Pharmacognostical Studies on Plantaginis Herba
On the Morphology of the Pollen Grains1)

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Electron microscopic studies on the pollen grains of some plant species of genus Plantago and commercially available folk medicine called “Shazensō” and “Shazenyōmatsu” were made. The following results were obtained.

In order to identify the original plant of “Shazensō” and “Shazenyōmatsu” in market, the pollen grains collected from six species of genus Plantago were subjected to electron microscopic examination.

It was found easy to distinguish each of the species belonging to genus Plantago according to the observations of the exterior configuration of their pollen grains with electron microscope. The configuration of the pollen grains from “Shazensō” and “Shazenyōmatsu” were the same as those of P. asiatica. The above facts showed that “Shazensō” was identical with P. asiatica and also that a great deal of pollen grains of P. asiatica were contained in “Shazenyōmatsu”.

All the pollen grains of these species of genus Plantago were shown to have operculum, although slight difference in ornamentation on operculum may exist.

The pattern of the pollen grains of genus Plantago was found to comprise tuberculata-spinoid. Many canals were seen on the surfaces.

The whole herb of Plantago asiatica collected in flowering period is called “Shazensō” in folk medicine and has long been used as a diuretic or an antitussive agent or as a drug for the disorder of digestion. This is described in like manner in J.P.4), “Shazenyōmatsu” prepared by grinding “Shazensō” is also in market.

Light microscopic study of the pollen configuration of thirteen plant species of genus Plantago, namely P. asiatica, P. albicans, P. cynops, P. depressa, P. formosa, P. hakusanensis, P. japonica, P. lanceolata, P. major var. kimurae, P. maritima, P. psyllium and P. saussuriae was reported by Ikuse,6) Academia Sinica,6) Erdtman,7) Huang8) and Shimakura.9) Ikuse et al.10) also studied the morphology of the pollen grains of P. asiatica with scanning electron microscope.

It is known that the pollen grains of the thirteen species are all of poly (ca. 10)-forate 4C type, but that there are various patterns of ornamentation such as verrucate,6,8) spinoid,6) granulate,8) slightly undulating8) and slightly undulating with scabrate,10)

1) A part of this study was presented at the 25th Annual Meeting of the Japanese Society of Pharmacognosy, Fukuoka, October 1978. Abstracts of Papers, p. 30.
2) Location: 5-6-1, Mitahara-Higashi, Gifu 502, Japan.
8) T.C. Huang, “Pollen Flora of Taiwan,” National Taiwan University Botany Department Press, Taipei, 1972, p. 154.
To establish the morphological identification of the pollen grains of the six species belonging to genus *Plantago* chosen and to identify the original plant of "Shazenō" and "Shazenōmatsu", the authors examined both exterior and interior (pollen wall and pore membrane) configurations thereof using electron microscope, and obtained the results described below.

**Experimental**

1. **Materials and Methods**


   *P. camtschatica* Cham.: Indigenous species, Erimo cape (Hokkaido) (collected July 30, 1967).

   *P. hakusanensis* Koidz.: Indigenous species, Mt. Tateyama (Toyama pref.) (collected Aug. 13, 1956).


   *P. lanceolata* L.: Cultivated species, Kawashima-cho (Gifu pref.) (collected May 29, 1978).


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**Fig. 1.** Pollen Grains of *Plantago* spp.


a, whole view; b, germinal pore; ca, canal; fr, fringe.
The pollen grains were prepared from the inflorescence of authentic species of genus Plantago and "Shazensō," or from leaves if not inflorescence, using the ultrasonic method formerly reported\(^{11}\) and then examined with a scanning electron microscope (JSM-S1). Two grams of specimens of "Shazenyōmatsu" were acetylated as previously reported,\(^{19}\) washed with water and heated to 90° for 30 minutes with the addition of 10% sodium hydroxide solution. Then the residue was washed with water repeatedly through centrifugal separator. After dehydration with a series of acetone solution (from 30 to 100%), 30 mg and 120 mg of pollen grain-containing samples were obtained from "Shazenyōmatsu-(G)" and "Shazenyōmatsu-(H)" respectively.

The interior configuration of the pollen grains of genus Plantago were examined with a transmission electron microscope (JEM-100B) as previously reported.\(^{11}\)

2. Exterior configuration of pollen grains

The pollen grains of the six species of genus Plantago were all of poly (ca. 10)-orate 4C\(^{4}\) type (Fig. 1). A number of tuberculata\(^{23}\) and canals were seen all over the surface of the pollen grains. Spinoids were also recognized at equal intervals of 0.4 to 0.6 \(\mu m\). Only on the pollen grains of P. virginica, were scattering many spinoids sporadically. The details of their configuration were as follows;

1) Plantago asiatica: The size of the pollen grains was 18.5-20.0-20.5×20.0-20.5-22.0 \(\mu m\) (Table I). The number of the spinoids per unit surface area (3 \(\mu m\) square) was 54-56. The diameter of the germinal pore was 3.3-3.4 \(\mu m\). The operculum was covered with granules bearing spinoids at the top (Fig. 1).

2) P. camtschatica: The size of the pollen grains was 15.7-17.1-18.6×15.9-17.1-18.6 \(\mu m\) (Table I). The number of the spinoids per 3 \(\mu m\) square was 62-71. The diameter of the germinal pore was 2.2-2.9 \(\mu m\). The operculum was covered with granules bearing spinoids at the top (Fig. 1).

3) P. hakusanensis: The size of the pollen grains was 20.2-22.1-23.8×20.2-22.3-24.0 \(\mu m\) (Table I). The number of the spinoids per 3 \(\mu m\) square was 54-56. The diameter of the germinal pore was 2.3-3.4 \(\mu m\). The operculum was covered with granules bearing spinoids at the top (Fig. 1).

4) P. lanceolata: The size of the pollen grains was 15.0-17.0-17.5×15.0-17.5-17.5 \(\mu m\) (Table I). The number of the spinoids per 3 \(\mu m\) square was 62-71. The diameter of the germinal pore was 2.2-2.9 \(\mu m\). The operculum was covered with granules bearing spinoids at the top (Fig. 1).

5) P. virginica: The size of the pollen grains was 20.0-20.0-20.0×20.0-20.0-20.0 \(\mu m\) (Table I). The number of the spinoids per 3 \(\mu m\) square was 54-56. The diameter of the germinal pore was 2.3-3.4 \(\mu m\). The operculum was covered with granules bearing spinoids at the top (Fig. 1).

6) P. hakusanensis: The size of the pollen grains was 20.0-20.0-20.0×20.0-20.0-20.0 \(\mu m\) (Table I). The number of the spinoids per 3 \(\mu m\) square was 54-56. The diameter of the germinal pore was 2.3-3.4 \(\mu m\). The operculum was covered with granules bearing spinoids at the top (Fig. 1).

\(^{11}\) M. Mizuno, F. Yamazaki and K. Taki, Shoyakagaku Zasshi, 32, 129 (1978).

The number of the spinoids per 3 μm square was 69–71. The diameter of the germinal pore was 3.0–3.2 μm. The operculum was covered with granules bearing spinoids at the top (Fig. 1).

(4) *P. japonica*: The size of the pollen grains was 18.0–19.0–21.0 × 18.0–19.2–20.8 μm (Table I). The number of the spinoids per 3 μm square was 101–108. The diameter of the germinal pore was 2.0–2.3 μm. The operculum was covered with granules bearing spinoids at the top (Fig. 1).

(5) *P. lanceolata*: The size of the pollen grains was 18.5–20.0–20.5 × 19.5–20.0–20.5 μm (Table I). The number of the spinoids per 3 μm square was 25–32. The diameter of the germinal pore was 2.1–2.7 μm. The operculum, which was surrounded with the fringe, was covered with many spinoids (Fig. 1).

(6) *P. virginica*: The size of the pollen grains was 22.0–22.5–24.0 × 22.0–23.0–28.0 μm (Table I). The number of the spinoids per 3 μm square was 65–70. The diameter of the germinal pore was 2.4–3.1 μm. The operculum was covered with spinoids which was similar to those on the pollen surface (Fig. 1).

Fig. 2. Pollen Grains from the Commercially Available "Shazenō"

A, Shazenō–(A); B, Shazenō–(B); C, Shazenō–(C); D, Shazenō–(D);
E, Shazenō–(E); F, Shazenō–(F).
a, whole view; b, germinal pore.
The pollen grains of the six specimens chosen were all of poly (ca. 10)-forate 4Cα type (Fig. 2). Each pollen grain was ornamented with tuberculatas, numerous spinoids scattering all over at regular intervals (about 0.4 μm), and many canals. The size of the pollen grains and the diameter of the germinal pore are shown at Table I. The operculum was covered with granules bearing spinoids at the top (Fig. 2).

8) “Shazenyōmatsu”: The pollen grains prepared from the two specimens chosen were all of poly (ca. 10)-forate 4Cα type (Fig. 3). Each pollen grain was ornamented with many tuberculatas, numerous spinoids scattering at regular intervals (about 0.4 μm), and with many canals. The size of the pollen grains and the diameter of the germinal pore are also shown at Table I. The operculum was covered with granules bearing spinoids at the top (Fig. 3).

3. Interior configuration of pollen grains

The pollen walls of the six species of genus Plantago were composed of tectum, columella, thin endexine, intine and non-continuous foot-layer, so called channeled nexine.13) Sexine presented tectate-imperforate structure (Fig. 4), and the thickness of pollen wall is as shown at Table II. The spinoid, which was 0.15 μm in length and 0.23 μm in diameter at base, was observed projecting on tectum. The surface of tectum was undulating at places. Canals penetrating the tectum were also seen in places. The pore membrane was composed of two layers, i.e., intine and the well-developed medine, on which operculum was placed bearing spinoid at the top of tectum. Operculum was quite similar to exine in structure, although it was smaller than exine in size (Fig. 5).

Results and Discussion

Pollen grains of the six species of genus Plantago were all of poly (ca. 10)-forate 4Cα type. The pattern comprises tuberculata-spinoid all over the surface, and many canals. The pollen grains were similar each other in dimension and pattern. Identification of each of the above species would be easy according to the practical electron microscopic examination on the pollen dimensions, diameter of the germinal pore, ornamentation of the operculum and the number of the spinoids per unit area. Thus, the pollen grain of P. asiatica was shown to have the size of 20.0-20.5 μm, germinal pore diameter of 3.3-3.4 μm, and 54-56 spinoids per 3 μm square. The ornamentation on the operculum was of granular type bearing spinoids at top. On the other hand, the pollen grains from “Shazensō” and “Shazenyōmatsu” were both bound to have the size of 19.5-20.3 × 20.0-20.6 μm, germinal pore diameter of 3.2-3.6 μm, and 53-58 spinoids per 3 μm square. The ornamentation on their operculum was of granular type bearing spinoids at top. These dimensions and characteristics were also seen on the pollen grains of

Fig. 4. Pollen Sections of Plantago spp.
A, P. asiatica; B, P. camtschatica; C, P. hakusanensis; D, P. japonica; E, P. lanceolata; F, P. virginica.
a, whole view; b, pollen wall; ca, canal; ch, channeled nexine; s, spinoid; t, tectum; c, columella; e, endexine; i, intine.

Fig. 5. Germinal Pore Membranes of the Pollen Grains of Plantago spp.
A, P. asiatica; B, P. camtschatica; C, P. lanceolata.
e, exine with spinoid; i, intine; m, medine; o, operculum.
**Table II. Interior Configurations of the Pollen Grains of Plantago spp.**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Tectum</th>
<th>Columella</th>
<th>Foot-layer</th>
<th>Endexine</th>
<th>Intine</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. asiatica</td>
<td>0.2-0.4</td>
<td>0.06-0.15</td>
<td>0.06-0.15</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>P. camtschatica</td>
<td>0.3-0.5</td>
<td>0.06-0.15</td>
<td>0.06-0.15</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>P. hakusanensis</td>
<td>0.3-0.5</td>
<td>0.06-0.15</td>
<td>0.06-0.15</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>P. japonica</td>
<td>0.2-0.3</td>
<td>0.06-0.15</td>
<td>0.06-0.15</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>P. lanceolata</td>
<td>0.2-0.3</td>
<td>0.06-0.15</td>
<td>0.06-0.15</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>P. virginica</td>
<td>0.2-0.4</td>
<td>0.06-0.15</td>
<td>0.06-0.15</td>
<td>0.03</td>
<td>0.3</td>
</tr>
</tbody>
</table>

unit: μm.

*P. asiatica*, which fact suggested that the original plant of commercially available “Shazensō” was *P. asiatica* and that the pollen grains contained in “Shazen'yōmatsu” in quantity was of those of *P. asiatica*.

Although operculum is seen only in *Cucurbita moschatula*,14) *C. maxima*,14) *Zea mays*,15) *Campanula persicifolia*16) and *Sorghum bicolor*,17) the ornamentations on their operculums are all similar to those on their pollen surfaces. However, the ornamentation on the operculum of the plants belonging to genus *Plantago* excepting *P. lanceolata* and *P. virginica* were apparently different from those on their pollen surfaces. Further microscopic observation of their pore membrane confirmed that the pollen grains of genus *Plantago* possessed operculum, and also that the pore was equivalent to exine in structure.

It is known10) that the pattern of ornamentation of the acetolysed pollen grains is clearer than that of the non-acetolysed. Thus, the pollen grains of *P. asiatica* was compared with the pollen grains that was acetolysed during the separation from “Shazen'yōmatsu”. However, no difference was observed between the ornamentation of acetolysed pollen grains and that of non-acetolysed pollen grains of *P. asiatica*. This is probably because the perine18) was dissolved into acetone, which was repeatedly used for the dehydration of the pollen grains after ultrasonic treatment.

Although various patterns of expressions were used on the ornamentation of the pollen grains from the plants of genus *Plantago* in the past, this was confirmed to be a pattern of tuberculata-spinoid as the result of the electron microscopic observation in this experiment.

It may thus be concluded that it would not be appropriate to distinguish each of the species of genus *Plantago* only from their interior configurations due to the marked resemblance of the structure of pollen wall of the six species of genus *Plantago* examined and also wide difference of thickness of tectum in individuals.

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18) J. Ueno, *Reports of Faculty of Science, Shizuoka University*, 1, 91 (1966).