Bangladesh J. Plant Taxon. **28**(2): 385–393, 2021 (December) DOI: https://doi.org/10.3329/bjpt.v28i2.57134

SEED MACRO- AND MICRO-MORPHOLOGY OF SOME SPECIES OF *KICKXIA* DUMORT, *SCROPHULARIA* L. AND *PLANTAGO* L. FROM SAUDI ARABIA

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Keywords: Seed morphology; SEM study; *Kickxia*; *Scrophularia*; *Plantago*; Saudi Arabia.

Abstract

The seed macro- and micro-morphological characters of 13 species belonging to three genera; *Kickxia* Dumort, *Scrophularia* L. and *Plantago* L., collected from different localities of Saudi Arabia, were studied using a Stereomicroscope and Scanning Electron Microscope for evaluating their taxonomic relationships. Three macro-morphological characters of seeds were shape, size, and color while three diagnostic micro-morphological characters were seed coat sculpture, the anticlinal and periclinal wall., The seed shape seems to be specific in *Plantago* L. (peltate, angled) than the two other genera while the color and seed size show little importance. The *Plantago* L. has a specific rugose sculpture while the other two genera have either tuberculate or alveolate. So *Plantago* L. was possessed no affinities to *Kickxia* Dumor or *Scrophularia* L.. This observation gives extra support to the earlier taxonomic views that suggested the retention of two genera in the traditional family Scrophulariaceae and maintaining *Plantago* L. in a separate monogeneric family Plantaginaceae Detailed analysis of seed characters of more species is needed to clarify the systematic relationships between the three genera. A key to the identification of studied species based on seed micro- and macro-morphological characters is provide.

Introduction

Scrophulariaceae is a large worldwide family (Miranda, 1988), with approximately 222 genera and 4480 species (Willis, 1973), mostly found in temperate zones of the northern hemisphere (Heywood, 1985).

The *Kickxia* Dumort (Scrophulariaceae) is one of the most important genera, with roughly 47 species (Mabberley, 1997). The genus is represented in Saudi Arabia by 10 species (Chaudhary, 2001), the majority of which are found in the south and west of the country. *Scrophularia* L. (Scrophulariaceae) is another major genus with over 300 species represented in both the ancient and modern worlds (Lersten and Curtis, 1997). It is represented by four species in Saudi Arabia (Chaudhary, 2001). Since ancient times, many species of this genus have been utilized as traditional medicine. The Plantaginaceae is a worldwide family of three genera: *Plantago*, *Littorella*, and *Bougueria*, which grow in a variety of environments (Bentham, 1846; Wettstein, 1891; Dahlgren, 1975; Cronquist, 1981; Heywood, 1993; Takhtajan, 1997).

Plantago L. (Plantaginaceae) is a perennial herb with a rosette of leaves at the base of the plant (Chiang *et al.*, 2002). The herb can be used to treat a variety of ailments. It is used to treat hypercholesterolemia and lower blood sugar levels (Haddadian *et al.*, 2014). It is also used to treat cancer (Souri *et al.*, 2008; Pourmorad *et al.*, 2006).It is represented by at least 12 species in Saudi

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Arabia (Chaudhary, 2001). Plantaginaceae and Scrophulariaceae have a tight link, according to several scientists (Takhtajan, 1980; Heywood, 1993; Mabberley, 1997). The notion of Plantaginaceae was broadened in Judd *et al.* (1999) and APG (2009) classifications to cover numerous taxa that were previously classified to the Scrophulariaceae based on molecular criteria. The existence of certain closely related families, such as Plantaginaceae, Orobanchaceae, and Acanthaceae, may further make the bounds of the Scrophulariaceae problematic.

The links between the traditional Plantaginaceae family and other families are still murky and need to be clarified (Heywood, 1993; Albach *et al*., 2004). The purpose of this study is to describe the seed morphological features in details to assess their utility in understanding the relationships among the three taxa analyzed (*Kickxia*, *Scrophularia* and *Plantago*). The findings are reviewed in considering the various classification systems.

Materials and Methods

The seeds of 12 species from two genera, *Kickxia* and *Scrophularia* of the family Scrophulariaceae and one species from *Plantago* of the Plantaginaceae were studied. The fresh seeds were collected from different localities of the Saudi Arabia as well as from preserved Specimens available at the Herbarium of Collage of Science, King Saud University (Table 1). The study was conducted at the Central Laboratory of Science and Medicine Department, King Saud University.

Table 1. List of studied species and their collected locations.

S/N	Name of Species	Localities
1	*Kickxia abhaica* D. A. Sutton	Wadi Alus, Rejal Almaa, Abha
2	*K. acerbiana* (Boiss.) Tackh. & Boulos	Yanbu-Omlog Road
3	*K. aegyptiaca* (L.) Nab.	Al sheheia, Alqassim
4	*K. corallicola* D. A. Sutton	Farasan Island, Jizan
5	*K. elatine* (L.) Dumort.	Herbarium of Ministry of Environment, Water & Agriculture
6	*K. hastata* (R. Br. Ex. Benth.) Dandy	Herbarium of Ministry of Environment Water, & Agriculture
7	*K. petiolate* D. A Sutton	Herbarium of Botany & Microbiology Department, Collage of Science, King Saud University
8	*K. pseudoscoparia* v. w. Smith & D. A. Sutton	Al sail Alsageer, Taif
9	*K. scalarum* D. A. Sutton	Herbarium of Ministry of Environment water & Agriculture
10	*K. spartioides* (Brouss. ex. Bush Janch.)	Herbarium of Botany & Microbiology Department, Collage of Science, King Saud University
11	*Plantago major* L.	Wadi Darak – Al Mandaq
12	*Scrophularia deserti* Del.	Bani Saad, Taif-Albaha Road
13	*S. peyronii* Post	Hebarium of Ministry of Environment. water & Agriculture

The external macro-morphological characters of the mature seeds were investigated with the aid of a Stereomicroscope. For seed size and width, the mean value of three seeds of each species were measured by a Micrometer.

For Scanning Electron Microscopic (SEM) examinations, mature dried seeds were selected, mounted on stubs using double side adhesive tape, coated in auto fine coater (JFC-1600) with gold by a Zeiss Scanning Electron Microscope, model (JEOL-JSM-6060 LV) at the electron microscope unite in the Central Laboratory of Science and Medicine Department, King Saud University. These were then examined and photographed with an accelerating voltage 15KV. Terminology of seed coat sculpturing basically following Stearn (1992) and Juan *et al.* (1997, 2000).

For numerical analysis we used the NTSYS-pc 2.2. software package according to the method of Rohlf, (2009) and then generated a cluster analysis of the similarity and dissimilarity matrix between the species under study to construct a Dendrogram.

Results and Discussion

The macro-morphological characters of the studied species presented in Table 2 revealed that the Seed Shape was varied from reniform in *Kickxia abhaica*, *K. scalarum* and *K. spartioides*, prismatic cylindrical in *K. aegyptiaca*, ovate in *K. elatine*, oblong ovate in the two *Scrophularia* species, peltate angled with rounded apical scar in *Plantago major*, rounded in *Kickxia petiolate* while ellipsoide in the remaining four species. The Seed Size ranges from large seeds (more than 500 µm long) in *Kickxia acerbiana* (866 x 591.6 µm) to small (Less than 500 µm) in *K. spartioides* (367.08 x 243.0 µm), *K. petiolata* (367.3 x 282.1 µm) followed by *K. hastata* (367.7 x 234.5 µm). The rest of the studied species possessed medium sized seeds (414-571 µm) long. The seed color showed low variation from brown in *Kickxia acerbiana*, *K. elatine* and *K. spartioides* to dark brown in the remaining studied species.

Table 2. The macro-morphological seed characters of the studied species.

S/N	Taxa	Seed shape	Size length x width	color
1	*Kickxia abhaica*	Reniform	571.7 x 358.1	Dark brown
2	*K. acerbiana,*	Ellipsoid	866.0 x 591.6	Brown
3	*K. aegyptiaca*	Prismatic/cylindrical	447.5 x 304.5	Dark brown
4	*K. corallicola*	Ellipsoid	440.4 x 283.8	Brown
5	*K. elatine*	Ovate	532.1 x 380.1	Brown
6	*K. hastata*	Ellipsoid	367.7 x 234.5	Dark brown
7	*K. petiolate*	Rounded	367.3 x 282.1	Dark brown
8	*K. pseudoscoparia*	Ellipsoid	558.3 x 381.9	Dark brown
9	*K. scalarum*	Reniform	416.1 x 336.8	Dark brown
10	*K. spartioides*	Reniform	367.1 x 243.0	Brown
11	*Plantago major*	Peltate angled with apical rounded scar	414.6 x 289.1	Dark brown
12	*Scrophularia deserti*	Oblong ovate	490.2 x 289.9	Brown
13	*S. peyronii*	Oblong ovate	504.9 x 402.5	Dark brown

The results also revealed that the variations of seed shape and size are of great importance in taxa delimitation while seed color of little importance. Also, the seed shape of *Plantago major* (peltate/angled with apical rounded scar) diagnostic and clearly separated it than the other two genera.

Esau (1977), Barthlott (1984), Werker (1997), Abdel Khalik and Maesen (2002), Akbari and Azizan (2006), Abdel Khalik (2010), Kaya *et al*. (2011), Abdel Khalik and Hassan (2012), Bona (2013), Ghimire *et al*. (2017) stated that, the seed morphology and anatomical characters are of taxonomic importance at both sub generic and sub familial levels.

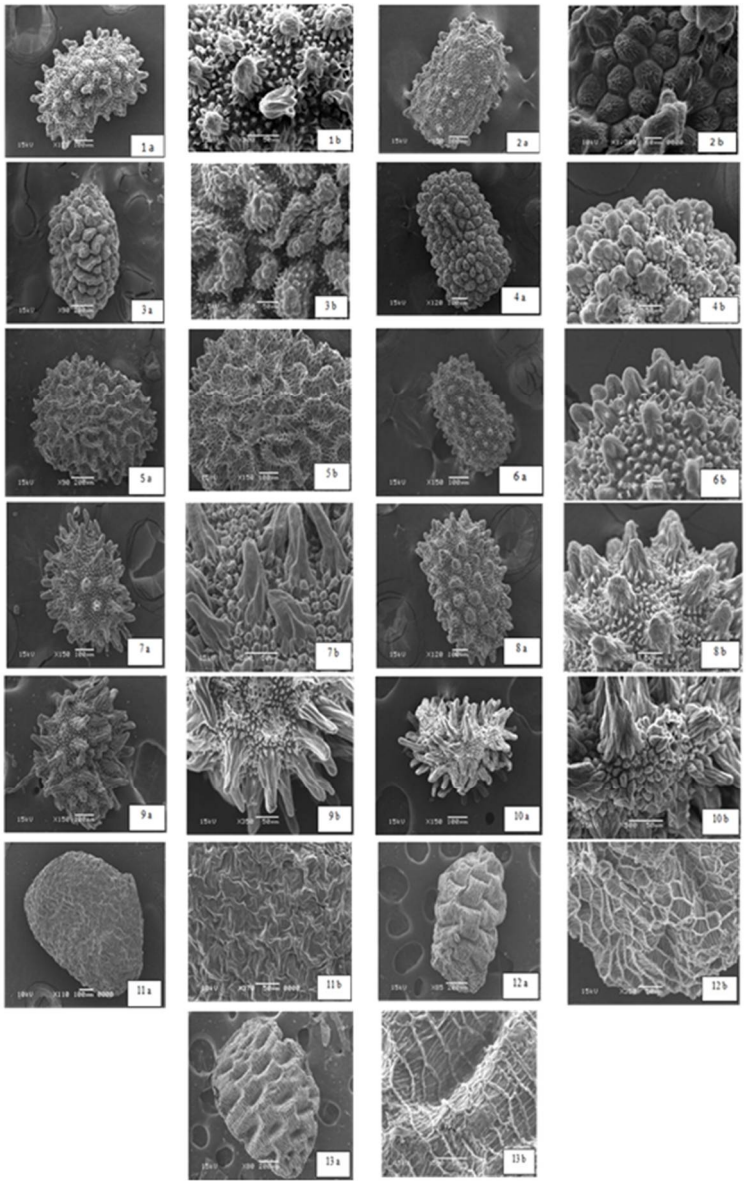
The micro-morphological characters investigated by SEM of the studied species presented in Table 3 and Figs 1-13 revealed that the Shape of the epidermal cells are penta-hexa-polygonal except in *Kickxia corallicola* which is ill-defied, while it is irregular in *Plantago major*.

Table 3. Micro-morphological seed characters of the studied species.

S/N	Taxa	Cell shape	Anticlinal wall	Periclinal wall	Seed coat sculpture
1	*Kickxia abhaica*	Pentagonal	Channeled	Raised with acute apex	Tuberculate/cristate (tubercles with obtuse conical apex).
2	*K. acerbiana,*	Pentagonal	Channeled	Raised with obtuse apex	Tuberculate with obtuse apex.
3	*K. aegyptiaca*	Polygonal	Levelled	Raised with broad apex	Tuberculate/ verrucose with broad base and globes cells
4	*K. corallicola*	ill-defined	Levelled	Raised with broad apex	Tuberculate /verrucose with broad base and globes cells
5	*K. elatine*	Hexagonal	Raised/Undulate	Concave	Reticulate/ ridged or undulate
6	*K. hastata*	Pentagonal	Levelled	Raised with obtuse apex	Tuberculate with obtuse apex.
7	*K. petiolate*	Pentagonal	Levelled	Raised with obtuse apex	Tuberculate tubercles conical
8	*K. pseudoscoparia*	Pentagonal	Levelled	Raised with acute apex	Tuberculate tubercles papillate
9	*K. scalarum*	Pentagonal	Levelled/ undulate	Raised with acute apex	Tuberculate tubercles conical, long
10	*K. spartioides*	Pentagonal	Levelled	Raised with acute apex	Tuberculate tubercles conical, long
11	*Plantago major*	Irregular	Slightly / Raised	Concave/ Striated	Rugose/reticulate
12	*Scrophularia deserti*	Hexagonal	Raised with wax	Flat/Ribbed	Alveolate-vesicles absent
13	*S. peyronii*	Penta-Hexagonal	Raised with wax	Flat/Ribbed	Alveolate-vesicles present

Anticlinal wall boundaries**:** These boundaries are well developed and indicated by channels in *Kickxia abhaica* and *K. acerbiana*, raised without wax *in K. elatine* and *Plantago major*, raised with wax in the two *Scrophularia* species, levelled in the remaining studied species.

Periclinal cell wall: Are flat ribbed in the two *Scrophularia* species, concave striated in *Plantago major*, concave without stria in *Kickxia elatine* while raised in the remaining studied species. It was raised with obtuse apex in *K. acerbiana*, *K. hastata* and *K. petiolate*, raised with acute apex in *K. abhaica*, *K. pseudoscoparia*, *K. scalarum* and *K. spartioides* while raised with broad base and globose cells in *K. aegyptiaca* and *K. corallicola*.



Figs. 1-13. SEM seed characters of studied species:1 a, b. *Kickxia abhaica*; 2 a, b. *K. acerbiana*; 3 a, b. *K. aegyptiaca*; 4 a, b *K. corallicola*; 5 a, b *K. elatine*; 6 a, b *K. hastata*; 7a, b *K. petiolate*; 8 a, b. *K. pseudoscoparia*; 9 a, b. *K. scalarum*; 10 a, b. *K. spartioides*; 11 a, b. *Plantago major*; 12 a, b. *Scrophularia deserti*; 13 a, b. *S. peyronii*. a-general seed shape, b- seed coat surface.

Seed coat sculpture: Scanning electron microscope (SEM) has been providing detailed and useful data on the fine structure of the seed coat in various genera of Scrophulariaceae (Canne, 1979, 1980; Elisens and Tomb, 1983; Sutton, 1988). Also, according to Molau (1990) the morphology of the seed coat provides a major diagnostic and significant features at tribes and subtribes of Scrophulariaceae.

All the studied *Kickxia* species except *K. elatine*, show tuberculate/ cristate seed coat sculpture with different tubercles or crista. The tubercles either small with obtuse apex or with broad base and globose cells or short papillated or long conical in shape.

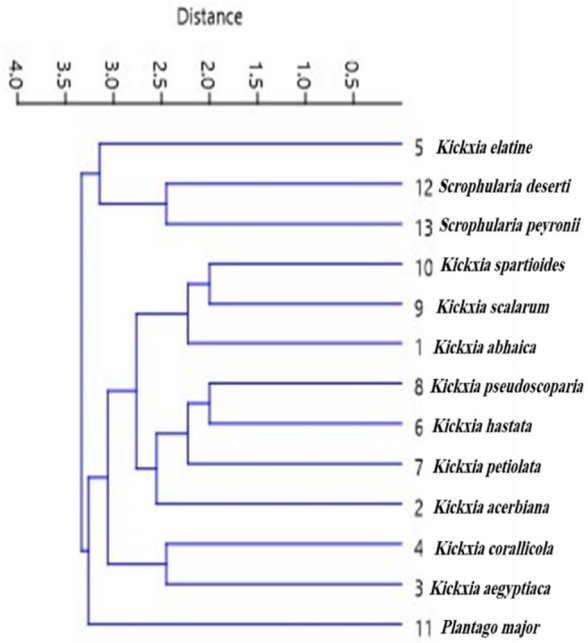


Fig. 14. Evolutionary Relationship Tree of 13 species based on seed phenotypes.

The presence of seed surface with developed protuberances seems to have adaptive advantages for these seeds. Barthlott (1981) stated that such seeds with tubercles are much less to be contaminated by pathogens than smooth ones. Also, the rough surface could help to control the temperature in sunlight and have hydration ability which could create microclimate for the seeds to avoid drying damage as indicated by Hedge (1970).

With respect to *Plantagomajor* and *Kickxiaelatine*, the seed coat characterized by rugose-reticulate sculpture. This result in accordance with Shehata and Loutfy (2006) with respect to *Plantago*. Pijl (1982) stated that, reticulate seed coat has often been related to water dispersion due to that these seeds having the ability to trap air, thus can float easily.

The results were cleared that, the seed coat sculpture could be useful in separation between the three studied genera. This in accordance with Attar *et al*. (2007) who stated that seed surface ornamentation can used for species delimitation in *Verbascum* (Scrophulariaceae).

The results cleared that; the seed coat sculpture as shown in the SEM micrographs varied in their appearance among the three studied genera. Only, the two *Scrophularia* species have seed coat with alveolate-reticulate Sculpture, the alveoli arranged in longitudinal rows with vesicles in *S. peyronii* and without vesicles in *S. deserti*. This result in agreement with Sutton (1988).

In conclusion, this study revealed that, some seed morphological characters are presented as a taxonomic criterion for genera delimitation. It was separated *Plantago major* than the two other studied genera based on seed shape and seed coat sculpture. Also, the dendrogram (Fig. 14) showed clearly that *Plantago major* was separated in independent cluster away from the rest of the

studied species through significant characters; seed shape, epidermis, cell shape, periclinal and anticlinal walls as well as seed sculpture. While *K. elatine* combined with the two *Scrophularia* species in one cluster. The results in accordance with Hamed *et al.* (2014) and confirms the earlier views of some authors (Wettstein, 1895) and others for maintaining *Plantago* as a separate monogeneric family (Plantaginaceae) while grouping the other two genera *Kickxi*a and *Scrophularia* in family Scrophulariaceae. However, this result contradicts with Albach *et al.* (2005) finding, who grouped *Kickxia* in tribe: Antirrhineae while *Plantago* in tribe: Plantagineae under new circumscribed family Plantaginaceae.

Despite the progression in the molecular and phylogenetic studies that has been made toward a new circumscription of Plantaginaceae and Scrophulariaceae, several important problems remain obscured and unsolved. For more accurate assignment of some genera of the two families, more studies on many species are still needed.

Based on these macro- and micro-morphological characters of seeds, an identification key to the studied species is prepared as follows.

Key to the species

	+		Seed length long (866 µm), seed shape ellipsoid	*Kickxia acerbiana*
+	+		Seed length short (367 µm)	
		●	Seed shape ellipsoid	*K. hastata*
	●	●	Seed shape rounded	*K. petiolata*
●	●	●	Seed shape reniform	*K. spartioides*
+	+	+	Seed length moderate size (414 – 571 µm)	
		1.	Seed shape prismatic/ cylindrical	*K. aegyptiaca*
		2.	Seed shape peltate, angled with apical rounded scar	*Plantago major*
		3.	Seed shape ovate	*K. elatine*
		4.	Seed shape oblong ovate	
			a. Seed coat sculpture alveolate with vesicles	*Scrophularia peyronii*
			b. Seed coat sculpture alveolate without vesicles	*S. deserti*
		5.	Seed shape reniform	
			● Anticlinal cell wall channel, seed coat sculpture with obtuse apex	*K. abhaica*
		●	● Anticlinal cell wall levelled, undulate, seed coat sculpture with long conical tubercles	*K. scalarum*
		6.	Seed shape ellipsoid	
			♦ Seed sculpture tubercles with broad base and globose cells	*K. corallicola*
		♦	♦ Seed sculpture with papillate tubercles	*K. pseudoscoparia*

Conclusion and Recommendation

The Morphological characteristics of the seed surface are important in defining, separating, and studying the evolutionary relationships between Taxa. This observation gives extra support to the taxonomic views that suggest the retention of the *Kickxia* in the family of Scrophulariaceae *s.l.* and maintaining *Plantago* in a separate monogenetic family of Plantaginaceae using a stereo and scanning electron microscope.

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(Manuscript received on 8 July 2021; revised on 3 December 2021)