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Macro and micromorphological characteristics of *Ruellia* L. species in the kingdom of Saudi Arabia

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Abstract
The current study uses Stereo and SEM microscopy, to present an accurate morphological description of the seeds of eight *Ruellia* species in the Kingdom of Saudi Arabia. Diagnostic features such as seed shape, dimensions, color, epidermal cells, and seed coat surface were studied. The ripe seeds showed some morphological differences; the seed shape was confined between orbicular disc shapes in *R. malacosperma*, *R.sp.sh2010*, *R. patula*, *R. tweediana*. (violat, white) and the oval shape *in R. brittoniana*, *R. sp. Taif.rose*, and the renal shape in *R. carolinensis.* The seed's color ranged from brown to dark brown, *R. brittoniana* and *R. tweediana.-rose* were distinguished with dark brown edges. The decoration on the outer seed coat (the stalk) is reticulate in two species, *R. patula* and *R.sp.sh2010*, with long, dense cylindrical hairs with annular long cylindrical rings at the edges of the seed, while the decoration was not evident in the rest of the species due to the presence of a dense layer of hairs.

Keywords: *Ruellia* L., seeds, morphological description, Stereo and SEM microscopy

Introduction

Ruellia L. Is one of the Acanthaceaea genera, which is represented in the Kingdom of Saudi Arabia with fourteen genera and thirty-five species (Al farhan *et al*., 2005) [1]. The seed's color, shape, and size change from one period to another due to the environmental and physiological conditions surrounding the seeds and their differences during the different stages of their life. They are relatively stable, so they are not considered essential characteristics in taxonomic studies, but they have limited taxonomic value among taxonomic units. They are used in a narrow field of studies. Taxonomic. Some scientists have used them to re-separate species (Ullah, 2019) [17]. So this study was presented to address the Ruellia species as the first specialized taxonomic study of the species of Ruellia at the Saudi Arabian level. The study will provide a morphological and anatomical description of the seeds of the species belonging to the genus Ruellia. The fruit is described as a capsule, striped or unstriated (Wheeler *et al*., 1992) [18]. The seeds are discoid, usually containing hygroscopic hairs (Deng *et al*., 2006) [11] with an asymmetrical base and rounded apex. With a distinct beak (Azevedo and Braz, 2018) [3], it is also unable to control water loss through transpiration. Still, the ornamentation of the outer layer of the seed increases its surface area from two to twenty times, which may be the primary means of controlling the internal temperature of the source (Barthlott, 1981) [6], as well as the secreted trichomes surrounding it of a resinous substance that is very effective in retaining water, and may contribute to the stability and protection of seeds until the germination stage (Daniel and McDade, 2014) [10]. When the fruits explode due to contact with water (Wortley *et al*., 2005) [19]. All *Ruellia* species are distinguished by their ability to spread their disc-shaped seeds to distances of up to 7 meters due to the effect of gyroscopic rotation, which helps them fly to distant places (Cooper *et al*., 2018) [9].

Meterial and Methods

Sample collection

The study adopted the seeds of plant samples collected from their natural environments (Table 1), at a rate of 10 seeds per plant sample.

Seed examination

The characteristics of the seeds (shape, color, size) were examined using a stereomicroscope; after that, the seeds were placed directly on small metal stups above a bar. The adhesive on both sides is coated with gold Coating using the JEC-550 Twin Coater device to study the shapes of the seeds and the characteristics of the delicate outer surface and the trimmings of the seed coat. The seeds were examined with different magnification powers by a scanning electron microscope in the Electron Microscope Unit - Central Research Laboratory- Female University City, to study the external characteristics of the seed, where the defining terms were used. Based on the method (Freitas Azevedo and Rodrigues de Moraes, 2019; Al-Hakimi and Latiff, 2015) [4, 5, 2].

Identification key

Generate an identification key based on the results of seed testing

Table 1: List of the *Ruellia* species studied and their localities in Saudi Arabia

Taxa	collection place	date of collection	GPS coordinates
Ruellia brittoniana	Abha	December, 2021	18°16'37.6"N 42°43'23.5"E
Ruellia carolinensis	Jazan	September, 2021	16°59'30.7"N 42°42'59.0"E
Ruellia malacosperma	Riyadh, Jazan, Madinah	September, 2021	24°43'07.9"N 46°37'24.4"E
Ruellia patula	Jazan	September, 2021	16°54'50.1"N 42°33'20.4"E
Ruellia.sp.sh2010	Aseer	December, 2021	19°07'44.5"N 41°55'42.2"E
Ruellia tweediana-rose	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E
Ruellia tweediana-Violet	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E
Ruellia tweediana-White	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E

Result

After examining the seeds of the eight *Ruellia* species, images of the seeds were inserted using a stereo microscope (Fig. 1) and SEM (Fig. 2). Ripe seeds showed some morphological differences in size, shape, color, and external decoration on the surface of the seed and the outer hairs. It is registered in Table (2) as follows:

1. *Ruellia malacosperma*

The seed is disc-shaped orbicular, and its dimensions range between 4.75×2.96 mm and dark brown in color. It has an orbicular apex and a cardiac base. The outer seed coat (the stalk) is characterized by a dense layer of hairs that impede access to identify the shape of the decoration on it. The bristles have a cylindrical shape, and they are long with rings, as in figures (1a and 2a).

2. *Ruellia patula*

The seed is disc-shaped orbicular, its dimensions range between 3.14 x 5.17 mm, brown in color, it has a triangular top with a bulge Triangle, the base of the seed is circular, orbicular, and it contains somewhat prominent protrusions called crested cells. Rings with annular long cylindrical in addition to tiny papillae on the surface of the seed (the stalk) as shown in figures (1b and 2b).

3. *Ruellia brittoniana*

The seed is elliptic in shape, its dimensions range between 2.81 x 1.24 mm, and it is brown with gray edges; it has a triangular Apex apex and a cordate base. The bristles are cylindrical and short with annular short cylindrical, as shown in figures (1c and 2c).

4. *Ruellia.sp.sh*2010

The seed is disc-shaped orbicular; its dimensions range between 4.56 x 2.45 mm, brown. It has an orbicular Apex and a cordate base and contains somewhat prominent protrusions called crested cells. With annular long cylindrical, in addition to tiny papillae hairs on the surface of the seed (the stalk) as shown in figures (1d and 2d).

5. *Ruellia carolinensis*

The seed is reniform, its dimensions are 1.85 x 2.87 mm, brown, has an orbicular top and a cordate base and contains somewhat prominent protrusions called crested cells. The filaments are cylindrical and short with annular short cylindrical, as shown in figures (1e and 2e).

6. *Ruellia tweediana -rose*

The seed has an elliptic shape, its dimensions are 3.52×2 mm, brown with gray edges, a triangular apex, an orbicular base, and somewhat prominent protrusions called crested cells. It hinders access to identify the shape of the decoration on it, and the filaments have a cylindrical shape and are short with annular short cylindrical rings, as shown in figures (1f and 2f).

7. *Ruellia tweediana -violat*

The seed has an orbicular disk shape; its dimensions range from 4×2.14 mm, brown in color, and a circular orbicular top the base of the seed is cordate and contains somewhat prominent protrusions called crested cells. In the form of decoration, the bristles are cylindrical and short with annular short cylindrical rings, as shown in the figures (1g and 2g).

8. *Ruellia tweediana -white*

The seed has an orbicular disk shape, its dimensions range between 2.40 x 3.92 mm, brown in color, it has a circular top with a prominent orbicular protrusion, a cordate base, and contains somewhat prominent protrusions called crested cells. The shape decoration on it and the bristles have a cylindrical shape and are short with annular short cylindrical rings, as shown in the figures (1h and 2h).

Table 2: Seed characteristics of *Ruellia* L. species in Saudi Arabia

Taxa	Trichomes site	Shape	Size(µm) length*width	sculpture	Color	Base	Apex
R. brittoniana	Entire	Elliptic	2.81×1.24	None	brownandgry in margin	Cordate	Triangle
R. carolinensis	Entire	Reniform	1.85×2.87	None	Brown	Cordate	Orbicular
R. malacosperma	Entire	Orbicular	4.75×2.96	None	dark brown	Cordate	Triangle
R.patula	Margins	Orbicular	3.14×5.17	Reticulat	Brown	Orbicular	Triangle with bluge
R.sp.sh2010	Margins	Orbicular	4.56×2.45	Reticulat	Brown	Cordate	Orbicular
R. tweediana-rose	Entire	Elliptic	3.52×2	None	brownandgry in margin	Orbicular	Triangle
R. tweediana-violet	Entire	Orbicular	4×2.14	None	Brown	Cordate	Orbicular
R. tweediana-wthite	Entire	Orbicular	2.40×3.92	None	Brown	Cordate	Orbicular with bluge

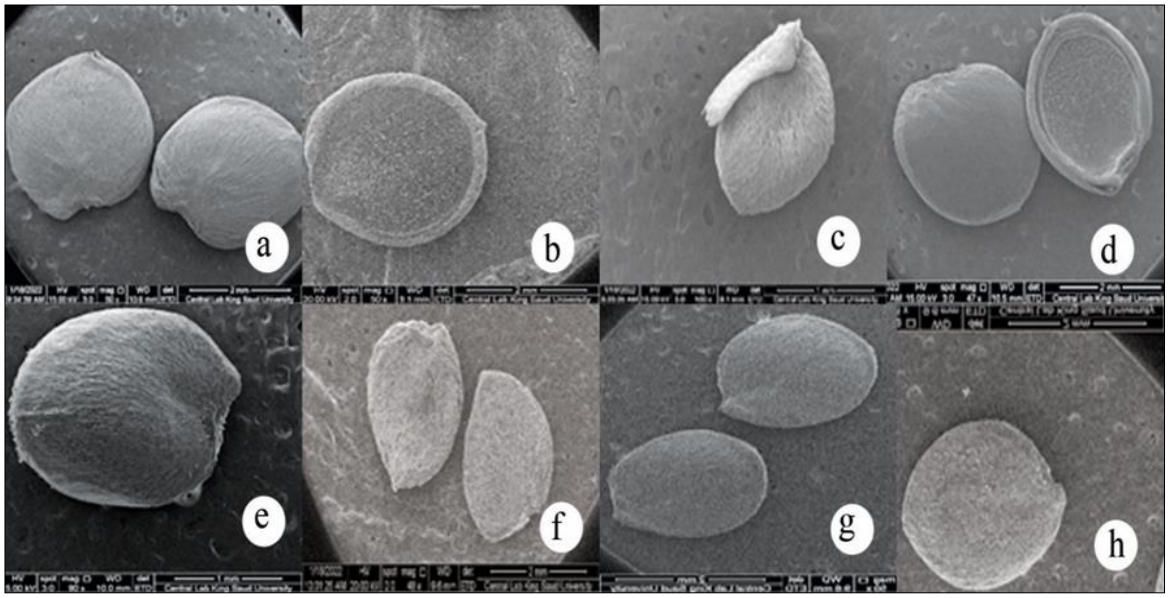

Fig 1

Scanning electron micrographs showing variation in seed morphological characteristics in *Ruellia* species; (a)*R.malacosperm*(50×) (b) *R.patula* (50×) (c) *R.brittoniana*(100×) (d) *R.sp.sh2010*(47×) (e) *R. carolinensis*(90×) (f) *R.tweediana-rose*(48×) (g) *R.tweediana-violat* (50×) (h) *R.tweediana-white*(48×)

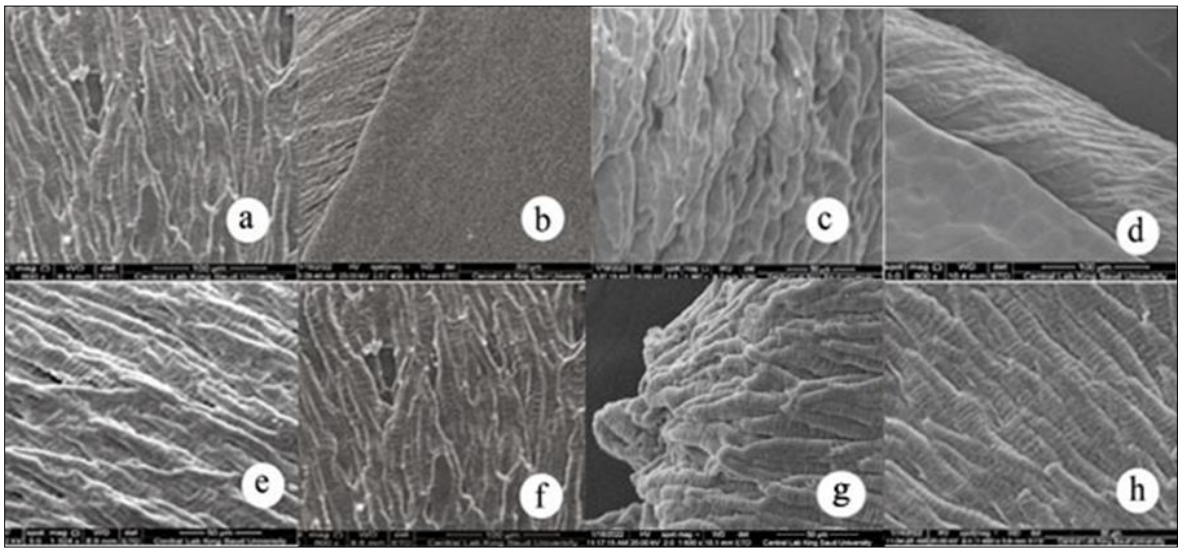

Fig 2

Scanning electron micrographs showing coat sculpture in *Ruellia* species seed: (a) *R. malacosperma* (800×) (b) *R. patula* (800×) (c) *R. brittoniana* (1367×) (d) *R.sp.sh2010* (800×) (e) *R. carolinensis* (1324×) (f) *R. tweediana-rose* (800×) (g) *R. tweediana -violat* (1600×) (h) *R. tweediana-white* (1600×)

An identification key for the species of *Ruellia* L. based on the seeds' characteristics

1. Seed shape

a. Treniform .....……*R. carolinensis*
b. renal tubule.....…….....……..... (2)

2. Seed color

a. brown with gray edges, heart base……*R. brittoniana*
b. b dark brown.....……*R. malacosperma*
c. brown with gray edges, round base…*R. tweediana-rose*
d. D- brown.....(3)

3. Seed external decoration

a. not clear ..... (4)
b. lattice, seed apex is triangular.....*R. Patula*
c. reticulate, seed apex is circular.....….*R.sp.sh2010*

4. Seed length and the apex shape

a. 4 mm long, with a round top..... *R. tweediana-volet*
b. 2.40 mm, circular in prominence apex.... *R. tweediana-white*

Discussion

The apparent seed characteristics of the studied species showed a diversity of the general shape of its seeds, so the oval shape appeared in the two types *R. brittoniana* and *R. tweediana-rose*, and the discoid shape appeared in both *R.sp.sh2010* and *R. tweediana- (violet* and white), *R. patula*, and *R. malacosperma*. These results are consistent with both (Braz and Azevedo, 2020; Monteiro, *et al*., 2020; Al-

Hakimi and Latiff, 2015) [20, 16, 2]. While the seeds of *R. carolinensis* was distinguished by the Reniform shape, this result agreed with (Freitas and Rodrigues, 2019) [13]. Seed colors ranged from brown in *R. carolinensis*, *R.sp.sh2010*, *R.patula*, and *R. tweediana-violet* and white to dark brown in *R.malacosperma*. Seeds of *R.brittoniana* and *R. tweediana-rose* were isolated. is brown with white to gray edges. These results were consistent with the result of (Chaudhary, 2000) [8] and (Greuter and Rodríguez, 2010) [14]. The latter also mentioned that the seeds acquire a gray color at their edges due to the dryness of the capsule and its distance from moisture. These gray tips are extended bristles. The ornamentation on the seed cover is not completely clear due to the density of hairs on its surface in all species except for the two species *R.sp.sh2010* and *R. patula*. The ornamentation is reticulated as the first record of the type of ornamentation, as the seeds are densely covered with hairs, but some species are wholly surrounded by short cylindrical hairs, as in The species *R.brittoniana R. malacosperma*, *R. tweediana-(*violet and white), *R. carolinensis*, and *R. tweediana-rose.*As for the rest of the species, a kind of papillary hairs spread in the middle in a small amount, but the long soft cylindrical hairs are concentrated at the edges only in the two species *R.sp.sh2010* and *R. patula*. These results were consistent with those of (Azevedo and Braz, 2018; Lester and Ezcurra, 1991) [3,15] and the latter added that when the seed is exposed to water, it secretes mucilage. It is possible that the mucilage secreted by the hair also has a protective function, it showed high resistance after treatment with its enzymes against the effect of the enzyme treated with it to try to remove the mucilage and hair for the purpose of knowing the nature of the decoration. The surface of the seed, but to no avail. The important presence of the hairs in a density of two layers is to retain water and protect the seed until germination (Azevedo and de Moraes, 2019) [4, 5].

Conclusion

The apparent seed characteristics of the studied species showed a diversity of the general shape of its seeds, ranging from oval to discoid. Seed colors ranged from brown to dark brown. The ornamentation on the seed cover is not completely clear due to the density of hairs on its surface, but short cylindrical hairs wholly surround some species. The seeds acquire a gray color at their edges due to the dryness of the capsule and its distance from moisture.

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