Difference in Micromorphological Pattern on Pollen Surface of Japanese Pear Cultivars

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The dry pollen surface of Japanese pear cultivars was investigated using the scanning electron microscope (SEM). Ridges, perforations and nanoprocesses were observed on the exine, and the micromorphological pattern combined with these characteristics appeared to vary with the cultivars. Four cultivars, Imamuraaki, Suisei, Kikusui and Choku which apparently differed from each other with regard to the micromorphology of pollen surface, were chosen for estimating the morphological differences quantitatively. Twelve characteristics could be selected and measured on 50 pollen grains in each cultivar, based on microphotographs. Discriminant analysis was carried out using these 12 characteristics. Of the total of 200 pollen grains examined, about 76 percent could be correctly assigned to each cultivar. It is thus suggested that the micromorphology of pollen surface is available for distinguishing and identifying Japanese pear cultivars.

Introduction

Because of the advances made in breeding systems, the number of cultivars of fruit trees as well as other crops has experienced a marked increase. Consequently some of the cultivars have many similar features, and it is sometimes difficult to distinguish and identify cultivars in using mainly visible characters such as fruit appearance, leaf shape, tree habit. It is thus necessary to seek new characters for distinguishing and identifying cultivars so as to avoid confusion. The recent development of the scanning electron microscope (SEM) has enabled us to observe the micromorphology of plant surface easily and clearly. The micromorphology of pollen (NOWICKE and SKVARLA 1979, ARGUE 1980, WALKER and WALKER 1980), trichome (HARDIN 1979) and seed coat (HILL 1976, CROW 1979) has been utilized for taxonomic purposes.

FOGLE (1977 a, 1977 b) observed the micromorphology of pollen surface in several fruit trees, and suggested that micromorphological differences in the pollen surface were useful for distinguishing and identifying species and clones. Variation in the micromorphology of pollen surface in fruit trees has also been reported in 72 grape cultivars (TOMPA and KOZMA 1978), 18 *Pyrus* species (WESTWOOD and CHALLICE 1978), nine olive cultivars (PACINI and VOSA 1979) and eight crabapple species and hybrids (MARTENS and FRETZ 1980).

In this study, difference in the micromorphology of pollen surface among Japanese pear cultivars was investigated and the numerical approach was used to estimate morphological differences quantitatively.

Materials and Methods

Four Japanese pear (*Pyrus serotina* Rehd. var. *culta* Rehd.) cultivars, Imamuraaki, Suisei, Kikusui and Choku were chosen for numerical approach of pollen morphology, on

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the basis of the preliminary findings in which these four cultivars apparently differed from each other with regard to the micromorphology of pollen surface.

Pollen collection was carried out by the following procedures. Flowers just before anthesis were collected from trees planted in the experimental orchards of the Fruit Tree Research Station, MAFF, in late April 1980. Anthers were removed from the collected flowers, and wrapped in paper bags. Besides, wrapping one anther in one paper bag was also done. After dehiscence of anthers at 25°C for two days, the paper bags containing dry pollen grains with anther walls were preserved in a desiccator at 20°C, until used.

The micromorphology of pollen surface was investigated by SEM. Dry pollen was placed on double adhesive tape attached to the brass stub, coated with gold in an EIKO IB-3 ion coater, and observed with a JEOL JSM-35 at 15 kV accelerating voltage.

In each cultivar 50 pollen grains were selected, in which two of three germinal furrows were recognized to locate symmetrically under SEM, as shown in the left figures of Fig.1 to Fig.4. For each pollen grain thus selected, the whole was photographed at 1500x magnification, and then the central region of the exine at 13000x. The former microphotographs were used for measurements of length of polar axis, diameter of equator and distance between germinal furrows, and the latter for measurements, at the central $5\mu\text{m} \times 5\mu\text{m}$, of number of nanoprocesses (termed tentatively), number of perforations, total area of perforations, number of ridges along the polar axis and number of ridges along the equator, by the use of a NAC Sonic-digitiser. The number of ridges along the polar axis was given by the average number of ridges on six latitudinal lines with interval of $1\mu\text{m}$ and number of ridges along the equator by the average on six longitudinal lines. In addition to these eight characteristics, shape of pollen grains, withdrawal of germinal furrows, average area of perforations and inclination of ridges were obtained in dividing diameter of equator by length of polar axis, distance between germinal furrows by diameter of equator, total area of perforations by number of perforations and number of ridges along the polar axis by number of ridges along the equator, respectively.

A total of 12 characteristics as described above were used for the discriminant analysis applying the Multivariate Analysis Program (MAP) in the Computing Centre for Research in Agriculture, Forestry and Fishery, MAFF.

Results and Discussion

Dry pollen of Japanese pear was elliptical and tricolpate, and had a finger print type exine. Ridges, perforations and nanoprocesses were observed on the exine, as in other *Pyrus* species (WESTWOOD and CHALLICE 1978). The pollen of Imamuraaki had a large number of clear ridges, and the interval between ridges was very short (Fig.1). Suisei had numerous and large perforations, and ridges were indistinct (Fig.2). Kikusui had clear ridges, and two or three ridges were closely located (Fig.3). Choju had a vague exine and conspicuous nanoprocesses (Fig.4). Thus, the micromorphology of pollen surface showed a specific pattern in each cultivar, though there was slight variation among individual pollen grains of the same cultivar and of an anther, probably due to the differences in developmental condition of pollen mother cells. In some plant species, the exine pattern was presumed to be controlled sporophytically by the pollen mother cell based on

Fig.1 and Fig.2. Scanning electron microphotograph of whole pollen grain, x 1500 (left) and exine pattern, x 13000 (right) in Japanese pear : 1. Imamuraaki had clear ridges (arrow), 2. Suisei has numerous perforations (arrow) : the scale is equal to 10 μm (left) and 1 μm (right).

Fig. 3 and Fig. 4. Scanning electron microphotograph of whole pollen grain, $\times 1500$ (left) and exine pattern, $\times 13000$ (right) in Japanese pear: 3. Kikusui has two or three closely located ridges, 4. Choju has conspicuous nanoprocesses (arrow): the scale is equal to $10\text{ }\mu\text{m}$ (left) and $1\text{ }\mu\text{m}$ (right).

the results of progeny tests (QUIROS 1975, RAO *et al.* 1979), and exine ontogeny studies (HESLOP-HARRISON 1968, ROGERS and HARRIS 1969, PANDEY and TROUGHTON 1974). The mechanism responsible for variation of exine patterns in Japanese pear will be explained, since investigation of the genetic behavior is now in progress.

To estimate the morphological differences quantitatively, a numerical method was developed using the 12 characteristics, of which the mean and standard deviation are given in Table 1. The cultivars could not be distinguished on the basis of any one characteristic; even in the total area of perforations which was the most useful characteristic to distinguish, only 50 percent of the total of 200 pollen grains examined could be correctly assigned to each cultivar. Thus, discriminant analysis, one of the multivariate analyses, was carried out for a better distinction of cultivars. The Mahalanobis' generalized dis-

Table 1. Mean and standard deviation of 12 characteristics in 50 pollen grains of four Japanese pear cultivars.

Characteristics	Cultivars			
	Imamuraaki mean $\pm$ S. D.	Suisei mean $\pm$ S. D.	Kikusui mean $\pm$ S. D.	Choju mean $\pm$ S. D.
Length of polar axis (μm)	44.16 $\pm$ 1.58	43.94 $\pm$ 2.06	43.40 $\pm$ 3.14	45.08 $\pm$ 2.07
Diameter of equator (μm)	22.95 $\pm$ 1.07	22.50 $\pm$ 1.73	22.53 $\pm$ 1.45	21.80 $\pm$ 1.59
Distance between germinal furrows (μm)	16.61 $\pm$ 1.67	14.42 $\pm$ 1.97	14.08 $\pm$ 1.89	14.96 $\pm$ 1.74
Number of nanoprocesses	1.8 $\pm$ 2.1	1.2 $\pm$ 2.3	2.3 $\pm$ 2.4	3.1 $\pm$ 3.2
Number of perforations	5.0 $\pm$ 10.7	27.7 $\pm$ 12.4	15.0 $\pm$ 14.6	10.7 $\pm$ 10.8
Total area of perforations (μm^2)	0.097 $\pm$ 0.245	1.007 $\pm$ 0.542	0.301 $\pm$ 0.327	0.249 $\pm$ 0.326
Number of ridges along the polar axis	14.8 $\pm$ 3.0	8.6 $\pm$ 4.9	10.1 $\pm$ 3.4	9.6 $\pm$ 4.5
Number of ridges along the equator	5.0 $\pm$ 2.9	3.3 $\pm$ 2.5	6.3 $\pm$ 2.6	3.7 $\pm$ 3.0
Shape of pollen grain	0.52 $\pm$ 0.03	0.51 $\pm$ 0.03	0.52 $\pm$ 0.04	0.48 $\pm$ 0.03
Withdrawal of germinal furrows	0.73 $\pm$ 0.09	0.64 $\pm$ 0.07	0.63 $\pm$ 0.08	0.69 $\pm$ 0.08
Average area of perforations (μm^2)	0.010 $\pm$ 0.011	0.036 $\pm$ 0.013	0.019 $\pm$ 0.011	0.021 $\pm$ 0.011
Inclination of ridges	4.29 $\pm$ 2.67	3.16 $\pm$ 2.27	2.29 $\pm$ 2.10	3.91 $\pm$ 3.31

Table 2. Mahalanobis' generalized distance among four Japanese pear cultivars.

Cultivars	Imamuraaki	Suisei	Kikusui
Suisei	12.03		
Kikusui	5.42	7.11	
Choju	6.26	6.02	3.57

Table 3. Classification of 200 pollen grains from four Japanese pear cultivars by the discriminant analysis.

Sample cultivars	Number of pollen grains assigned to :			
	Imamuraaki	Suisei	Kikusui	Choju
Imamuraaki	41	2	3	4
Suisei	1	40	4	5
Kikusui	10	1	33	6
Choju	3	5	5	37

Fig. 5. Scatter diagram of 200 pollen grains by the discriminant analysis with reduction of dimensionality.

● : Imamuraaki

▲ : Suisei

□ : Kikusui

× : Choju

A : Centroid of Imamuraaki

B : Centroid of suisei

C : Centroid of Kikusui

D : Centroid of Choju

tance among each cultivar is shown in Table 2. The scatter diagram of each cultivar by application of the discriminant analysis with reduction of the dimensionality is shown in Fig. 5. Individual pollen grains of Imamuraaki and Suisei were scattered mainly in the forth quadrant and the third, respectively, whereas those of Kikusui and Choju were scattered broadly in the first and second quadrants. Moreover, the distance between centroids of Imamuraaki and Suisei was the longest, and that of Kikusui and Choju was the shortest. The classification of individual pollen grains in each cultivar by the discriminant analysis is shown in Table 3. Of the total of 200 pollen grains examined, about 76 percent could be correctly assigned to each cultivar ; 82, 80, 66 and 76 percent to Imamuraaki, Suisei, Kikusui and Choju, respectively.

The result indicates that the micromorphology of pollen surface may provide a valuable key for the distinction of Japanese pear cultivars. FOGLE (1977 b) set up the identification key based on pollen size and exine characteristics in order to distinguish the 59 examined clones of four fruit trees. MARTENS and FRETZ (1980) set up the dichotomous key based on pollen exine feature in order to distinguish the eight examined crabapple species and hybrids. The distinction and identification, using not only key reference but also numerical analysis, is effective especially in case of showing slight variation within the same cultivar. The application of the discriminant analysis to pollen morphology was reported in *Ambrosia* (ROBBINS *et al.* 1979) and *Picea* (BIRK and PEGLAR 1980) to distinguish species. But there was no information about the distinction of cultivars, using the numerical method for micromorphology, to the best of authors' knowledge. In this study, about 76 percent of total number of pollen grains examined could be correctly assigned to each cultivar, which proves that this method can be developed to cultivar level in Japanese pear. Through the further investigation of numerical analysis for the pollen morphology, it will become more reliable to distinguish and identify the large number of Japanese pear cultivars.

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ニホンナシ (*Pyrus serotina* Rehd. var. *culta* Rehd.) の
花粉表面微細形態における品種間差異

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ニホンナシにおける品種の識別・同定に役立つ新形質を探索するため、今村秋、翠星、菊水、長寿の4品種の乾燥花粉の表面微細形態を走査型電子顕微鏡を用いて観察した。

ニホンナシの花粉外壁の様子は、指紋状であり、ウネ、微散孔、微細突起が外壁上に観察された。これらを組み合わせた微細形態が、品種により差異のあることが認められた。しかし、この微細形態は同一品種内である程度の変異がみられ、この変異は同一葯内においても観察された。

次に、形態的な差異を量的に評価するため、花粉形態の数値化を試みた。走査型電子顕微鏡写真をもとに、各品種50花粉ずつ、次の12特性を選出した。まず、花粉粒の、1. 極軸の長さ、2. 赤道直径、3. 発芽溝間の距離を測定した。次に、同じ花粉の花粉外壁中央部5 μ m四方内の、4. 微細突起の数、5. 微散孔の数、6. 微散孔の総面積、7. 極軸に沿ったウネの数、8. 赤道に沿ったウネの数を測定した。そして、9. 花粉粒の形(赤道直径に対する極軸の長さの比)、10. 発芽溝の引っ込み(発芽溝間の距離に対する赤道直径の比)、11. 微散孔1個当りの平均面積(微散孔の総面積を微散孔の数で除した商)、12. ウネの傾き(極軸に沿ったウネの数に対する赤道に沿ったウネの数の比)を計算した。

これらの12特性は、どれも単独では品種を十分に識別できず、最も良い結果は、「微散孔の総面積」を用いた場合で、供試花粉全体の50%が正しい品種に識別された。そこで、これら12特性を使って、判別分析を行った。その結果、各品種50花粉のうち、今村秋では82%、翠星では80%、菊水では66%、長寿では74%の花粉が正しい品種に識別され、供試花粉全体で約76%の花粉が正しい品種に識別された。また、次元の減少を伴う判別分別を行って得られた、各々の花粉の二次元散布図では、今村秋と翠星はそれぞれ他の群と区別できたが、菊水と長寿はお互いに混在していた。

結論として、ニホンナシの花粉表面は、品種特有の微細形態をもっていることが明らかになり、それらに基づいた品種の識別・同定の可能性が示唆された。