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Phylogenetic implication of seed coat sculpturing in subtribe Agrimoniinae (Rosaceae)

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장미과 짚신나물아족 종피형태의 계통분류학적 고찰

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ABSTRACT: Seed morphology in the subtribe Agrimoniinae (Rosaceae) was examined using scanning electron microscopy to identify distinct characters and evaluate their evolution in a phylogenetic framework for five genera in the subtribe: *Agrimonia* L., *Aremonia* Neck. ex Nestl., *Hagenia* J.F. Gmel., *Leucosidea* Eckl. & Zeyh., and *Spenceria* Trimen. All genera have one or two mature achenes in a fruiting hypanthium. In the seed coats, the cell shape, size, wall features, and sculpturing vary across genera. Of most significance is the presence of papillae structures in both *Agrimonia* and *Aremonia.* Through the mapping of papillae features onto phylogenetic trees, either one or two changes in seed coats are hypothesized. The phylogenetic tree inferred from four nuclear and six chloroplast regions of sequence data suggests that at least two steps of papillae sculpturing on seed coats are required. On the other hand, in the phylogenetic tree of a low-copy nuclear gene, one independent evolutionary step is postulated to explain the current character states. In the latter hypothesis, the seed coat sculpturing also supports a monophyletic relationship for cosmopolitan *Agrimonia* and European endemic *Aremonia*. The seed coat sculpturing provides valuable information for inferring phylogenetic relationships at the generic level in the subtribe Agrimoniinae.

Keywords: *Agrimonia*, Agrimoniinae, *Aremonia*, *Hagenia*, *Leucosidea*, seed coat, *Spenceria*

적 요: 장미과 Agrimoniinae(짚신나물아족)의 5속(*Agrimonia* L., *Aremonia* Neck. ex Nestl., *Hagenia* J.F. Gmel., *Leucosidea* Eckl. & Zeyh., and *Spenceria* Trimen.)의 종피를 주사전자현미경으로 관찰하여 계통분류학적으로 유용한 형질이 있는지 조사하였다. 또한, 관찰된 종피의 형질들을 이미 수행된 분자계통학적 연구에서 제시된 5속의 계통분류학적 관계를 설명하는 가설들에 적용하여 종피 형질의 계통분류학적 진화를 고찰하였다. 짚신나물아족의 5속 모두 하나의 열매화통에 하나 또는 두 개의 성숙한 수과를 가지고 있었고, 종피는 표피세포의 모양, 크기, 세포벽의 돌출 정도, 세포표면의 돌기의 유무 등에서 다양한 형질상태가 관찰되었다. 특히, 세포표면의 유두상 돌기(papillae)는 2속 *Agrimonia*(짚신나물속)과 *Aremonia*에서만 관찰되었다. 유두상 돌기가 없는 것이 원시형질이라는 가정하에 유두상 돌기가 나타나는 형질변화를 이미 수행된 분자계

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통학적 연구에서 고찰하였다. 4개의 핵과 6개의 엽록체 DNA의 염기서열에 기초한 계통수에서는 적어도 2회의 형질변화가 요구되며, 저복사수 핵 유전자의 염기서열 계통분석의 계통수에서는 단 1회의 형질변화가 일어나는 것으로 나타났다. 종합하면, 종피의 유두상 돌기의 출현은 *Agrimonia*(짚신나물속)과 *Aremonia* 을 단일계통 분류군으로 설명하는 공통진화형질이라고 할 수 있고 이러한 가설은 저복사수 유전자의 염기서열의 계통분석을 지지하고 있다. 이렇게 종피 형질은 장미과 Agrimoniinae(짚신나물아족)의 속간의 계통분류학적 이해에 매우 유용한 형질임을 밝힌다.

주요어: 짚신나물, 짚신나물아족, *Aremonia*, *Hagenia*, *Leucosidea*, 종피, *Spenceria*

Subtribe Agrimoniinae (Rosaceae) comprises four narrowly endemic and monotypic genera (*Aremonia* Neck. ex Nestl., *Hagenia* J.F. Gmel., *Leucosidea* Eckl. & Zeyh., *Spenceria* Trimen) and one widely distributed, speciose genus (*Agrimonia* L.) (Kalkman, 2004; Potter et al., 2007). Most species in the subtribe occur in temperate regions and exhibit dynamic and variable features despite its small size (ca. 25 species) (Kalkman, 2004; Chung et al., 2010). The plants vary in growth form (herbaceous, woody), inflorescence type (racemes, panicles), floral structure (bisexual, unisexual), and pollen morphology (Table 1; Kalkman, 2004; Chung et al., 2010). Moreover, the species display different levels of ploidy (2*x*, 4*x*, 6*x*, and 8*x*), that correlate with geographic distribution patterns (Chung, 2008). These variable characters within such a small taxonomic group provide a great opportunity to study their dynamic evolutionary patterns in a phylogenetic framework.

Subtribe Agrimoniinae belongs to tribe Sanguisorbeae in subfamily Rosoideae, and variable reproductive characters have provided hypotheses regarding the classification and phylogenetic relationships among genera in Rosaceae (Kalkman, 1988; Morgan et al., 1994; Potter et al., 2002; Chung et al., 2010). For example, traditional subfamily classification was established based on a combination of basic chromosome numbers and fruit types: Rosoideae (x = 7 (8, 9); achenes, drupelets), Amygdaloideae (x = 8; drupes), Spiraeoideae (x = 9 (17); follicles, capsules), and Maloideae (x = 17; pomes). In addition, variable pollen sculpturing patterns provided useful information for infra-sectional classification. Hebda and Chinnappa (1994) proposed reclassification in the tribes Dryadeae, Potentilleae, and Sanguisorbeae based on striate, microperforate pollen grains, while Chung et al. (2010) and Lee et al. (2011) postulated inter/infra-generic relationships inferred by pollen sculpturing features in the tribe Sanguisorbeae.

In flowering plants, seed morphology has provided useful features for delineating infra-generic classification (Wyatt 1984; Buss et al., 2001; An and Hong, 2003; Hassan et al., 2005; Minuto et al., 2006; Muñoz-Centeno et al., 2006; Jacobs et al., 2008; Jin and Park, 2008; Kong et al., 2011), and the great variability of seed surface characters has the potential to explain phylogenetic relationships (Juan et al., 2000; Muñoz-Centeno et al., 2006). Buss et al. (2001) revealed close phylogenetic relationships between *Centropogon* and *Siphocampylus*; *Legenere* and *Downingia*; and *Hippobroma* and *Lobelia* sect. *Tylomium* based on their seed morphologies. The relationships were further supported by the geographic distribution of each group. In the rose family, although diverse fruit types have received much attention from botanists (Kalkman, 1988; Morgan et al., 1994), only a few studies on seed morphology have been conducted. For example, Latif (2004) found that analyses of seed coat patterns using scanning electron microscopy (SEM) are useful to distinguish species within the taxonomically ambiguous genus *Sanguisorba*.

In order to characterize the seed morphology of the genera in Agrimoniinae and utilize it for classification and systematics, we investigated all five genera in the subtribe using SEM. Seed morphological characters were mapped onto the phylogenetic

Table 1. Major characters of subtribe Agrimoniinae (Kalkman, 2004; Chung et al., 2010).

Genus (No. of species)	Habit	Inflorescence	Flower sexuality	Ploidy level	Pollen Exine pattern	Geographic distribution
Agrimonia (19)	Herb	Raceme	Bisexual	4*x*, 6*x*, 8*x*	Striate	Temperate N. & S. America, Eurasia
Aremonia (1)	Herb	Raceme	Bisexual	6*x*	Striate	Central Europe
Hagenia (1)	Tree	Panicle	Unisexual/ Dioecious	6*x*	Microverrucate	Central Africa
Leucosidea (1)	Shrub	Raceme	Bisexual	2*x*	Striate	Southern Africa
Spenceria (1)	Herb	Raceme	Bisexual	2*x*	Striate	Central Asia

trees constructed in previous studies (Potter et al., 2007; Chung, 2008). Evolution of seed characters in a phylogenetic framework will contribute to an understanding of character evolution in the subtribe.

Materials and Methods

Fifteen species from five genera in Agrimoniinae were sampled: *Agrimonia* L. (11 species) *Hagenia abyssinica* J.F. Gmel. (1 species), *Leucosidea sericea* Eckl. & Zeyh. (1 species), and *Spenceria ramalana* Trimen (1 species) (Table 2). Achenes were removed from hypanthia and pericarps were peeled off from the achenes under stereomicroscopy. Dried seeds are then mounted on aluminum stubs using double-sided conductive tape. The seeds were then coated with 8 nm of gold/palladium and examined in a Hitachi 2460N SEM at 25 kV, with a 20 mm working distance and consistent spot size. All SEM sample preparations and observations were made at the University of Oklahoma Samuel Noble Electron Microscopy Laboratory.

Results

All genera have one or two mature achenes in a fruiting hypanthium; and all seeds are circular on the top and bottom views, and pentagon-like with converged, pointed tips on the side views (Fig. 1A, B, C). The cell shape, size, and wall features are variable across genera and species. In particular,

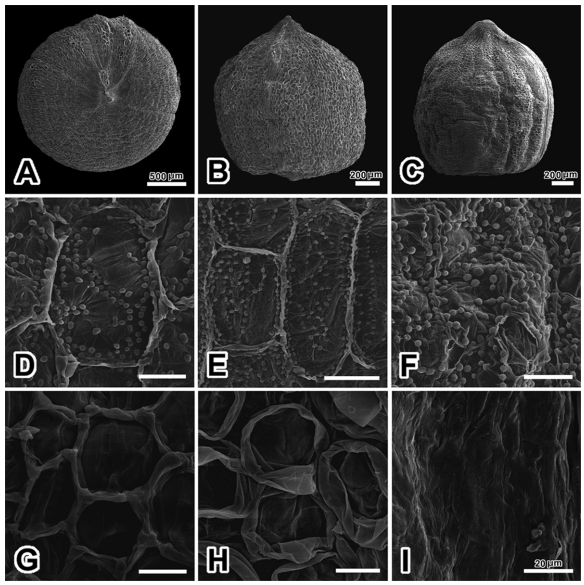

Fig. 1. SEM photos of seed in Agrimoniinae. A. *Agrimonia coreana* (*Chung 2007-5*, OKL), top view; B. *A. rostellata* (*Chung 20*, OKL), side view; C. *Hagenia abyssinica* (*J. W. Ash 1388*, MO), side view; D-I. seed sculpturing. D. *Agrimonia coreana* (*Chung 2007-5*, OKL); E. *A. procera* (*A. K. Skvortsov s.n.*, GH); F. *Aremonia agrimonoides* (*G. Gigo s.n.*, DAO); G. *Hagenia abyssinica* (*J. W. Ash 1388*, MO); H. *Leucosidea sericea* (*Balkwill & Balkwill 9343*, MO); I. *Spenceria ramalana* (*C. Schneider 3316*, A). Scale bars D-I = 20 μm.

Table 2. Seed coat features in the subtribe Agrimoniina studied.

Taxon	Cell shape	Cell size (length × width, μm)	Cell wall height (μm)	Papillae sculpturing	Voucher specimen (Herbarium)
Agrimonia L.	Polygonal	ca. 20-30 × 20-70	ca. 2-4	Present	*Agrimonia coreana* Nakai, Korea, *Chung 2007-5* (OKL); *A. eupatoria* L., *Georgia, M. Merello et al. 2035* (MO); *A. gryposepala* Wallr., USA, *Chung 39* (OKL); *A. microcarpa* Wallr., USA, *Nelson 26674* (SUCH); *A. nipponica* Koidz., Korea, *Chung 2007-9* (OKL); *A. parviflora* Aiton, USA, *Chung 31* (OKL); *A. pilosa* Ledeb., Japan, *Chung 2007-7 (*OKL); *A. pubescens* Wallr., USA, *Chung 18* (OKL); *A. procera* Wallr., *France, A. K. Skvortsov s.n.*, (GH); *A. rostellata* Wallr., USA, *Chung 20* (OKL); *A. striata* Michx., USA, *Chung 1* (OKL)
Aremonia agrimonoides (L.) DC.	Polygonal	ca. 20-30 × 20-70	ca. 1-2	Present	Switzerland, *G. Gigo s.n.*, (DAO)
Hagenia abyssinica J.F. Gmel.	Polygonal-Circular	ca. 20-30 × 20-30	ca. 2-3	Absent	Tanzania, *J. W. Ash 1388* (MO)
Leucosidea sericea Eckl. & Zeyh.	Circular	ca. 20 × 20	ca. 5-15	Absent	South Africa, *Balkwill & Balkwill 9343* (MO)
Spenceria ramalana Trimen	-	-	-	Absent	China, *C. Schneider 3316* (A)

the seed coat sculpturing is greatly variable among genera (Table 2).

***Agrimonia* L.:** Eleven species were observed in the genus: *A. coreana* (Asia), *A. eupatoria*, (Europe)), *A. gryposepala* (North America), *A. microcarpa* (North America), *A. nipponica* (Asia), *A. parviflora* (North America), *A. pilosa* (Eurasia), *A. procera* (Europe), *A. pubescens* (North America), *A. rostellata* (North America), *A. striata* (North America). All species of the genus have seeds with polygonal, reticulum-shaped cells (ca. 20-30 × 20-70 μm) with protruded cell walls (ca. 2-4 μm high) and prominent papillae structures throughout the cell bodies (ca. 1-2 μm in diameter) (Fig. 1D, E).

***Aremonia* Neck. ex Nestl.:** *A. agrimoniides* was observed from the European monotypic genus *Aremonia*. Its seed shape is similar to *Agrimonia* except having a flattened top. Like that of *Agrimonia*, the seed coat of *A. agrimoniides* is composed of polygonal, reticulum-shaped cells (ca. 20-30 × 20-70 μm) with cell wall protrusions (ca. 1-2 μm high) shorter than those in *Agrimonia*. *A. agrimoniides* also exhibits papillae structures throughout the cell bodies (ca. 1-2 μm in diameter) (Fig. 1F).

***Hagenia* J.F. Gmel.:** *H. abyssinica* in this African genus. The seed shape of *H. abyssinica* is similar to *Agrimonia* (Fig. 1C), but its cell shape and sculpturing are greatly different from the features observed in the other taxa. Some cells are polygonal, but most of them are more circular than reticulum (ca. 20-30 × 20-30 μm) with thicker and more protruded walls (ca. 2-3 μm high). Papillae structures are absent on cell surfaces (Fig. 1G).

***Leucosidea* Eckl. & Zeyh.:** The other African monotypic genus, *L. sericea*, was also investigated. Overall the seed characteristics (seed and cell size, wall, and sculpturing patterns) of *Leucosidea* overlap those of the other African genus, *Hagenia* (Fig. 1G vs H). However, cells in *Leucosidea* are more circular and smaller in size than those in *Hagenia* (ca. 20 × 20 μm). The thinner cell walls are taller (ca. 5-15 μm high) although they may appear pressed (Fig. 1H). As in *Hagenia*, cells on the surface of *Leucosidea* seeds lack papillae structures (Fig. 1H).

***Spenceria* Trimen:** *Spenceria* is a monotypic genus with only one Asian endemic species, *S. ramalana*. The cell shape of *Spenceria* seeds is not evident since their cell walls are greatly pressed with a relatively smooth surface possibly suggesting that cell walls were not well developed. The cell surfaces likewise do not exhibit any papillae structures (Fig. 1I).

Discussion

The seed coat surfaces of taxa within the subtribe Agrimoniinae exhibit variability in cell size, shape, wall features, and sculpturing patterns. Although the levels of polyploidy typically exhibit positive correlation with cell sizes in flowering plants, that is polyploidy with higher ploidy levels has larger-sized cells (Stebbins, 1971), the correlation is not found within the genus *Agrimonia* (tetraploidy, 4*x*: *A. coreana*, *A. eupatoria*, *A. microcarpa*, *A. nipponica*, *A. parviflora*, *A. rostellata*; octoploidy, 8*x*: *A. gryposepala*, A. *pilosa*, *A. procera, A. pubescens*, *A. striata*) nor within the subtribe. Although the diploid genus *Leucosidea* (2*x*), an endemic African genus, exhibits smaller cells than those in tetraploid and octoploid *Agrimonia* (4*x* and 8*x*), the cell size of the hexaploid *Hagenia* (6*x*), another endemic African genus, is similar to the cell size of *Leucosidea* (Fig. 1). Since the strong correlation between ploidy levels and fruit size (the higher ploidy levels, the larger fruits) or distribution patterns has been

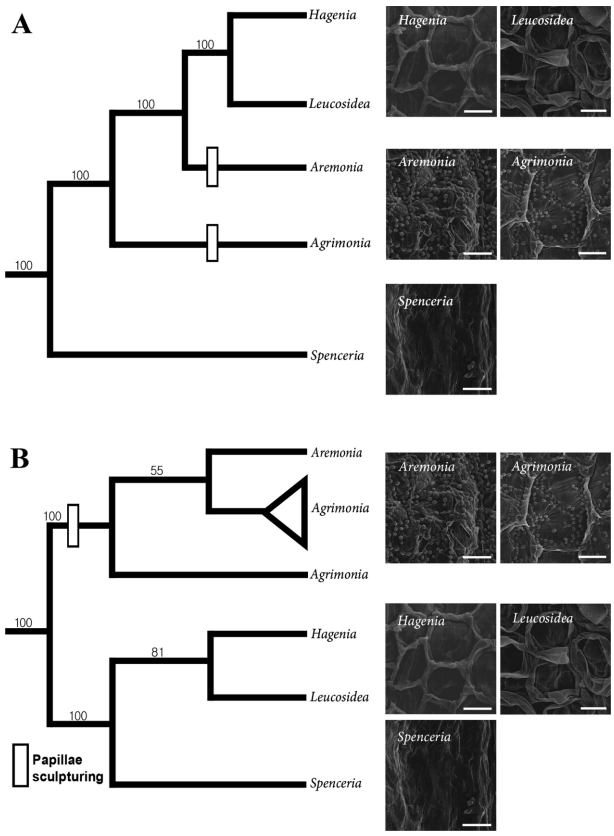

Fig. 2. Alternative hypotheses on seed coat sculpturing in Agrimoniinae. A. Phylogentic tree is adopted from Potter et al. (2007). In this tree, at least two changes of papillae sculpturing on seed coat are required; B. In the phylogentic tree of the low-copy nuclear gene, WAXY data (Chung, 2008), one evolutionary event on the seed coat sculpturing is necessary to explain current character states. Numbers above the branches Bayesian clade credibility, and all the SEM photos from the same vouchers as in Fig. 1. Scale bars = 20 μm.

detected in the genus *Agrimonia* (Chung, 2008), a more detailed study on cytological and morphological features is needed to understand the consecutive changes in cellular formation and structuring within the genus.

Of most significance is the presence of papillae structures in both *Agrimonia* and *Aremonia,* which is a key feature in distinguishing them from the other genera in Agrimoniinae. When the seed sculpturing characters are mapped on the phylogenetic trees previously reported (Potter et al., 2007; Chung, 2008), two alternative hypotheses on seed coat sculpturing in Agrimoniinae are postulated (Fig. 2). In the phylogenetic tree inferred from four nuclear and six chloroplast regions of sequence data (Potter et al., 2007), at least two changes of papillae sculpturing on seed coats are required. On the other hand, in the phylogenetic tree of the low-copy nuclear gene, WAXY data (Chung, 2008), one evolutionary step is required to explain the current character states. In the phylogenetic tree inferred from WAXY data, the European monotypic genus *Aremonia* forms a clade with all *Agrimonia* species, and the seed coat sculpturing supports the monophyly of *Agrimonia* and *Aremonia* (Chung, 2008). Under the assumption of papillae structures derived, the seed coat character is a synapomorphy for the *Agrimonia* + *Aremonia* clade.

A more detailed morphological study on seeds, especially an anatomical study, will reveal more informative characters for a better and more thorough understanding of this biologically diverse lineage (see Oh et al., 1993; Shepherd et al., 2005; Heo and Suh, 2008; Fagúndez et al., 2010; Jung et al., 2010).

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