Morphological Difference on Leaf Surface and Pollen Grains in Genus *Artemisia*

Carola LODARI, Kazumi HATTORI and Yuzo FUTSUHARA

Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, 464

The leaf surface of 12 *Artemisia* species was examined under scanning electron microscope in order to verify the possibility of identifying the species and the affinities among them through morphological features. Roundish glandular trichomes were found on the leaf surface of *A. princeps*, *A. montana*, *A. monophylla*, *A. keiskeana*, *A. absinthium*, *A. Genipi*. Big T-shaped trichomes were observed on the vein of *A. princeps* 'Okinawa' line. All the species had characteristic small T-shaped trichomes. The examination of the pollen morphology was done for 9 *Artemisia* species. All the species had tricolporate pollen grains with reduced exine ornamentation belonging either to the small or medium size class. The results of the investigation suggest the possibility of using new morphological parameters for botanical classification.

KEY WORDS: *Artemisia*, trichome, pollen morphology, scanning electron microscope, ultrastructure.

Introduction

Since very long ago the officinal plants are part of the sphere of the Oriental culture. In recent years, the renewed interest for the traditional medicine stimulated the research aimed scientific understanding of the materials used in the Kanpo method, that developed in Japan from the traditional medicinal science of China, and to the outlining of the possibilities of exploitation of these materials on the industrial scale. Some species belonging to the genus *Artemisia* are also to be found among the plants useful for the production of herbal remedies.

The genus *Artemisia* finds its center of origin in Eurasia and comprises about 280 species. It is widely distributed in the cold-temperate zone of the northern hemisphere and scarce in the tropical and subtropical zones or in the southern hemisphere. About 30 species are growing in Japan, some of which, being rare or insufficient in the natural environment are here cultivated due to the increased demand of raw vegetable material and alimentary purposes.

The genus *Artemisia* shows a great variety of forms and hybrids and therefore on the taxonomic level problems can sometimes be met with in the identification of the taxa. Traditionally, using the floral characters as parameters for the establishment of a natural classification, the genus has been divided into four sections, that is *Abrotanum*, with glabrous receptacle and central flowers fertile, *Absinthium*, with hairy receptacle and central flowers fertile, *Dracunculus*, with globose receptacle and central flowers sterile, *Seriphidium*, with glabrous receptacle and all flowers hermaphrodite. Some taxonomists now regard these sections as subgenera (KELSEY and SHAFIDAZEH, 1978, McARTHUR *et al.* 1981, LING, 1982). Attempts to classify the genus through karyological characteristics were also made but no relationship was recognized between the four taxonomical sections and the relative chromosome numbers. The species here investigated have following chromosome numbers: *A. princeps* Pamp., $2n=34$, *A. japonica* Thunb., $2n=36$, *A. keiskeana* Miq., $2n=18$, *A. stelleriana* Bess.,

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2n=18, *A. capillaris* Thunb., 2n=18, *A. maritima* L. ssp. *monogyna* Wall. et kit., 2n=18, 36, 54, *A. absinthium* L., 2n=18, *A. sinanensis* Yabe, 2n=18, *A. montana* (Nakai) Pamp., 2n=52, *A. schmidtiana* Maxim., 2n=18, *A. pedunculosa* Miq., 2n=18, *A. scoparia* Waldst. et Kit., 2n=16, 36, *A. Genipi* Web., 2n=18, 36, *A. monophylla* Kitam., 2n=unknown (ARANO, 1963, KAWATANI and OHNO, 1964, APPENDINO *et al.* 1982).

The morphological investigation on pollen grains represents an important step for the phylogenetic interpretation on the generic as well as on the specific level (ERDTMAN, 1972).

The present work intends to provide additional information concerning the morphological aspects of some *Artemisia* species growing in Japan and at the same time, keeping as a reference the traditional classification method, it is an attempt to find new parameters for the identification of the species. Therefore the investigation of the trichome type and structure and the pollen size, shape and surface carried out to verify the specificity of these characters and their usefulness in plant recognition was made into the main object of this research.

Materials and Methods

The morphological investigation was carried out on 13 *Artemisia* species native to or cultivated in Japan and one Italian species *A. Genipi*. The sample materials were supplied by the Toyama Medicinal and Pharmaceutical University or collected in the natural environment. *A. Genipi* was planted from seed in the greenhouses of Nagoya University.

The leaf surface examination was done for the following species: *A. princeps*, *A. japonica*, *A. keiskeana*, *A. stelleriana*, *A. capillaris*, *A. maritima* ssp. *monogyna*, *A. absinthium*, *A. monophylla*, *A. sinanensis*, *A. montana*, *A. schmidtiana*, *A. Genipi*. Within the species *A. princeps* wild lines from Nagoya, Izu and Okinawa were also examined.

Small portions of fresh leaves were fixed in 3% glutaraldehyde with 0.05M phosphate buffer (pH 7.2) for 24 hours at 4°C, dehydrated in acetone series and critical-point dried. After gold coating the specimen were observed under JSM F-7 scanning electron microscope (SEM).

For the investigation under light microscope the specimen were fixed in glutaraldehyde, dehydrated in alcohol series, embedded in basic resin with glycolmethacrylate. Then 5 micron thick sections were cut with rotary microtome and stained with toluidine blue.

The pollen investigation was carried out for the following species: *A. princeps*, *A. keiskeana*, *A. stelleriana*, *A. montana*, *A. pedunculosa*, *A. absinthium*, *A. japonica*, *A. capillaris*, *A. scoparia*. Florets were collected from the fresh plants and kept in dessicator glass for several weeks. The pollen grains were then sampled, gold coated and observed SEM.

Results

In the microscopical study of the single trichome type of the leaves a primary distinction was made between glandular and non-glandular trichomes.

Glandular trichomes in relation with secretion of sesquiterpene lactone were observed on both leaf surfaces of *A. princeps* (Plate 1. A.) and its lines, *A. montana*, *A. monophylla*, *A. keiskeana*, *A. absinthium* and *A. Genipi*. These appendages generally arising from a small

depression of the epidermis consist of a foot supporting a large roundish multicellular head. *A. princeps*'s glandular hairs showed the biggest and most differentiated structure, the other species having slightly smaller sized hairs. Glandular trichomes were present on young and old leaves as well as on plant organs different from the leaves. In particular, the investigation of the floral region of *A. princeps* showed a high concentration of small glandular trichomes on the head flower bracts and florets. In *A. Genipi* a high density of these trichomes was also found on the surface of the seeds.

Beside the glandular trichomes in some species, in all the *Artemisia* species observed another trichome type was found that can be called T-shaped trichome for its particular structure consisting of a stalk projecting from a foot cell in the epidermis and branched in two long ramifications. The investigation of the T-shaped trichomes showed how these trichomes are characteristic for each species although in some cases similarities exist that permit their grouping. *A. princeps* (Plate 1. B.), *A. montana*, *A. monophylla*, *A. stelleriana* showed the biggest trichome type that has a long slender stalk, 3, 4-celled with the upper part branched in two elongated arms. The ramification appears to be a ribbon-like structure tightly rolled up, especially towards the tips, and quite twisted.

T-shaped trichomes with slender 2, 3-celled stalks were found in *A. absinthium* (Plate 1. C.) and *A. maritima*, yet the arms showed a different conformation being like an unrolled ribbon longitudinally oriented in *A. absinthium* and, on the other hand, twisted in *A. maritima*.

A. capillaris (Plate 1. D.), *A. keiskeana*, *A. schmidtiana* had 2, 3-celled stalked trichomes with an enlarged basal cell of the stalk. The arms of *A. capillaris* and *A. schmidtiana* showed some collective tendency for the longitudinal orientation.

Very short-stalked trichomes with 1 or 2 basal cells were found in *A. japonica* (Plate 1. E.), *A. sinanensis* and *A. Genipi*. In the latter species the ramifications were also longitudinally oriented and could show a great difference in length.

In all the *A. princeps*'s lines surveyed similar T-shaped trichomes were present. Yet in the Okinawa line another kind of trichome was observed in the region of veins (Plate 1. F.). These trichomes were also T-shaped but were remarkably bigger than the previous ones since they had a very long stalk consisting of several, sometimes more than five, elongated cells that became progressively smaller in the distal part.

The microscopical investigation of the pollen of the nine *Artemisia* species observed showed that the grains of all the specimen had three longitudinal equatorial apertures, oblate or sub-spheroidal shape and belonged to the size class of either the small pollen grains or the medium size pollen grains. The exine layer was scarcely sculptured, the ornamentation being characterized by the presence of granules and spinules of very short length, less than one micron.

The pollen grains of *A. stelleriana* were the biggest, being about 29 micron in length and therefore falling within the medium size class together with *A. princeps*, *A. keiskeana*, *A. absinthium*. The remaining species, *A. montana*, *A. pedunculosa*, *A. japonica*, *A. capillaris* and *A. scoparia*, had pollen grain length inferior to 25 micron thus falling into the small size class. The shortest pollen grain length, around 17 micron, was found for the pollen grains

of *A. capillaris* and *A. scoparia*, two species that showed almost identical pollen grains. Among the species of the medium size class *A. princeps* had the thinnest pollen grains with a breadth of indicatively 15.5 micronb (Plate 2.).

From polar view all the pollen grains appeared fossaperturate and in *A. montana* and *A. pedunculosa* the mesocolpia were more prominent.

The superficial ornamentation, although basically similar for all the specimen, also showed some slight intraspecific differences being, for instance, the longest spinules those of *A. keiskeana*. Furthermore in *A. princeps* it was observed that the pollen grains at different maturation stages gradually change from an oblong into a spheroidal shape. At this latter stage the operculum becomes clearly visible on the aperture region. For all the species it was noticed that the pollen tends to form only small aggregations of pollen grains.

Discussions

The microscopical investigation of the leaf surface could detect altogether the presence of three main trichome types, namely the glandular trichomes, the T-shaped trichomes and the big T-shaped trichomes of the vascular region. Each *Artemisia* species observed showed to have characteristic T-shaped trichomes differing from the other species in shape, size and orientation of the arms. It is therefore believed that the fine structure of the single trichome can give unambiguous information about the identity of one species and about the affinities possibly existing among the different species.

There were many works about pollen morphology. MAAS (1977) reported that pollen ultrastructures of strawberry appeared to correspond with ploidy level. In the genus *Mimulus* (ARGUE, 1980), *Ambrosia* (ROBBINS, *et al.* 1979) and *Citrus* (KOZAKI and HIRAI, 1986), the morphological data of pollen was useful for characterizing each species. Moreover, in the olive (PACINI and VOSA, 1979) and Japanese pear (MATSUTA, *et al.* 1986), every cultivar possessed its own morphological characteristics.

The investigation of the pollen showed, as expected, great homogeneity among the species. Yet the overall appearance of the pollen grains due to their variation in size and hsape, more than the ornamentation pattern of the pollen surface, proves to be useful for the recognition of the plants on the specific and generic levels as well.

The observations carried out on the trichomes and the pollen have thus provided new morphological data that can be of practical advantage not only for mere taxonomic purposes but also, for instance, for drug control analyses or allergenic pollen studies. The comparative examination of the morphological data could also contribute to clarifying the phylogenetic relationship among the species. So far, although high morphological similarities were observed among some species belonging to the same section, a clear correlation between the trichome and pollen traits and the traditional systematic sections could not be found due to the frequent overlapping in the other sections.

In the wide *Artemisia* genus the chromosome number of about 150 species is now known.

Karyotype analyses were undertaken by ARANO (1963) to find out the possibility to classify the genus through karyological characteristics. Studies, on a larger scale, by KAWATANI and OHNO (1964) stated the existence of seven groups of chromosome numbers, that is

Plate 1 (Bars indicate 100 microns)

A—Glandular trichome of *A. princeps*

B—T-shaped trichome of *A. princeps*

C—T-shaped trichome of *A. absinthium*

D—T-shaped trichome of *A. capillaris*

E—T-shaped trichome of *A. japonica*

F—Big T-shaped trichome on vascular region of *A. princeps*

'Okinawa' line

Plate 2 (Bars indicate 10microns)

A—Pollen of *A. princeps*

B—Frontal view of pollen of *A. princeps*

C—Equatorial view of pollen of *A. keiskeana*

D—Equatorial view of pollen of *A. capillaris*

E—Equatorial view of pollen of *A. montana*

F—Pollen surface of *A. montana*