



Seed morphological variations within some monocotyledons taxa

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Abstract

Seeds belonging to seven monocot species belonging to three families; Asparagaceae, Asphodelaceae and Colchiaceae; are investigated using both stereo- and scanning microscopes. From the studied seed characters, the data obtained revealed that the tri-locular monocot taxa shared most of the floral characters, in spite of the differences in the inflorescences. Seed macro-morphological characters aid some information which can be used in the identification of the species, but the micro-characters are more valuable in the taxonomy of the taxa. Hilum shape and length beside the micropyle position are valuable characters in monocot classification. In this work, the hilum length varied greatly from 0.3 mm in both *Leopoldia camosa* and *Asphodelus ramosus* to 1.5 mm in *Colchium cornigulata*. With three different shapes; linear, oval or even circular; and two micropyle position either lateral or central. These characters can help in the discrimination of the studied taxa and consider from the first observable ones within the monocots.

Keywords: asparagaceae, asphodelaceae, colchiaceae, monocotyledons, seeds, taxonomy

Introduction

Monocotyledons are diverse group of plants objected to many opinions about their categorization. Monocot taxonomic studies exhibit wide range of physiological and morphological directions, as they have great economic and ornamental uses. In the old systems of classifications (Bentham & Hooker, 1883 and Engler & Prantl, 1888) ^[10] all the tri-carpelled superior ovaries are considered under family Liliaceae. This consideration has been changed later on by Cronquist (1981) ^[8] who gathered 15 families under order Liliales in a broader sense. Dahlgren *et al.* (1985) ^[9] gathered 27 families under superorder Liliopsida with five orders under it; Discorales, Asparagales, Melanthiales, Burmanniales and Liliales. In fact the clear discrimination of the taxa is not achieved and till recent years taxa between the two orders Liliales and Asparagales are confused. Most recent taxonomic works relied on molecular investigations and analyses and these did not give clear separations and identifications of them. Accordingly, more descriptive taxonomic works must do to give easier tool to recognize each group.

The use of seed characters in plant taxonomy and species discrimination proved to be useful in many taxonomical works starting from the mid nineteenth century. Wofford (1981) ^[20] commented that external morphology of seed is distinctive and valuable for species distinction. Heywood (1971), Barthlott (1984) ^[3] and Kaya *et al.* (2011) announced that after the invention of the high resolution microscopes many seed characters, especially size, color, shape, ornamentation and micropyle position became very important in solving many taxonomic problems. Within the monocot families the distinction of the Asparagales, Liliales, Dioscoreales, and Pandanales is unclear (APG IV, 2016) as all of them has tri-carpellate superior ovary with many ovules in axial placentation. The three syncarpous ovaries have a single style and three stigmas.

In an ongoing study we seek to provide basic information about the morphological characters of the seeds gathered from seven species belonging to three monocotyledonous families to clarify the similarity between them as a tool in the identification of the taxa. This work has many objectives, (1) to examine the seeds obtained from some taxa and enumerate the variability between them; (2) to determine infraspecific variation of the seed characters; (3) to analyze taxonomic significance of characters of seed variations of the studied taxa; (4) to compile an identification key to the studied taxa based on seed micro-morphology.

Materials and Methods

Seven species belonging to three families; Asparagaceae, Asphodelaceae and Colchicaceae; are subjected in this work (table 1). Herbarium sheets examined carefully from the both ALEX and CAI herbaria (ALEX=Alexandria university, CAI= Cairo University) to confirm the species identifications. The taxa nomenclature and the synonym of each species obtained from the International Plant Names Index and World Checklist of Selected Plant Families; IPNI; (2022) as listed in table 2.

Mature seeds, from each available taxa, have been examined, measured and photographed using Stereomicroscope and Scanning Electron microscope. Seed color, shape and size measured as well as the hilum lengths. Further characters are not clear under the Stereomicroscope and examined by the SEM.

Mature seeds chosen, and stuck onto Aluminum stubs using double stick cello tape, coated by Gold in a polaron JFC-1100E coating unit, examined and photographed under 15Kev, with JEOL JSM-IT200 SEM. The terminology used in the seed ornamentation is that Barthlott (1981) ^[2].

Results

The results obtained are summarized in table 3 and illustrated in Photos from 1-21. The descriptions of the studied seed characters are given below.

Order Asparagales

1. Family Asparagaceae, Subfamily Asparagoideae

1. *Asparagus stipularis* Forssk.

This species has black indehiscent berry fruit. The berries have a spherical structure, about 6 mm across, and hold small, nearly globular, hard, black seeds (3.3X 2.9 mm) with a smooth, glossy surface under the stereomicroscope, but with careful examination under SEM the testa has straight, elevated anticlinal walls and flat pitted periclinal walls with reticulate epicuticular ornamentation and few wax depositions (2ry.sculpture) (Photos.1, 2 & 3). There is 1-3 seeds per fruit, but often only 1 is present at maturity. The hilum 0.7 mm with lateral micropyle.

2. *Leopoldia comosa* (L.) Parl.

Leopoldia comosa has fruits loculicidal dehiscent capsule opened by valves, contained three parts, each part has three ovules. The ovules are small, oval (2.4X1.7mm), hard, black with smooth surface under the stereomicroscope. Under SEM investigations the anticlinal walls appeared straight and elevated, while the periclinal walls are highly convex with striate 1ry ornamentations. No 2ry.ornamentations or wax depositions appeared. The hilum circular 0.3mm long with central micropyle (Photos, 4, 5 &6).

3. *Scilla peruviana* L. (commonly within Liliaceae)

Individuals of this species have loculicidal dehiscent capsules opened by valves composed of three parts. Each part has four black, hard small triangular seeds (1.9X1.5mm) with smooth surfaces under the stereomicroscope. SEM investigations revealed undulate sunken anticlinal walls with flat smooth periclinal walls. There is 2ry. Epicuticular wrinkled ornamentation and no wax depositions over the testa. The hilum oval (0.5mm) with central hilum (Photos 7, 8 & 9).

2. Family Asphodelaceae, Subfamily Asphodeloideae

4. *Asphodelus fistulosus* L.

Fruits within this species are rounded loculicidal dehiscent capsules opened by valves, composed of three parts, each with two ovules. The seeds are oval (2.8X1.5mm) dark brown, hard and rough texture under the stereomicroscope. SEM investigation revealed straight sunken anticlinal walls and highly convex smooth periclinal walls. The overall looking of the testa like the papillate form due to the elevation of the periclinal walls without wax depositions. The seeds have irregular pores and depressions in the testa (Photo 11). The hilum linear (0.4mm) with lateral micropyle (Photos 10, 11 & 12).

5. *Asphodelus ramosus* L.

Fruits are globular loculicidal dehiscent capsules opened by valves, composed of three parts. Each part composed of one seed. The seeds are pyramidal in shape (5.1X2.3 mm), hard, olive green testa appeared glossy under the stereomicroscope. SEM investigations show straight superficial anticlinal walls and flat striate periclinal walls with no wax depositions. The testa has longitudinal straight line (Photo 14) and supercuticular reticulate secretions (Photo 15). The hilum linear (0.3mm) with lateral micropyle (Photos 13, 14 & 15).

6. *Asphodelus tenuifolius* Cav.

Individuals of this species have globular loculicidal dehiscent capsules opened by valves into three compartments. Each part contains two hard, dark brown wrinkled oval seeds (2.3X1.2mm) and appeared irregular under the stereomicroscope investigation. SEM examinations showed straight sunken anticlinal walls and convex concentric periclinal walls. The periclinal walls have circles in regular manner and without any secondary sculpture or wax depositions. The testa has sunken pores and deepest areas (Photo 17). The hilum oval (0.5mm) with central elevated micropyle (Photos 16, 17 & 18).

Order Liliales

Family Colchiaceae

7. *Colchicum cornigerum* Tackh.

This species has globular loculicidal dehiscent capsules opened by valves into three compartments. Each part contains four hard, brown tough nearly globular seeds (2.5X2.2mm) as seen under the stereomicroscope

investigation. SEM examinations showed undulate sunken anticlinal walls and flat, almost smooth periclinal walls. The periclinal walls have irregular arrangements and without any secondary sculpture but few granulated wax depositions are recorded. The hilum linear (1.5mm) with lateral micropyle (Photos 19, 20 & 21).

Key to the investigated species according to seed characters

- 1-Berry fruits with single seed-----*Asparagus stipularis*
 1-Loculicidal dehiscent capsules with 3 seeds
 2-Oval shape 2.4X1.7mm----- *Leopoldia comosa*
 2-Pyramidal shape 5.1X2.3mm----- *Asphodelus ramosus*
 1-Loculicidal dehiscent capsules with 6 seeds
 2-Smooth periclinal wall----- *Asphodelus fistulosus*
 2-Concentric periclinal wall----- *Asphodelus tenuifolius*
 1-Loculicidal dehiscent capsules with 12 seeds
 2-Triangular shape 1.9X1.5mm----- *Scilla peruviana*
 2-Globose shape 2.5X2.2mm----- *Colchicum cornigerum*

Table 1: Taxonomic status of the studied taxa according to APG IV family list (2016)

Order	Family	Subfamily	Tribe	Genus	Species
Asparagales	Asparagaceae	Asparagoideae	--	<i>Asparagus</i>	<i>stipularis</i> Forssk.
				<i>Leopoldia</i>	<i>comosa</i> (L.) Parl.
				<i>Scilla</i>	<i>peruviana</i> L.
	Asphodelaceae	Asphodeloideae	---	<i>Asphodelus</i>	<i>fistulosus</i> L.
					<i>ramosus</i> L.
					<i>tenuifolius</i> Cav.
Liliales	Colchicaceae			<i>Colchicum</i>	<i>cornigerum</i> Tackh.

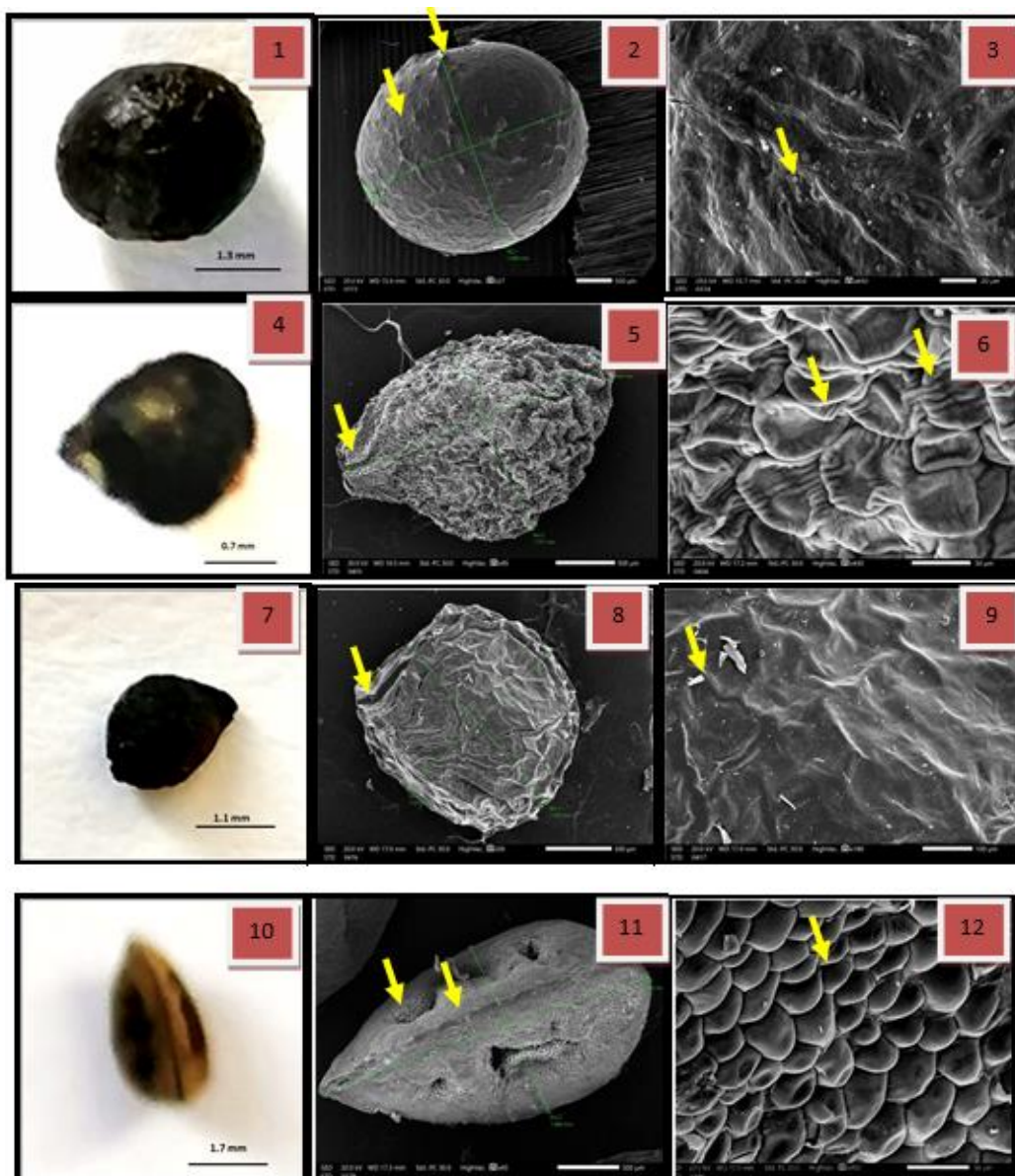
Table 2: Collection data, localities, source of the specimens, Synonyms, and confirmation of nomenclature

No	Species	Localities	Source of Sp.	Synonyms	Nomenclature Confirmation
1	<i>Asparagus stipularis</i> Forssk.	Sinai	ALEX	<i>A. broussonetii</i> . <i>A. horridus</i> .	IPNI (2022)
2	<i>Leopoldia comosa</i> (L.) Parl.	El-Omayed, Coastal region	ALEX	<i>Hyacinthus comosus</i> L. <i>Muscari comosum</i> (L.) Mill	IPNI (2022)
3	<i>Scilla peruviana</i> L.	El-Omayed, Coastal region	ALEX	<ul style="list-style-type: none"> ▪ <i>Basaltogeton peruvianum</i> (L.) Salisb. ▪ <i>Hyacinthus peruvianus</i> (L.) Vilm. ▪ <i>Melomphis peruviana</i> (L.) Raf. ▪ <i>Oncostema peruviana</i> (L.) Speta ▪ <i>Scilla hemisphaerica</i> (L.) Boiss. 	IPNI (2022)
4	<i>Asphodelus fistulosus</i> L.	Siwa	ALEX	<ul style="list-style-type: none"> ▪ <i>Asphodeloides ramosa</i> Moench ▪ <i>Ophioprason fistulosum</i> (L.) Salisb. ▪ <i>Verinea fistulosa</i> (L.) Pomel 	IPNI (2022)
5	<i>Asphodelus ramosus</i> L.	El-Arish, Sinai	CAI	<ul style="list-style-type: none"> ▪ <i>Asphodelus affinis</i> Parl. ▪ <i>Asphodelus africanus</i> Jord. ▪ <i>Asphodelus albus</i> Boiss. ▪ <i>Asphodelus audibertii</i> Req. ex Schult. & Schult.f. ▪ <i>Asphodelus crinipes</i> Jord. ▪ <i>Asphodelus fuscatus</i> Jord. & Fourr. ▪ <i>Asphodelus infestus</i> Parl. ▪ <i>Asphodelus jacobi</i> Sennen & Mauricio ▪ Others 	IPNI (2022)
6	<i>Asphodelus tenuifolius</i> Cav.	Baheig Burg El Arab road	ALEX	<ul style="list-style-type: none"> ▪ <i>Anthericum annuum</i> Pourr. ex Willk. & Lange ▪ <i>Ornithogalum flavum</i> Forssk. ▪ <i>Asphodelus bornmuelleri</i> Gand. ▪ <i>Asphodelus canariensis</i> C.Sm. & Buch ▪ <i>Asphodelus clavatus</i> Roxb. ex Lindl. ▪ <i>Asphodelus clavosus</i> Don ex Steud. ▪ <i>Asphodelus fistulosus</i> subsp. <i>nilotica</i> Ravenna 	IPNI (2022)

				<ul style="list-style-type: none"> ▪ <i>Asphodelus maroccanus</i> Gand. ▪ <i>Asphodelus serrulatifolius</i> Sennen ▪ <i>Verinea tenuifolia</i> (Cav.) Pomel 	
7	<i>Colchicum cornigerum</i> Tackh.	Mersa-Matruh-El-Dabaa	ALEX	No synonyms	IPNI (2022)

Table 3: Summary of seed characters as seen by both Stereomicroscope & SEM

Taxa	<i>Asparagus stipularis</i>	<i>Leopoldia comosa</i>	<i>Scilla peruviana</i>	<i>Asphodelus fistulosus</i>	<i>A. ramosus</i>	<i>A. tenuifolius</i>	<i>Colchicum cornigerum</i>
Type of Fruit	Berry	Capsule	Capsule	Capsule	Capsule	Capsule	Capsule
No. of Seeds/Ovary	1	3	12	6	3	6	12
Colour	Black	black	Black	Dark Brown	Olive Green	Dark Brown	Dark Brown
Shape	Globular	Oval	Triangular	Oval	Pyramid	Oval	Globular
Size (mm)	3.3x2.9	2.4x1.7	1.9x1.5	2.8x1.5	5.1x2.3	2.3x1.2	2.5x2.2
Anticlinal wall	Shape	Straight	Straight	Undulate	Straight	Straight	Undulate
	Relief	Elevated	Sunken	Sunken	Sunken	Superficial	Sunken
Periclinal wall	State	Flat	Convex	Flat	Convex	Flat	Convex
	1ry Orn.	Pitted	Striate	Smooth	Smooth	Striate	Concentric
	2ry Orn.	Reticulate	--	Wrinkled	--	--	--
Hilum	Wax	+	-	-	-	-	+
	Length (mm)	0.7	0.3	0.5	0.4	0.3	0.5
	Shape	Linear	Circular	Oval	Linear	Linear	Oval
Micropyle Position	Linear	Circular	Oval	Linear	Linear	Oval	Linear
	Lateral	Central	Central	Lateral	Lateral	Central	Lateral



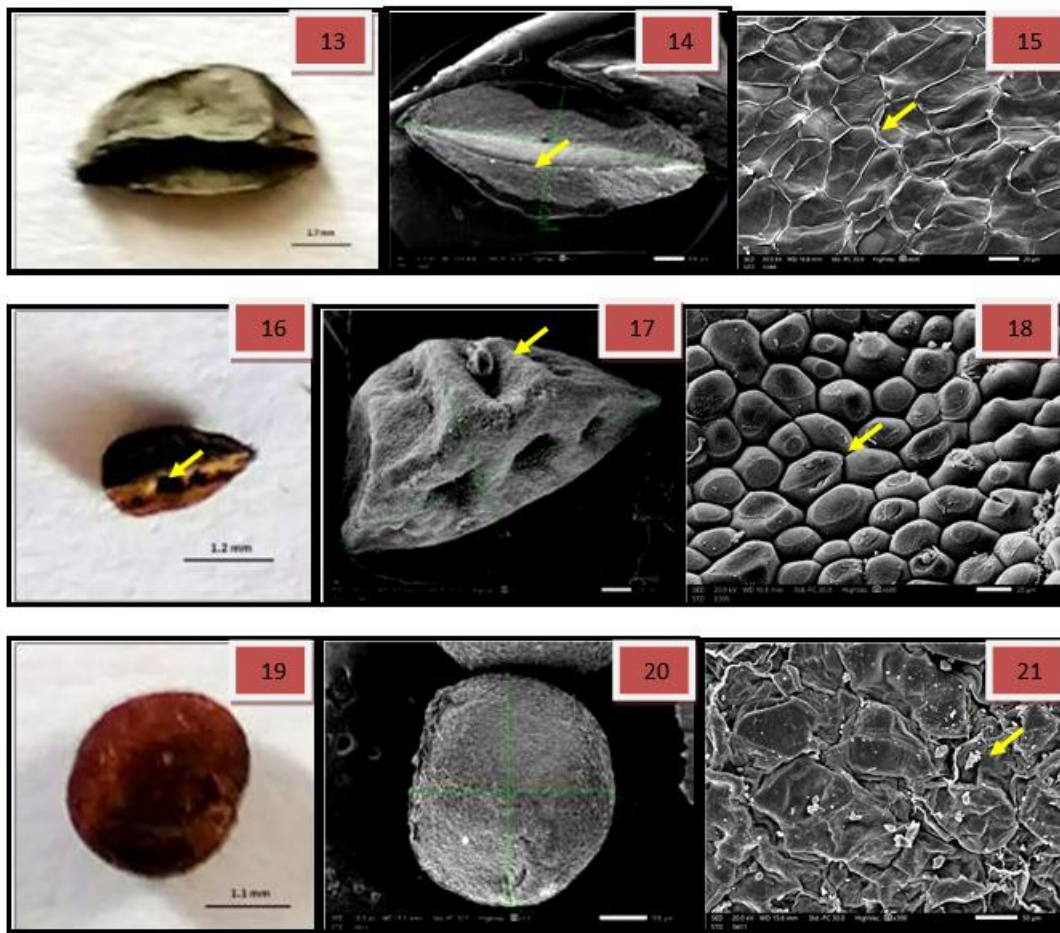


Fig 1: Photographs of the studied seeds: 1, 4, 7, 10, 13, 16 & 19 stereomicroscope photographs, arrow in 16 indicates to the grooves in the testa. Photos 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21 are SEM photographs. 1-3 *Asparagus stipularis*, arrows in 2 indicate to reticulate supercuticular secretion and hilum, arrow in 3 indicates to wax globules; 4-6 *Leopoldia comosa*, arrow in 5 indicates to the hilum, arrows in 6 indicate to the striate and convex periclinal walls; 7-9 *Scilla peruviana* arrow in 8 indicates to the hilum, arrow in 9 indicates to the supercuticular wrinkled secretions; 10-12 *Asphodelus fistulosus*, arrows in 11 indicate to the holes in the testa and the line in the middle, arrow in 12 indicates to the high convex smooth periclinal wall; 13-15 *Asphodelus ramosus* arrow in 14 indicates to the midline in the testa, arrow in 15 indicate to the supercuticular reticulate secretions; 16-18 *Asphodelus tenuifolius*, arrow in 17 indicates to the hilum and micropyle, arrow in 18 indicates to the concentric ornamentation of the periclinal wall; 19-21 *Colchicum cornigerum* arrow in 21 indicates to the wax granules.

Discussion

Monocot plants are diverse in taxonomic study and exhibit wide range of tolerance against the environmental factors. In fact classification of any group is subjective and depends on both the tools used and data analyses. Taxonomists differ in the relative importance they give to particular characters and in the degree of difference between related taxa they deem sufficient to constitute family or ordinal rank. Class Liliopsida i.e. the monocotyledons characterize by having one cotyledon in the seed and under this class the seeds have great role in their classification and phylogeny.

The study of seed characters proved to be useful in solving the taxonomic problems since the invention of the Scanning Electron Microscopes. In fact, most of the taxonomic works on the monocots are on the grasses i.e. family Poaceae (Clayton and Renvoize, 1986; Watson *et al.*, 1992; Watson and Dallwitz, 1994; Clark *et al.*, 1995; Kellogg, 2015; Whipple, 2017; Hodkinson, 2018 and Mandal and Chatterjee, 2020) [7, 18, 17, 6, 12, 11, 15] this due to the high economic importance of the grasses. At the same time taxonomic works on the other monocot groups are few. Accordingly, this work carried out to investigate the relation between the available species obtained from three families; Asparagaceae, Asphodelaceae and Colchiaceae; on the bases of seed macro- and micro-morphological characters.

The data obtained revealed that *Asparagus stipularis* is the only taxa with berry fruits with one seed/fruit, this may attributed to their small flowers. Bawa *et al.*, 2019 [4] indicated to the correlations between the size of flowers and the number of seeds per ovary. This cannot apply in the studied taxa, as both *Scilla peruviana* has small flowers and their fruits have twelve seeds in each capsule, while *Colchium cornigerum* has large flowers with 12 seeds/capsule as well. In this study the number of seeds/fruit grouped the studied taxa into four groups, 1, 3, 6 or 12, in spite of the taxonomic category of each group.

All the studied taxa have loculicidal dehiscent capsules; except *Asparagus stipularis* which has berry fruits; but the seed shape, color and testa ornamentations varied between them. Meanwhile, the hilum length and micropyle position can be diagnostic to the studied species. These characters proved to be important in the taxonomy of some taxa (Turki *et al.*, 2014) ^[16]. They found four different shapes and colors within the *Trigonella* seeds as well as different sizes and testa ornamentations which used in the classification of the genus. Meanwhile the hilum shape and micropyle positions proved to be good taxonomic features in the identification of many taxa. Mahdy *et al.* (2019) ^[14] recognized different hilum shapes within *Solanum* species can be used in their classifications. In this work, the hilum length varied greatly from 0.3 mm in both *Leopoldia camosa* and *Asphodelus ramosus* to 1.5mm in *Colchium corniculata*. With three different shapes; linear, oval or even circular; and two micropyle position either lateral or central. These characters can help in the discrimination of the studied taxa and consider from the first observable ones within the monocots.

Conclusion

From the studied seed characters, the data obtained revealed that the tri-locular monocot taxa shared most of the floral characters, in spite of the differences in the inflorescences. Seed macro-morphological characters aid some information which can be used in the identification of the species, but the micro-characters are more valuable in the taxonomy of the taxa. Hilum shape and length beside the micropyle position are valuable characters in monocot classification.

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