% (12 regenerated trees).

TABLE 4. Viability (%) of pollen grains from 26 peach regenerated trees.

Group no.	Range (%)	Regenerated tree no.	Mean (%)	Standard error
1	≤ 64	4, 21, 22	62.0 a	1.09
2	65 – 69	15, 16, 24	66.67 b	1.09
3	70 – 74	2, 5, 6, 8, 12, 17, 18, 25	72.38 c	0.67
4	≥ 75	1, 3, 7, 9, 10, 11, 13, 14, 19, 20, 23, 26	77.17 d	0.54

*Means followed by different letters are significantly different at $P < 0.01$ using L.S.D. test.

Concerning the pollen tube length, it was found that pollen tube length of the 26 peach regenerated trees was not significantly different $P > 0.05$ (Table 5).

TABLE 5. Least squares from analysis of variance of pollen tube length for 26 peach regenerated trees.

Source of variation	df	Mean square
Replication	4	8997.2
Regenerated trees	25	$21878.7^{n.s}$
Error	100	18485.6

n.s.: not significant at $P > 0.05$.

These results are in agreement with the conclusions of Martens and Fretz (1980), Fogle (1977a, b), and Mass (1977) that the pollen grain morphology can be useful for taxonomic purposes. So it can be used to distinguishing between these 26 peach regenerated trees.

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التركيب الدقيق لحبوب لقاح أشجار الخوخ المكاثرة عن طريق زراعة الأنسجة

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المستخلص. أجريت دراسة بالمهجر الإلكتروني الماسح والميكروسكوب العادي على حبوب لقاح ٢٦ شجرة خوخ تم إكثارها عن طريق زراعة الأنسجة (زراعة الجنين). وقد تضمنت التقديرات التي أظهرت تغيرات مورفولوجية، طول وعرض ونسبة الطول إلى العرض لحبة اللقاح.

وأوضحت الدراسة أن حبوب لقاح جميع الأشجار كانت إهليلجية ثلاثية الفتحات وبها تجويفان غائران ممتدان بطول الحبة، كما أن الطبقة الخارجية من حبة اللقاح كانت شبكية وبها ثقوب نصف دائرية أو غير منتظمة. وظهر أن الفروق معنوية بين تقديرات الطول، والعرض ونسبة الطول إلى العرض لحبوب اللقاح. وتبين أن هناك فروقًا معنوية عالية بين حيوية حبوب لقاح الأشجار. أما بالنسبة لتقدير طول أنبوبة اللقاح فلم تظهر فروق معنوية بين الأشجار. وبذلك ثبت جدوى استخدام تلك التقديرات في التفرقة بين أشجار الخوخ المكاثرة عن طريق زراعة الأنسجة.