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## A Contribution to the Taxonomy of Four Taxa of *Sanguisorba* (*Rosoideae-Rosaceae*)

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**Abstract:** Macromorphological characters, SEM of seed coat surface criteria, seed coat anatomy and seed protein electrophoresis aspects, were used to re-assess the taxonomic relationships between four taxa of *Sanguisorba* L. The characters were analyzed by using SPSS Division 10. The dendrograms produced were discussed and showed a close relationship between *Sanguisorba minor* (L.) Scop and *Sanguisorba muricata* (Spach) Greml. Greml.

**Key words:** *Rosaceae*, *Sanguisorba*, SEM, seed protein, anatomy

### INTRODUCTION

The *Rosaceae* is a large sub-cosmopolitan family of about 95 genera and 2825 species. It is located mainly in the temperate and warm areas of the northern hemisphere<sup>[1]</sup>. However, Heywood<sup>[2]</sup> stated that the family consists of 122 genera and 3370 species.

The classification of the *Rosaceae* itself, raises many problems. Opinions differ as to the relation of the family with other families, the delimitation of its subfamilies, tribes, genera and even species<sup>[2]</sup>. The classification of the *Rosaceae* into its minor categories was first achieved by Focke<sup>[3]</sup>. Today, only four of Focke's subfamilies are recognized, these are: *Prunoideae*, *Rosoideae*, *Spiraeoideae* and *Maloideae*. The phylogeny of the *Rosaceae* was treated by Kalkman<sup>[4]</sup>.

*Sanguisorba* L. (*Rosoideae*) is a small natural genus of about 10 species, that are present mainly at the temperate zones of the Northern Hemisphere<sup>[1]</sup>.

However, the delimitation of this genus as regarding its minor categories has been problematic. Briq<sup>[5]</sup> stated that *Sanguisorba muricata* can be considered as a subspecies of *Sanguisorba minor*, while Greml<sup>[6]</sup> retained it as a distinct taxon.

The significance of seed structure in taxonomic and phylogenetic studies has been emphasised by many authors<sup>[7-12]</sup>. SEM of seed coat surface is useful in the identification and classification of various taxa<sup>[13-15]</sup>. A comparison of surface scan patterns of the seed coat has efficiently been used in studying species of some genera including *Vigna*<sup>[16]</sup>, the *Abutilaeae*<sup>[17]</sup>, the *Vicieae*<sup>[18]</sup> and *Ranuncul*<sup>[32]</sup>.

On the other hand, seed proteins are highly stable, being unaffected by environmental conditions<sup>[19]</sup>. Thus polyacrylamide gel electrophoresis in the presence of

sodium dodecyl sulfate (SDS-PAGE) have provided a valid source of taxonomic evidence and were used to address taxonomic relationships at the generic and specific levels, for example *Vigna*<sup>[20]</sup>, *Phaseolus*<sup>[21]</sup>, *Sesbania*<sup>[22]</sup> and *Nigella*<sup>[23]</sup>.

The present study aims at using seed characters (macromorphological and micromorphological ones) including seed coat anatomy and SEM of seed coat surface, together with characters from vegetative morphology, seed storage protein profiles and numerical taxonomic methods to help in clarifying, delimiting and re-assessing the relations between 4 taxa of *Sanguisorba* viz., *S. minor* (L.) Scop, *S. muricata* (Spach) Greml, *S. officinalis* L. and *S. tenuifolia* Fisch.ex Link.

### MATERIALS AND METHODS

Seeds of the examined species and their sources are listed in Table 1. Macromorphological aspects were collected from relevant literature<sup>[1,24-28]</sup>.

The detailed morphological features of the seeds were examined by SEM using different magnifications. The seeds were mounted with colloidal silver or carbon on copper stubs and coated with a thin layer of gold in polaron E 5000 sputter coater. The specimens were then examined by a JEOL scanning microscope (SEM) at the National Research Center Dokki, Cairo. The magnification power was expressed by (x) for each SEM photograph.

In addition, transverse sections were made in the seed coats of the studied taxa by a hand microtome at 15-20 U at the Faculty of Science-Ain Sahms University. Description and terminology presented by Corner<sup>[10]</sup> has been used to study the anatomical features of the seed coat sections.

For SDS-PAGE electrophoresis, three replicates of 0.1 g of seeds were mixed, each with an equal weight of

Table 1: Taxa studied and their sources

Taxon	Source
A) <i>Sanguisorba officinalis</i> L.	Ort.
B) <i>Sanguisorba minor</i> (L.) Scop.	Sw.
C) <i>Sanguisorba muricata</i> (Spach) Greml.	Sw.
D) <i>Sanguisorba tenuifolia</i> Fisch. ex Link.	Jap.
(Ort.): Orto Botanico D'ElI Universita Dipadova 35100 Padova, (Italy)	
(Jap.): Tsukuba Medicinal Plant Research Station National Institute of Health Sciences I Hachimandai, Tsukuba, IBARAKI, 305 (Japan)	
(Sw.): Botanischer Garten der universität Zurich Zolli ker strasse 107 CH -8008 Zurich, (Switzerland)	

pure, clean, sterile fine sand and powdered using mortar and pestle. Extraction of proteins was carried out using Tris-EDTA Extraction, Buffer (pH<sub>8</sub>).

The slurry was boiled for eight minutes and centrifuged at 12.000 rpm for 20 min. The supernatant was kept at -20°C until use.

For Tris-EDTA extracted proteins, electrophoresis was run in 15% gel concentration, but in Tris-Glycine running buffer (pH<sub>8.3</sub>) at 150 Volts for about 3-4 h. Using a low molecular weight protein of Sigma as a marker in the run. Gel was then stained in Comassie prelliant blue R-250 for 30 min destined. The banding profile of the examined species was photographed. The number of bands was scored and the recording data was computerized and analyzed by Gel Work I/D advanced soft ware, UVP Corbiration England at the Molecular Cytogentic Lab., Department of Genetic, Faculty of Agriculture, Ain Shams University.

For the data analysis, the total number of the recorded characters (57), were scored, combined together in four sets of data and coded for creating the data matrix of computation all characters combined.

The relationships between the taxa studied, expressed by average taxonomic linkage, have been demonstrated as phenogram, based on the analysis of the recorded characters using SPSS Diversions 10.

## RESULTS AND DISCUSSION

### Selected macromorphological features of the studied taxa

***Sanguisorba officinalis* L.:** Habit; Herb, leaf compound pinnate, ovate to lanceolate, leaf margin toothed, leaf apex acute, number of sepals 4, number of petals 0, stamin number many, ovary number 1-3 and fruit achene.

***Sanguisorba minor* (L.) Scop:** Habit; herb, leaf compound pinnate, round to oblong, leaf margin toothed, leaf apex

notched, number of sepals 4, number of petals 0, stamin number many, Ovary number 1-3 and fruit achene.

***Sanguisorba muricata* (Spach) Greml:** Habit; herb, leaf compound pinnate, round to oblong, leaf margin toothed, leaf apex acute, number of sepals 4, number of petals 0, stamin number many, ovary number 1-3 and fruit achene.

***Sanguisorba tenuifolia* Fisch.ex Link:** Habit; herb, leaf compound pinnate, lanceolate, leaf margin toothed, leaf apex notched, number of sepals 4, number of petals 0, stamin number many, over number 1-3 and fruit achene.

### Anatomical aspects of the seed coat of the studied taxa *Sanguisorba officinalis* L.

**Testa:** Outer epidermal cells ill-defined, some possess thick conical shaped hairs. Mesophyll is several layers of square shaped cells, the inner layer is highly lignified and shows brown pigmentation.

### *Sanguisorba minor* (L.) Scop

**Testa:** Outer epidermal cells ill-defined, some possess thick conical shaped hairs. Mesophyll is several layers of lignified square shaped cells with brown pigmentation.

### *Sanguisorba muricata* (Spach) Greml

**Testa:** Outer epidermal cells are ill-defined, some possess short conical hairs. Mesophyll is 4 layers of highly lignified square shaped cells with brown pigmentation.

### *Sanguisorba tenuifolia* Fisch.ex Link

**Testa:** Outer epidermal cells are illdefined, some possess short dome shaped hairs. Mesophyll is several layers of square shaped cells, the inner layer is highly lignified with brown pigmentation.

### The seed coat ultrastructure

***Sanguisorba officinalis* L.:** The overall seed coat pattern is reticulate to ruminant. The epidermal cells are irregularly shaped and polymorphic. Anticlinal walls are wavy, very thick, highly raised and highly striated. Periclinal walls are concave and highly striated. Long pointed tubercular hairs are seen on certain locations. (Fig. 1)

***Sanguisorba minor* (L.) Scop:** The overall seed coat pattern is irregularly reticulate. The epidermal cells are irregularly shaped and polymorphic. Anticlinal walls are slightly wavy, very thick, highly raised and highly striated. Periclinal walls are concave and striated. Medium sized conically shaped hair like structures are seen on some parts of the achene. (Fig. 1)

Table 2: Total characters and their codes used in the numerical analysis

No	Characters		A	B	C	D	
<b>Morphological characters</b>							
1	General characteristics	Habit	Herb	1	1	1	1
2			Tree	0	0	0	0
3	Leaf characteristics	Leaf blade	Type compound pinnate	1	1	1	1
4			Round-oblong	0	1	1	0
5		Shape	Lanceolate	0	0	0	1
6			Ovate to lanceolate	1	0	0	0
7		Leaf margin	Toothed	1	1	1	1
8		Leaf apex	Acute	1	1	1	0
9			Notched	0	1	0	1
10	Floral characters	No. Sepals	4	1	1	1	1
11		No. Petals	0	1	1	1	1
12		Stamen No.	many	1	1	1	1
13		Ovary No.	1-3	1	1	1	1
14	Fruit characters	Achene		1	1	1	1
<b>Seed coat anatomy (TS)</b>							
15	Testa	Outer epid. hairs	Conical	1	1	0	0
16			Short conical	0	0	1	0
17			Dome shaped	0	0	0	1
18		Mesophyll	lignified	0	1	1	0
19			Inner layer lignified	1	0	0	1
20		Number of layer of cells	4 Layers	0	0	1	0
21			Several	1	1	0	1
<b>Seed coat scanning</b>							
22	Overall seed coat pattern	Irregularly reticulate		0	1	0	0
23		Reticulate to ruminant		1	0	0	0
24		Reticulate to honey combed		0	0	1	0
25		Reticulate to favularite		0	0	0	1
26	Epidermal cells	Shape	Irregularly	1	1	0	1
27		Monohexagonal to irregularly		0	0	1	0
28		Size	Polymorphic	1	1	0	0
29			Monomorphic	0	0	1	1
30		Diagnostic features	Conically shaped hair like str.	0	1	1	0
31			Presence of long tubercular hairs	1	0	0	0
32			Presence of dense irregularly hairs	0	0	0	1
33	Anticlinal walls	Undulation	Slightly wavy	0	1	1	1
34			Wavy	1	0	0	0
35		Thickness	Very thick	1	1	1	1
36		Height	Highly raised	1	1	1	1
37		Texture	Highly striated	1	1	1	1
38	Periclinal wall	Surface	Concave	1	1	1	0
39			Flat	0	0	0	1
40		Texture	Highly striated	1	0	0	1
41			Striated	0	1	1	0
No.	Mol. Wt. (KD)			A	B	C	D
<b>Seed protein electrophoresis</b>							
42	58.5			1	0	0	0
43	51.8-510			0	1	1	0
44	49.4			1	0	0	0
45	44.4			0	0	1	0
46	43.7			0	1	0	0
47	37.3			0	0	0	1
48	36.8-36.2			1	1	0	0
49	35.7			0	0	1	1
50	33.5			0	1	1	0
51	24.4			1	0	0	0
52	22.0			0	1	1	0
53	20.0			0	0	0	1
54	19.5			1	0	0	0
55	18.3-18.5			0	1	1	0
56	16.3-16.5			1	1	1	1
57	15.1			0	0	0	1
Total No. of bands				6	7	7	5

0 = absent, 1 = present, A= *S. officinalis*, B= *S. minor*, C= *S. muricata*, D = *S. tenuifolia*

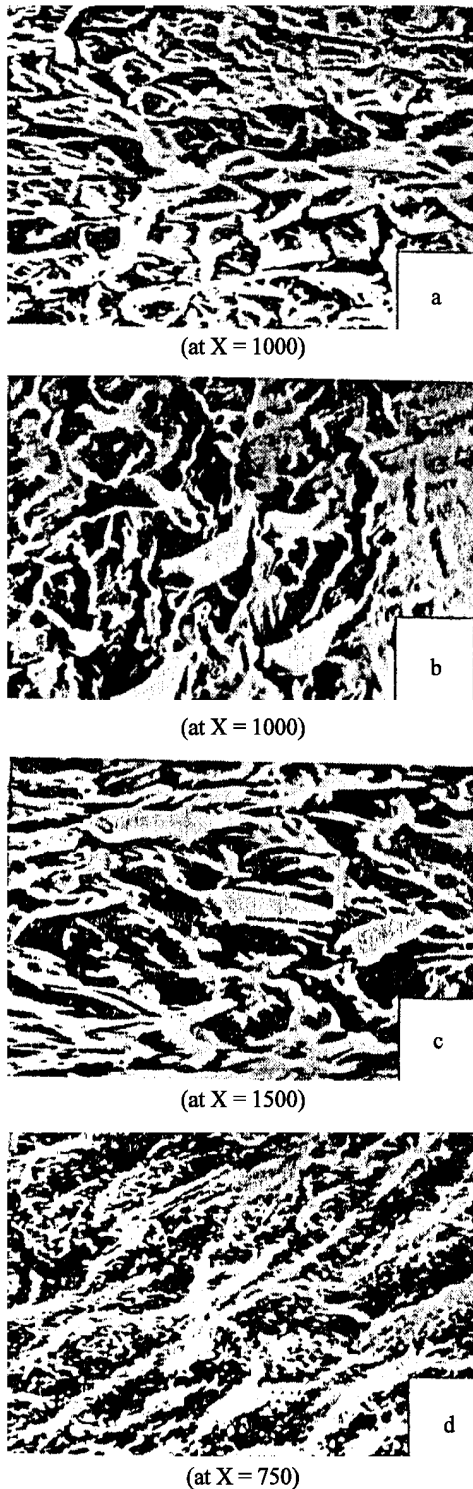


Fig. 1: Seed micromorphology of studied taxa of *Sanguisorba*  
 a) *Sanguisorba officinalis*  
 b) *Sanguisorba minor*  
 c) *Sanguisorba muricata*  
 d) *Sanguisorba tenuifolia*

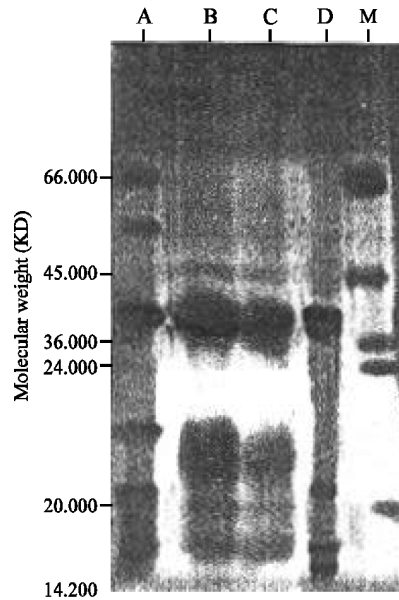


Fig. 2: Electrophoretic banding profiles of seed proteins extracted in Tris-EDTA buffer of four species of *Sanguisorba*. (A) *S. officinalis*, (B) *S. minor*, (C) *S. muricata*, (D) *S. tenuifolia*

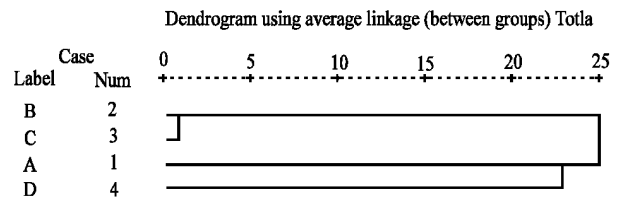


Fig. 3: UPGMA-phenogram based on 57 attributes (all characters), illustrating similarity between the studied taxa

***Sanguisorba muricata* (Spach) Greml:** The overall seed coat pattern is reticulate to honey combed. The epidermal cells are monohexagonal to irregularly shaped, and monomorphic. Anticlinal walls are slightly wavy, highly raised, highly striated and very thick. Periclinal walls are concave and striated. The medium sized conically shaped hairs like structure are seen on some parts of the achene (Fig. 1).

***Sanguisorba tenuifolia* Fisch. ex Link:** The overall seed coat pattern is reticulate to favulariate. The epidermal cells are irregularly shaped and monomorphic. Anticlinal walls are slightly wavy, highly raised, highly striated and very thick. Periclinal walls are flat and highly striated. Pointed hair dense irregularly shaped pointed hairs are seen on certain locations (Fig. 1).

**The electrophoretic banding patterns of the different taxa:** In Fig. 2 the distribution of protein bands in the different taxa based on their molecular weight as present (1) or absent (0) Table (2).

*Sanguisorba minor* and *S. muricata* were found to have the highest number of bands (7), whereas the lowest number (5) was found in *Sanguisorba tenuifolia*. The highest molecular weight protein (58.5 KD) was found in *Sanguisorba officinalis* and the lowest molecular weight (15 KD) was found in *Sanguisorba tenuifolia*.

**The numerical analysis of the recorded characters are summarized as follows:**

The phenogram was produced by cluster analysis and based on 57 characters from macro and micro morphology, seed coat anatomy and seed protein electrophoresis (Fig. 3). It revealed the following: *Sanguisorba tenuifolia* Fisch. ex Link was split off at similarity level of 25. The two species [*Sanguisorba minor* (L.) Scop (B) and *Sanguisorba muricata* (Spach) Greml (C)] were grouped together and clustered with *Sanguisorba officinalis* L. at similarity level of 11. The latter two species were separated from each other at similarity level of only one.

The close similarity between *Sanguisorba muricata* and *Sanguisorba minor* can add extra evidence to the views<sup>[5,6,29]</sup>. These authors favored considering *S. muricata* as a mere sub species of *S. minor*.

Finally, seed coat ultrastructural criteria and also seed anatomical aspect showed that *Sanguisorba* L. constitute a monophyletic lineage. The fact was shown previously by Morgan *et al.*<sup>[30]</sup> and Robertson *et al.*<sup>[31]</sup>.

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