



## Seed coat sculpture and epidermal study on some taxa of Zygothyllaceae from eastern region of Saudi Arabia

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### Abstract

Seed characters and leaf epidermis of 11 taxa of Zygothyllaceae collected from Saudi Arabia's eastern region are examined by scanning electron microscope to investigate the different characters which can be used for identification and differentiation between them. The morphology of seed for the studied taxa has different characters in color, size, hilum position and shape. Seed coat sculpture are of eight different patterns. The micro-morphological leaf characters are different within the studied taxa in stomata, cuticular ornamentation and trichomes. The results obtained recorded 16 types of trichomes, 4 glandular and 12 non-glandular and 5 types of cuticular ornamentation. The dendrogram obtained from the numerical analysis showed that, the studied taxa could be divided into two major clusters, the first of which includes four taxa of *Tribulus*, while the second cluster comprises the studied taxa of *Fagonia* and *Tetraena*.

**Key Words:** Cuticular ornamentation; epidermis; leaves; scanning electron microscope; stomata; trichomes

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### Introduction

Zygothyllaceae is a family of flowering plants belongs to Zygothyllales that is widely distributed across the world and especially in arid and semi-arid regions (Sheahan, 2007). Most species of Zygothyllaceae are perennial herbs or small shrubs, rarely annuals, except about 26 species recorded under 4 genera; *Bulnesia*, *Guaiacum*, *Larrea* and *Porliera*; are shrubs or trees with hard wood (Sheahan, 2007). Many species of Zygothyllaceae have important ecological and economic values, as they are used for medicinal purposes, as fodder for livestock, and as a source of food and fuel. The family comprises about 25 genera and 250 species (Agnew, 1980). Then Sheahan & Chase (1996, 2000), recorded about 285 species and 27 genera.

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Recently Mabberley (2008), Christenhusz & Bynge (2016) reduced the numbers of genera to 22 and approximately 285 species.

According to Migahid (1988), Zygophyllaceae in Arabia is represented by five genera; *Fagonia*, *Zygophyllum*, *Tribulus*, *Peganum*, and *Seetzenia* including 23 species. According to Thomas (2020), Zygophyllaceae comprises 6 genera, including *Tetraena*, *Balanites*, *Fagonia*, *Tribulus*, *Seetzenia* and *Zygophyllum*, with 22 species and 9 varieties. The family was represented by 6 genera and 15 species in Saudi Arabia's eastern region (Mandaville, 1990). The genus *Tetraena* is represented in Saudi Arabia by six species, two subspecies and six varieties (Beier et al. 2003; Alzahrani & Albokhari, 2017a, b; Alzahrani, 2019)

Seed coat sculpture and epidermal study are important characters that can help in the identification of plant species as the morphology of the seed coat structure and epidermal characters are fixed and mostly unaffected by environment (Heywood, 1971; Cole & Behnke, 1975; Barthlott, 1981, 1984). The characteristics of seed morphology analysis and seed coat pattern sculpture are very important for taxonomic relationships and species identification (Bartholtt, 1981; Gammarra et al. 2007; Koul et al. 2000), it has been successfully used to solve systematic and evolutionary problems between different species (Gunn, 1981; Zeng et al., 2004). Few research on seed coat characteristics and their taxonomic importance for the Zygophyllaceae. Soliman et al. (2010) studied seed coat of *Fagonia schweinfurthia* and *Zygophyllum simplex* from Saudi Arabia; Abdel Khalik & Hassan (2012) studied the seed coat and trichomes of 13 *Fagonia* species from Egypt; Semerdjieva, et al. (2014) studied pollen and seed morphology of *Tribulus terrestris* from Bulgaria, while Semerdjieva & Tsvetkova, (2017) studied pollen and seed morphology of *Zygophyllum fabago* and *Peganum harmala* from Bulgaria. Recently, Mohajel Kazemi et al., (2019) investigate the seed of *Zygophyllum fabago* from Iran.

The epidermal features as stomata, trichomes and cuticle ornamentation are very important in taxonomic studies (Stace, 1969; Metcalfe & Chalk, 1950, 1979; Palmer & Tucker, 1981; Naik & Nigrude, 1981; Ogunkunle & Oladele, 2000; Adedeji, 2004; Adedeji & Illoh, 2004). The different types of trichomes have taxonomic importance for identifying and differentiated between species (Rao & Ramayya, 1977). The characters of cuticular are important and used to help identify similarities between taxonomically challenging taxa (Whang et al., 2001). For Zygophyllaceae species few studies are performed on various trichome types, cuticle ornamentation, and stomata. Fahn & Shimony (1996) studied the occurrence of glandular trichomes in three species of *Fagonia*. Abdel Khalik & Hassan (2012) studied the different types of trichomes for 13 *Fagonia* species from Egypt, also Semerdjieva (2014) studied the stomata and trichome types on *Tribulus terrestris* from Thracian Lowland floristic region. The aim of this work is to examine the seed coat and epidermal features of some taxa of Zygophyllaceae from Eastern Saudi Arabia, some of these species will be studied for the first time.

## Materials and Methods

Eleven taxa belonging to nine species and three genera of the family Zygophyllaceae growing in eastern region of Saudi Arabia were collected from different location (Table 1). The taxa were identified by using regional flora (Mandaville, 1990; Chaudhary 2001; Alzahran & Albokhari, 2017 a, b and Thomas 2020). With the use of a binocular stereo microscope and incident light. Five to seven seeds were examined for their morphological characters as: color, shape, size, hilum, and surface texture. Scanning electron microscope (SEM) was used to examine and photograph the seed coat and leaf surface (cuticular ornamentation, stomata, and trichome types).

Two leaves and seeds were mounted on metal stubs, coated by golden, examined and photographed by JEOL- SEM at accelerating voltages of 7 and 10 KV. Metcalfe & Chalk (1979) Fahn & Shimony (1996) and Perveen et al. (2016) provided the terminology for trichome types and epidermal features, respectively, while the terminology of Barthlott (1981, 1990) and Stearn (1983) were used to characterize the SEM characters of the seed coat. For the purposes of numerical analysis, seed and epidermis characters for the examined taxa were collected and data matrix was created, then the relationships between the studied taxa were shown as dendrograms by using PRIMER 6, version 6.1.6 analysis using agglomeration of Schedule measure Euclidean distance, using complete linkage between groups (Fig 1).

## Results

### Seed Characters

Seed morphology (Table 2; Plate I)

The main seed color in the studied taxa is brown with different degree except in *Tribulus terrestris* var. *parvispinus* is off-white. The seeds are greenish brown in *Fagonia bruguieri* and *Tetraena hamiensis* var. *qatarensis*; dark brown in *Tetraena hamiensis* var. *hamiensis* and brown in the rest. Seed shape of the studied taxa recorded three main types; obovate in *Fagonia* species, oblong in *Tetraena* species and fusiform in *Tribulus* taxa with terminal or subterminal hilum. The hilum is terminal in the studied taxa of *Tribulus* and *Tetraena* but subterminal in species of *Fagonia*. The size varied greatly among studied taxa; it ranged from 1.3 - 4.8 × 0.95 – 1.9 mm. The smallest seed ratio 1.11 mm. present in *Fagonia ovalifolia*, while the largest ratio 3.41 mm. recorded in *Tribulus macropterus* var. *arabicus*. The outer surface of the studied taxa ranged from warty in *Fagonia* sp., and smooth in *Tetraena* taxa and *Tribulus* taxa as seen under stereomicroscope. The seed characterise by small membranous margin in five studied taxa: *Fagonia olivieri*, *F. indica*, *F. ovalifolia*, *F. bruguieri* and *Tetraena hamiensis* var. *mandavillei*.

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**Table 1.** Collection data of the taxa included in the present study, nomenclature according to; Chaudhary, (2001); Beier et al. (2003); Alzahran & Albokhari, (2017).

<b>Taxa</b>	<b>Locality and date</b>
<i>Fagonia olivieri</i> DC.	Rayan – Dammam, 3/2017
<i>Fagonia indica</i> Burm.f.	Rayan – Dammam, 3/2017
<i>Fagonia ovalifolia</i> Hadidi	Rayan – Dammam, 3/2017
<i>Fagonia bruguieri</i> DC.	Rayan – Dammam, 3/2017
<i>Tetraena hamiensis</i> (Schweinf.) Beier & Thulin var. <i>hamiensis</i> .	Rayan – Dammam, 3/2018
<i>Tetraena hamiensis</i> var. <i>qatarensis</i> (Hadidi) Alzahrani & Albokhari	Rayan – Dammam, 3/2018
<i>Tetraena hamiensis</i> var. <i>mandavillei</i> (Hadidi) Alzahrani & Albokhari	Rayan – Dammam, 3/2018
<i>Tribulus pentandrus</i> Forssk. var. <i>pentandrus</i>	Rawda – Dammam, 4/2017
<i>Tribulus macropterus</i> Boiss. var. <i>arabicus</i> (Hosni) Al-Hemaid & Jac. Thomas	Second Industrial area in Dammam, 4/2017
<i>Tribulus terrestris</i> L. var. <i>terrestris</i>	Rayan – Dammam, 3/2018
<i>Tribulus terrestris</i> var. <i>parvispinus</i> (C.Presl) Al- Hemaid and Jac. Thomas	Rawda – Dammam, 4/2017

### Seed Coat Pattern Sculpture (Table 3; Plate II)

The external epidermal cells of the seed coat different from irregular polygonal for *Fagonia bruguieri*, *Tetraena hamiensis* var. *mandavillei*, *T. hamiensis* var. *hamiensis* and *Tribulus pentandrus* var. *pentandrus*; irregular isodiametric in *Fagonia ovalifolia*, or even elongated cell in *Fagonia olivieri* and *Tetraena hamiensis* var. *qatarensis*, and weakly defined in the rest of the studied taxa.

The anticlinal wall shape within the studied taxa are of five types; straight in *Fagonia olivieri*, *F. indica* and *Tetraena hamiensis* var. *hamiensis*; straight to sinuous in *Fagonia bruguieri* only; straight-undulate in *Tetraena hamiensis* var. *mandavillei*, *T. hamiensis* var. *qatarensis* and *Tribulus pentandrus* var. *pentandrus*; undulate in *Fagonia ovalifolia* and undulate -folded in the rest taxa. The anticlinal wall texture recorded three types; smooth to fold in *Tetraena hamiensis* var. *qatarensis*, *Tribulus terrestris* var. *terrestris*, *T. macropterus* var. *arabicus* and *T. terrestris* var. *parvispinus*, rough in *Fagonia bruguieri* and *Tribulus pentandrus* var. *pentandrus* and smooth in the rest. The anticlinal wall thickening varied from thin to thick, it is thin in *Fagonia ovalifolia*, *F. bruguieri*, *Tetraena hamiensis* var. *qatarensis* and *Tribulus macropterus* var. *arabicus*, while thick in the rest of the studied taxa. The anticlinal wall for the studied taxa was raised or slightly raised in all the taxa except in *Fagonia bruguieri*, *Tetraena hamiensis* var. *hamiensis* and *Tribulus terrestris* var. *parvispinus* are flat. The periclinal wall characters can be

good diagnostic and useful. Four shape types for periclinal wall are recorded for the studied taxa; flat in *Fagonia indica*, *Fagonia bruguieri* and *Tetraena hamiensis* var. *hamiensis*; flat to convex in *Fagonia olivieri*; concave in *Fagonia ovalifolia* and *Tribulus terrestris* var. *parvispinus* and convex in the rest studied taxa. The periclinal wall texture have two main types; rough in *Tribulus pentandrus* var. *pentandrus*, and smooth with two sub-type (smooth-warty and smooth – folded) in the remainder.

Eight different types of seed coat patterns are recorded:

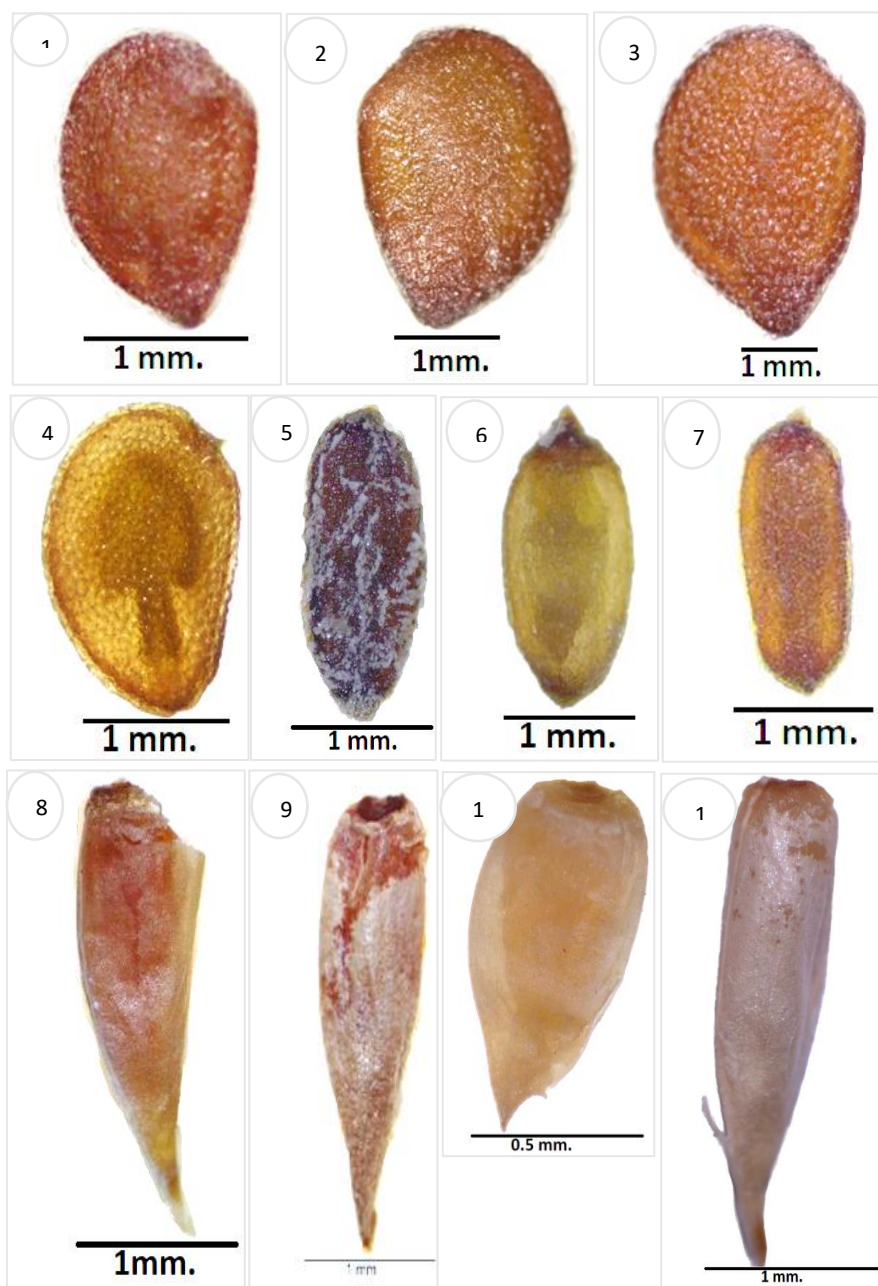
- 1- Lineolate in *Fagonia olivieri* (Plate II, Fig. 1).
- 2- Puncticulate with globular particles in *Fagonia indica* (Plate II, Fig. 2).
- 3- Colliculate in *Fagonia ovalifolia* (Plate II, Fig. 3).
- 4- Irregular colliculate in *Fagonia bruguieri* (Plate II, Fig. 4.)
- 5- Puncticulate in *Tetraena hamiensis* var. *hamiensis* (Plate II, Fig. 5).
- 6- Rugose in *Tetraena hamiensis* var. *qatarensis* (Plate II, Fig. 6).
- 7- Reticulate, with two subtypes:
  - a- Reticulate-areolate in *Tetraena hamiensis* var. *mandavillei* (Plate II, Fig. 7).
  - b- Irregular reticulate in *Tribulus pentandrus* var. *pentandrus* (Plate II, Fig. 8).

Irregular foveolate recorded in *Tribulus terrestris* var. *terrestris*, *T. macropterus* var. *arabicus* and *T. terrestris* var. *parvispinus* (Plate II, Fig. 9-11).

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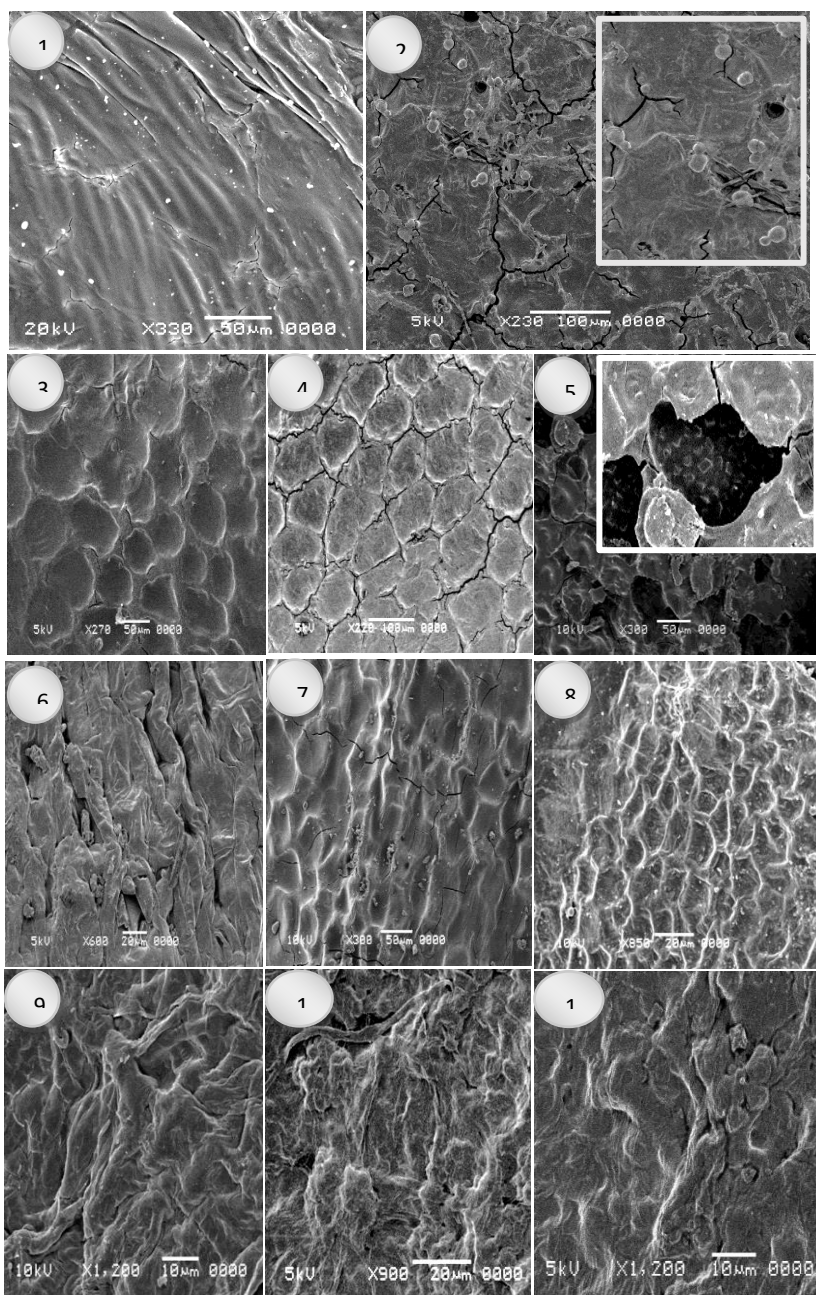
**Table 2.** Macro morphological characters of seed of studied taxa

Taxa		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguieri</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i>	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var. <i>mandavillei</i>	<i>Tribulus pentandrus</i> var. <i>pentandrus</i> .	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> . var. <i>terrestris</i> .	<i>Tribulus terrestris</i> var. <i>parvispinus</i>
color 1-brown 2-greenish brown 3-dark brown 4-off-white		1	1	1	2	3	2	1	1	1	4	1
shape 1-obovate 2-broad obovate 3-oblong 4-fusiform		1	2	2	1	3	3	3	4	4	4	4
hilum 1-terminal 2-subterminal		2	2	2	2	1	1	1	1	1	1	1
seed length (mm.)	standard	±0.2 2	±0.29	±0.4 5	±0.11	±0.1 9	±0.23	±0.23	±0.64	±0.3 2	±0.3 2	±0.3 7
	average	2.2- 2.8 2.5	2.3-3 2.7	1.3- 2.5 2.03	2-2.3 2.13	2.4- 2.9 2.6	1.9-2.5 2.2	1.8-2.4 2.03	2.3-4 2.98	4-4.8 4.4	2-2.8 2.4	2.1-3 2.7
seed width (mm.)	standard	±0.0 7	±0.29	±0.1 9	±0.08	±0.0 7	±0.38	±0.11	±0.08	±0.1 9	±0.3 9	±0.2 5
	average	1.8-2 1.9	1.5-2.2 1.9	1.5-2 1.8	1.3-1.5 1.4	1-1.2 1.1	0.8-1.8 1.18	0.8-1.1 0.95	0.9-1.1 1.03	1-1.5 1.3	1-2 1.4	0.8- 1.5 1.2
ratio l/w		1.30	1.39	1.11	1.49	2.3 4	1.83	2.13	2.90	3.41	1.68	2.37
Surface 1-smooth 2-warty 3-with glands		2	2	2	2	3	1	3	1	3	1	3
membranous margin 1-present 2-absent		1	1	1	1	2	2	1	2	2	2	2



**Plate I.** Seed morphology of 11 taxa of Zygodphyllaceae as revealed by light microscope: **1.** *Fagonia olivieri*; **2.** *Fagonia indica*; **3.** *Fagonia ovalifolia*; **4.** *Fagonia bruguieri*; **5.** *Tetraena hamiensis* var. *hamiensis*; **6.** *Tetraena hamiensis* var. *qatarensis*; **7.** *Tetraena hamiensis* var. *mandavillei*; **8.** *Tribulus pentandrus* var. *pentandrus*; **9.** *Tribulus macropterus* var. *arabicus*; **10.** *Tribulus terrestris* var. *terrestris* and **11.** *Tribulus terrestris* var. *parvispinus*.

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**Plate II.** Seed coat pattern Sculpture of 11 taxa of Zygophyllaceae as revealed by SEM.: 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Fagonia ovalifolia*; 4. *Fagonia bruguieri*; 5. *Tetraena hamiensis* var. *hamiensis*; 6. *Tetraena hamiensis* var. *qatarensis*; 7. *Tetraena hamiensis* var. *mandavillei*; 8. *Tribulus pentandrus* var. *pentandrus*; 9. *Tribulus macropterus* var. *arabicus*; 10. *Tribulus terrestris* var. *terrestris* and 11. *Tribulus terrestris* var. *parvispinus*.



## Leaf epidermal micro-characters

### I. Stomatal characters and cuticular ornamentation (Table 4; Plate III)

The stomata within the studied taxa are of two types: anomocytic and anisocytic. The stomata are superficial in *Fagonia indica* and *Tribulus terrestris* var. *terrestris*; semi-depressed in *Tetraena hamiensis* var. *qatarensis*, *T. hamiensis* var. *mandavillei* and *Tribulus pentandrus* var. *pentandrus*; depressed in *Fagonia olivieri* and *F. bruguieri*; at a level in the rest. Outline of stomata varying between elongated and sub-orbicular, the elongated type recorded in five taxa (*Fagonia ovalifolia*, *F. bruguieri*, *Tetraena hamiensis* var. *mandavillei*, *Tribulus macropterus* var. *arabicus* and *T. terrestris* var. *parvispinus*), while the sub-orbicular present in the rest.

Three types of the aperture shape are recorded: elliptic, oblong and ovate or oblong ovate; the elliptic type is the main shape found in seven studied taxa, oblong found in *Fagonia indica* and *Tribulus macropterus* var. *arabicus*, and ovate or oblong ovate in *Tetraena hamiensis* var. *qatarensis* and *Tribulus terrestris* var. *terrestris*. Aperture width is wide in most of the studied taxa while narrow in *Tribulus pentandrus* var. *pentandrus* and *T. macropterus* var. *arabicus*. The stomatal rim has three types; flat in *Tetraena hamiensis* var. *hamiensis* and *Tribulus terrestris* var. *terrestris*, raised in *Fagonia olivieri*, *F. indica*, *F. ovalifolia* and *Tribulus pentandrus* var. *pentandrus* and slightly raised in the remainder. The inner stomatal rim shows three types; sinuolate, sinuolate erose and smooth. Smooth recorded only in *Fagonia bruguieri*, sinuolate in *Fagonia olivieri*, *F. indica*, *Tetraena hamiensis* var. *mandavillei* and *Tribulus macropterus* var. *arabicus* where the sinuolate erose in the remainders. The peri-stomatal rim is absent in four studied taxa, the rest taxa recorded three types for peri-stomatal rim; overlapping-stout, overlapping and stout; overlapping-stout as in *Fagonia olivieri*, *F. ovalifolia* and *F. bruguieri*, overlapping in *Tetraena hamiensis* var. *qatarensis*, *T. hamiensis* var. *mandavillei* and *Tribulus terrestris* var. *parvispinus* and stout as in *Tribulus terrestris* var. *terrestris* only.

Five types of cuticular ornamentation are recorded; 1- Slightly favulariate plane in *Tetraena hamiensis* var. *hamiensis* and *T. hamiensis* var. *qatarensis* (Plate III, Fig. 5-6), 2- Favulariate plane in *Fagonia olivieri*, *F. indica*, *F. ovalifolia*, *Tetraena hamiensis* var. *mandavillei* and *Tribulus terrestris* var. *parvispinus* (Plate III, Fig. 1-3,7,11), 3- Irregular reticulate to favulariate Plane in *Fagonia bruguieri* (Plate III, Fig. 4), 4- Favulariate-granulate in *Tribulus pentandrus* var. *pentandrus* and *T. macropterus* var. *arabicus* (Plate III, Fig. 8-9), 5- Irregular reticulate in *Tribulus terrestris* var. *terrestris* (Plate III, Fig. 10).

### II. Trichomes types (Table 5; Plate IV)

Trichomes are important taxonomic tools used of the diagnoses between different species (Adedeji et al., 2007). The studied taxa of Zygophyllaceae recorded

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about 15 types: 4 glandular and 11 non-glandular. The species show great variation in trichomes types; some taxa recorded glandular trichomes only as, *Fagonia indica* and *F. bruguieri* while others recorded non-glandular trichomes only as in *Tetraena hamiensis* var. *hamiensis*., *T. hamiensis* var. *qatarensis*, *T. hamiensis* var. *mandavillei*, *Tribulus pentandrus* var. *pentandrus*, *T. terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*. Some types of trichome are specific for the taxa recorded on it as; capitate trichomes with long unicellular stalk are specific for *Tribulus macropterus* var. *arabicus*, non-glandular trichome with long blunt apical cell and short basal cell for *Tribulus terrestris* var. *parvispinus*. Also, there are some trichomes specific for the studied genera as; glandular with unicellular stalk and head are specific for the species of *Fagonia*, non-glandular broad unicellular with acute apex are recorded in *Tetraena* species only non-glandular trichome with unicellular acute apical cell and short basal cell and non-glandular with multicellular acute or curved apical cells and short basal cell are specific for studied species of *Tribulus*.

In the leaves of the taxa under study, the following trichome types were noted:

### a- Glandular

- 1- Unicellular stalk and unicellular head. (Plate IV, Fig. 1a) in *Fagonia olivieri*, *F. indica*, *F. ovalifolia* and *F. bruguieri*.
- 2- Unicellular stalk and multicellular head. (Plate IV, Fig. 2 a) in *Fagonia indica* and *F. bruguieri*.
- 3- Multicellular stalk and multicellular head. (Plate IV, Fig. 3a) in *Fagonia indica*, *F. ovalifolia* and *Tribulus terrestris* var. *terrestris*.
- 1- Clavate trichomes with cupule cell. (Plate IV, Fig. 4a) in *Fagonia ovalifolia* and *F. bruguieri*.

### b- Non-glandular

- 5- Unicellular papillose (Plate IV, Fig. 5b) in *Fagonia olivieri*, *F. ovalifolia* and *Tribulus macropterus* var. *arabicus*.
- 6- Capitate trichomes with long unicellular stalk (Plate IV, Fig. 6b) in *Tribulus macropterus* var. *arabicus*.
- 7- Broad unicellular with acute apex. (Plate IV, Fig. 7b) in *Tetraena hamiensis* var. *hamiensis*, *T. hamiensis* var. *qatarensis* and *T. hamiensis* var. *mandavillei*.
- 8- Unicellular with acute apical cell and short basal cell (Plate IV, Fig. 8b) in *Tribulus pentandrus* var. *pentandrus*, *T. macropterus* var. *arabicus*, *T. terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*.
- 9- Unicellular with curved apical cell and short basal cell (Plate IV Fig. 9b) in *Tribulus macropterus* var. *arabicus* and *T. terrestris* var. *parvispinus*.
- 10- Unicellular with blunt apical cell and short basal cell. (Plate IV, Fig. 10b) in *Tribulus macropterus* var. *arabicus*.

- 11- Unicellular with globose base and long tapered end (sting). (Plate IV, Fig. 11b) in *Tribulus terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*.
- 12- Unicellular with long broad acute apical cell and short basal cell. (Plate IV, Fig. 12b) in *Tribulus pentandrus* var. *pentandrus*, *T. macropterus* var. *arabicus*, *T. terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*.
- 13- Multicellular acute apical cell with regular cell wall and short basal cell. (Plate IV, Fig. 13b) in *Tribulus pentandrus* var. *pentandrus*, *T. macropterus* var. *arabicus*, *T. terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*.
- 14- Multicellular acute apical cell with irregular cell wall and short basal cell. (Plate IV, Fig. 13b) in *Tribulus pentandrus* var. *pentandrus*, *T. macropterus* var. *arabicus*, *T. terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*.
- 15- Multicellular curved acute apical cell with short basal cell. (Plate IV, Fig. 14b) in *Tribulus pentandrus* var. *pentandrus*, *T. macropterus* var. *arabicus*, *T. terrestris* var. *terrestris* and *T. terrestris* var. *parvispinus*.

Key for the studied taxa:

- 1- Seed obovate or broadly obovate with sub-terminal hilum and warty surface.....**2**
  - Seed oblong or fusiform with terminal hilum and smooth or the surface with glands .....**5**
- 2- Seed obovate and stomatal level depressed ..... **3**
  - Seed broad-obovate and stomatal level superficial or at level.....**4**
- 3- Seed coat sculpture lineolate with elongated epidermal cell, stomatal outline sub-orbiculate and glandular and non-glandular trichomes are present ..... ***Fagonia olivieri***
  - Seed coat pattern sculpture irregular colliculose with irregular polygonal epidermal cell, stomatal outline elongated and glandular trichomes only present..... ***Fagonia bruguieri***
- 4- Seed coat sculpture punctulate with glandular particles and ill-defined epidermal cell, straight anticlinal wall and flat periclinal wall; stomatal level superficial with sub-orbicular outline, oblong aperture shape and absent peristomatal rim; glandular trichomes only recorded ..... ***Fagonia indica***
  - Seed coat sculpture colliculate with irregular isodiametric epidermal cell, undulate anticlinal wall and concave periclinal wall; stomatal level at level with elongated outline, elliptic aperture shape and overlapping-stout peri-stomatal rim; glandular and non-glandular trichomes present ..... ***Fagonia ovalifolia***
- 5- Seed oblong; stomata semi depressed and non-glandular broad unicellular with acute apex trichomes present .....**6**
  - Seed fusiform; stomata superficial or at level, non-glandular multicellular acute or curved apical cell with short basal cell trichomes present .....**8**

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- 6- Seed brown; seed coat with reticulate-oreolate sculpture, stomata elongated with sinuolate inner stomatal rim and favulariate plane cuticular ornamentation..... ***Tetraena hamiensis* var. *mandavillei***
- Seed greenish brown or dark brown; seed coat with puncticulate or rugose sculpture, stomata sub-orbicular with Sinuolate erose inner stomatal rim and slightly favulariate plane cuticular ornamentation recorded .....**7**
- 7- Seed greenish brown; seed coat with puncticulate sculpture, irregular polygonal outer epidermal cell shape, straight, smooth and flat anticlinal wall, stomatal aperture elliptic with flat stomatal rim with the absence of peri-stomatal rim..... ***Tetraena hamiensis* var. *hamiensis***
- Seed dark brown with rugose sculpture, elongated outer epidermal cell shape, straight-sinuose, smooth folded and raised anticlinal wall, stomata aperture oblong with slightly raised stomatal rim and overlapping peri-stomatal rim ..... ***Tetraena hamiensis* var. *qatarensis***
- 8- Seed coat pattern irregular reticulate with irregular polygonal epidermal cells, straight undulate, rough anticlinal wall and rough periclinal wall. Stomata semi-depressed with raised stomatal rim..... ***Tribulus pentandrus* var. *pentandrus***
- Seed coat sculpture irregular favularate with ill-defined epidermal cells, undulate-folded, smooth folded anticlinal wall and smooth or smooth-warty periclinal wall. Stomata superficial or at-level with slightly raised or flat stomatal rim .....**9**
- 9- Seed with thin anticlinal wall and smooth periclinal wall, stomatal aperture oblong and narrow, inner stomatal rim Sinuolate, peri-stomatal rim absent, favulariate granulate cuticular ornamentation and capitate trichomes with long unicellular stalk ..... ***Tribulus macropterus* var. *arabicus***
- Seed with thick anticlinal wall and smooth-warty periclinal wall, stomatal aperture shape elliptic or oblong ovate and wide, inner stomatal rim Sinuolate erose, peri-stomatal rim present, favulariate plane or irregular reticulate cuticular ornamentation ; capitate trichomes absent.....**10**
- 10- Seed off-white, anticlinal wall raised, periclinal wall convex, stomata superficial, suborbicular with oblong aperture shape and flat stomatal rim and irregular reticulate cuticular ornamentation..... ***Tribulus terrestris* var. *terrestris***
- Seed brown, anticlinal wall flat, periclinal wall concave, stomata at-level, elongated with elliptic aperture shape and slightly raised stomatal rim, favulariate plane cuticular ornamentation..... ***Tribulus terrestris* var. *parvispinus***

**Table 4.** Stomatal characters and cuticular ornamentation of studied taxa.

Taxa		Characters										
		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguieri</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i> .	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var. <i>mandavillei</i>	<i>Tribulus pentandrus</i> var. <i>pentandrus</i> .	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> var. <i>terrestris</i> .	<i>Tribulus terrestris</i> var. <i>parvispinus</i>
Stomatal characters	Level: 1- Superficial 2- Semi-depressed 3- Depressed 4- At level	3	1	4	3	4	2	2	2	4	1	4
	Outline: 1- Elongated 2- Sub-orbicular	2	2	1	1	2	2	1	2	1	2	1
	Aperture shape: 1- Elliptic. 2- Oblong 3- Ovate 4- Oblong ovate	1	2	1	1	1	3	1	1	2	4	1
	Aperture width: 1- Narrow 2- Wide 3- ± wide	2	2	2	2	3	2	2	1	1	2	2
	Stomatal rim: 1- Slightly raised 2- Raised 3- Flat	2	2	2	1	3	1	1	2	1	3	1
	Inner stomatal rim: 1- Sinuolate erose	2	2	1	3	1	1	2	1	2	1	1

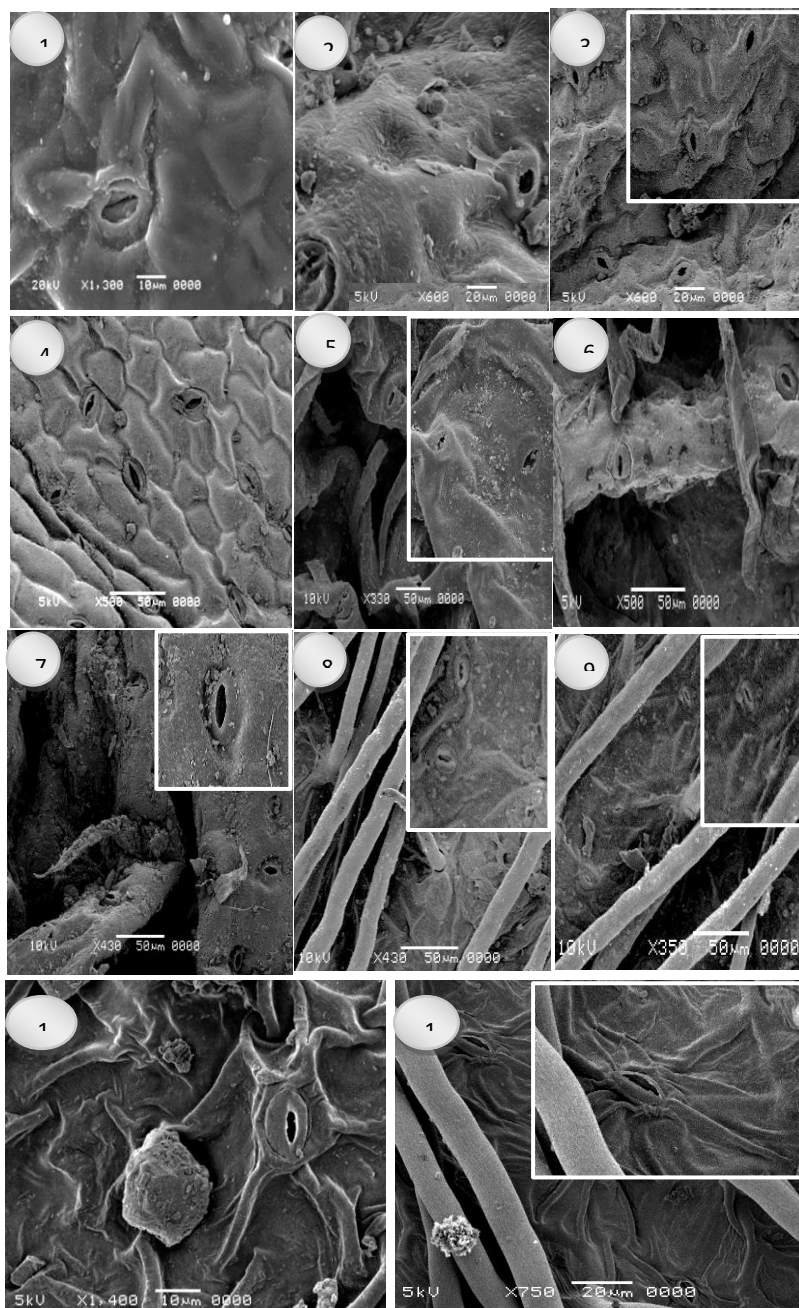
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	2- Sinuolate 3- Smooth											
	Peri-stomatal rim: 1- Overlapping-stout 2- Overlapping 3- Stout 4- Absent	1	4	1	1	4	2	2	4	4	3	2
	Cuticular ornamentation 1. slightly favulariate 2. favulariate 3. irregular reticulate to favulariate 4. favulariate-granulate 5. irregular reticulate	2	2	2	3	1	1	2	4	4	5	2

**Table 5.** Trichome types of the studied taxa. 1= present, 2= absent.

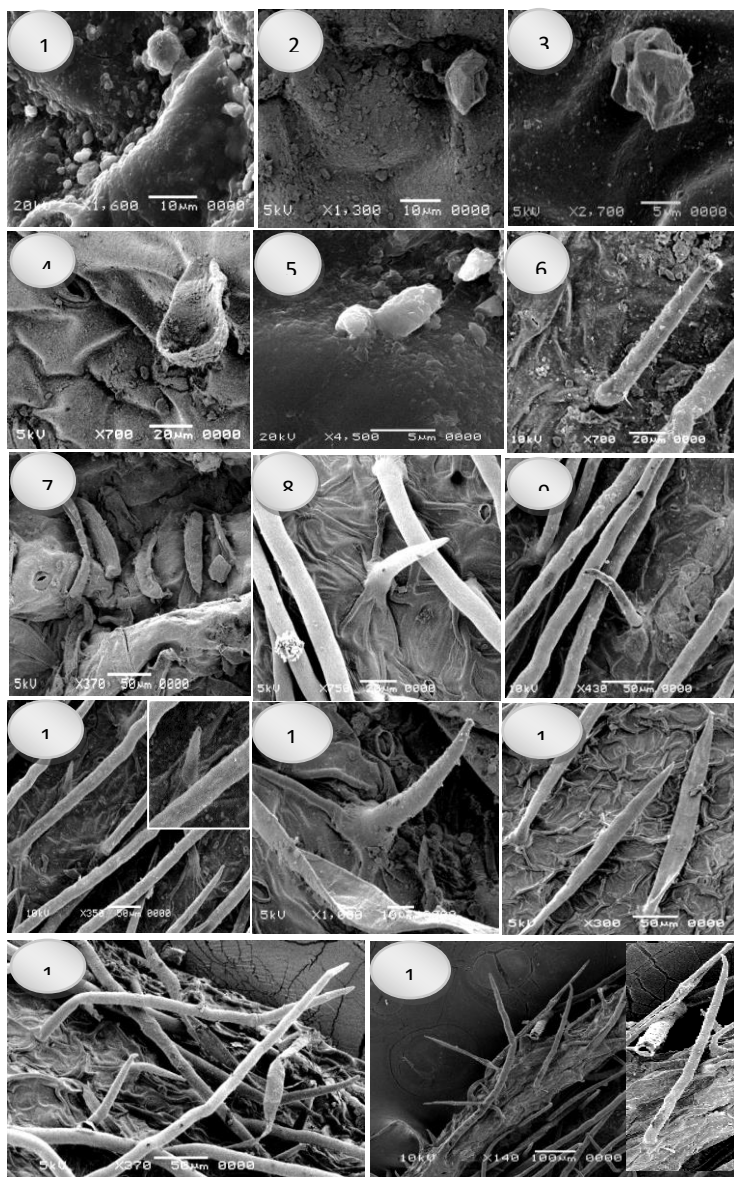
Trichome types		Taxa										
		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguieri</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i> .	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var. <i>mandavillei</i>	<i>Tribulus pentandrus</i> var. <i>pentandrus</i> .	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> var. <i>terrestris</i> .	<i>Tribulus terrestris</i> var. <i>parvispinus</i>
Glandular	1	1	1	1	1	2	2	2	2	2	2	2
	2	2	1	2	1	2	2	2	2	2	2	2
	3	2	1	1	2	2	2	2	2	2	1	2
	4	2	2	1	1	2	2	2	2	2	2	2
Non-glandular	5	1	2	1	2	2	2	2	2	1	2	2
	6	2	2	2	2	2	2	2	2	1	2	2
	7	2	2	2	2	1	1	1	2	2	2	2
	8	2	2	2	2	2	2	2	1	1	2	1
	9	2	2	2	2	2	2	2	2	1	2	1
	10	2	2	2	2	2	2	2	2	1	2	2
	11	2	2	2	2	2	2	2	2	2	1	1
	12	2	2	2	2	2	2	2	1	1	1	1
	13	2	2	2	2	2	2	2	1	1	1	1
	14	2	2	2	2	2	2	2	1	1	1	1
	15	2	2	2	2	2	2	2	1	1	1	1

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**Plate III.** Stomatal features and cuticular ornamentation of 11 taxa of Zygophyllaceae as revealed by SEM.: **1.** *Fagonia olivieri*; **2.** *Fagonia indica*; **3.** *Fagonia ovalifolia*; **4.** *Fagonia bruguieri*; **5.** *Tetraena hamiensis* var. *hamiensis*; **6.** *Tetraena hamiensis* var. *qatarensis*; **7.** *Tetraena hamiensis* var. *mandavillei*; **8.** *Tribulus pentandrus* var. *pentandrus*; **9.** *Tribulus macropterus* var. *arabicus*; **10.** *Tribulus terrestris* var. *terrestris* and **11.** *Tribulus terrestris* var. *parvispinus*.



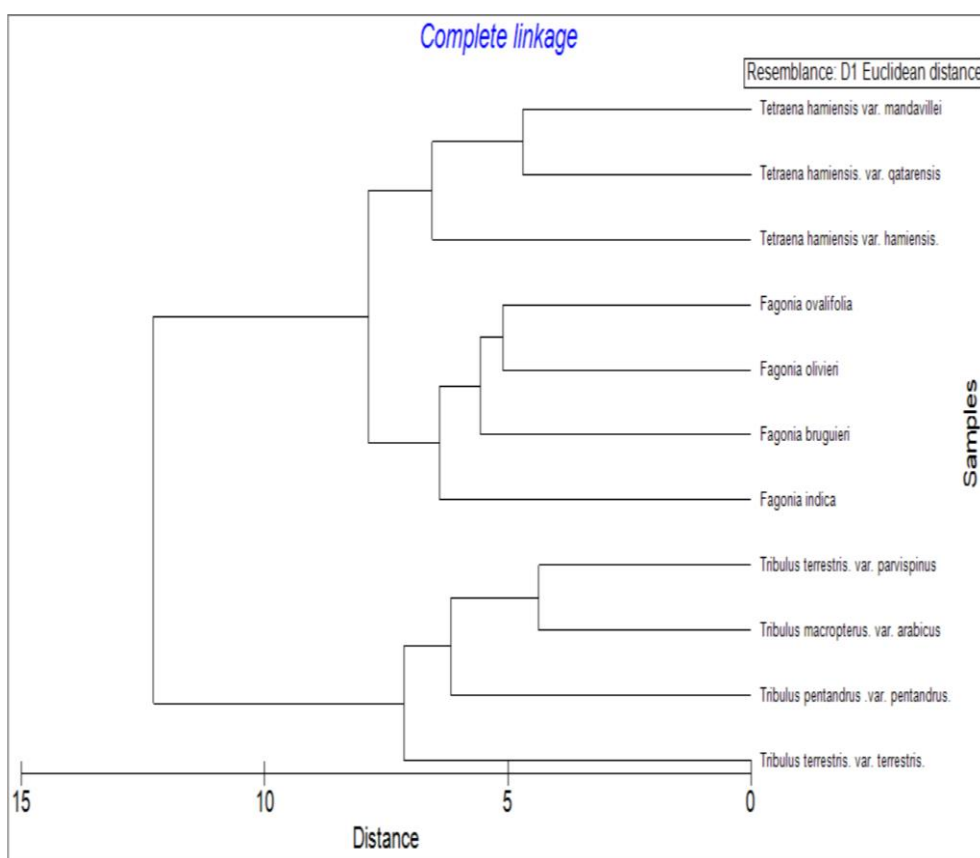


**Plate IV.** Main types of non-glandular and glandular trichomes for 11 taxa of Zygothylaceae as revealed by SEM. a. non-glandular: b. glandular **1.** *Fagonia olivieri*; **2.** *Fagonia indica*; **3.** *Fagonia ovalifolia*; **4.** *Fagonia bruguieri*; **5.** *Fagonia olivieri*; **6.** *Tribulus macropterus* var. *arabicus*; **7.** *Tetraena hamiensis* var. *qatarensis*; **8.** *Tribulus macropterus* var. *arabicus*; **9.** *Tribulus terrestris* var. *parvispinus*; **10.** *Tribulus macropterus* var. *arabicus*; **11.** *Tribulus terrestris* var. *parvispinus*; **12- 13.** *Tribulus terrestris* var. *terrestris* and **14.** *Tribulus terrestris* var. *parvispinus*.

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### Numerical analysis (Fig.1)

All the characters of seed coat sculpture and epidermis, including stomatal characters, cuticular ornamentation, and trichome types, were used in the numerical analysis of 11 taxa of Zygophyllaceae for identification and classification purposes. The clustering method was employed, and the results revealed that the studied taxa were grouped into two major clusters. The first cluster (I) consisted of the four studied taxa of *Tribulus*, while the second cluster (II) incorporated the studied taxa of *Fagonia* and *Tetraena*. The second cluster (II) was further divided into two groups: “G1,” which contained the four taxa of *Fagonia*, and “G2,” which incorporated the studied taxa of *Tetraena*. Group one “G1” was subdivided into two subgroups: subgroup “Sb. G1,” which included only one taxon, *Fagonia indica*, and subgroup “Sb. G2,” which had three taxa, *Fagonia bruguieri*, *Fagonia olivieri*, and *Fagonia ovalifolia*.



**Fig. 1.** Numerical dendrogram showing the interrelationships between 11 taxa of Zygophyllaceae based on 34 characters of Seed coat sculpture and epidermis by using PRIMER program.

## Discussion

The seeds of Zygophyllaceae are characterized by a hard and impermeable seed coat, which allows them to survive under extreme environmental conditions (Fernández-Mazuecos et al., 2013). The seeds are distinguished by their size, shape, color and surface ornamentation and these coordinate with the data obtained in this work. Brown is the main seed color in the studied taxa, but with varying degrees of darkness. The seed shape is categorized into three types including obovate in the studied *Fagonia* species, oblong in *Tetraena* and fusiform in *Tribulus* taxa which specified its species. The seed size vary greatly from the smallest ratio observed in *Fagonia ovalifolia* to the largest in *Tribulus macropterus var. arabicus* and the outer surface of the seeds vary from warty to smooth depending on the taxon, also the eight different types of seed coat pattern recorded for the studied taxa can be used as a criterion for distinguishing them. These findings agree with previous studies by Zhang et al. (2013); Abdel Khalik & Hassan (2012); Semerdjieva et al. (2014); Semerdjieva & Tsvetkova (2017).

In the identification and classification of Zygophyllaceae, stomatal characteristics and cuticular ornamentation considered as important features. The present study found two types of stomata, anomocytic and anisocytic, with varying level, shape, aperture width, rim type, and peri-stomatal rim type. Cuticular ornamentation was also observed in five types across the taxa. As well the trichome with about 15 types is considered as a diagnostic tool for distinguishing the species within Zygophyllaceae. Certain types of trichomes are specific to certain taxa or genera. These findings are consistent with previous studies by Abdel Khalik and Hassan (2012) and Semerdjieva (2014).

Zygophyllaceae has been challenging to classify due to its variability and phenotypic plasticity (Zohary, 1972; Shamso et al., 2013). Various characters, such as stipule characters, foliate number and shape, fruit characters, and number of stamens have been used to distinguish different genera. Some authors have attempted to divide the family into subfamilies, tribes, and sections, with varying placements for *Fagonia*, *Tetraena*, and *Tribulus* species.

Engler, (1896, 1931) divided the family into seven subfamilies, 8 tribes, and 4 sub-tribes, while El Hadidi (1975) separated *Tribulus* from Zygophyllaceae and placed it into Tribulaceae. Sheahan & Chase (1996, 2000) placed the three genera in Zygophyllaceae but in different subfamilies. In this study, numerical analysis depended on seed and epidermal characters place the three genera in two main clusters; cluster one contains taxa of *Tribulus* while cluster two contains the taxa of *Fagonia* and *Tetraena*, which agrees with Sheahan & Chase, (1996, 2000) and Abdel khalik (2012) classification. The dendrogram created in this study, showed that *Fagonia indica* has been separated from the other *Fagonia* studied taxa in a single subgroup and *Fagonia bruguieri* separated from the *Fagonia olivieri* and *Fagonia ovalifolia*, which agrees with El Hadidi (1972); Beier et al. (2004) and

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Abdel Khalik & Hassan (2012). *Tribulus terrestris* var. *terrestris* is separated from the other studied taxa of *Tribulus*; which agrees partially with El-Hadidi (1978) and Abdel Khalik (2012). This study is the first to analyse seed coat sculpture and epidermal characters for Saudi Arabian *Tetraena* species.

### Conclusion

The seed in the studied taxa have different characters in color, size, hilum position, shape, stomata, cuticular ornamentation and trichome types. Seed coat sculpture recorded eight types. All characters were used for numerical analysis. The results separated the *Tribulus* from *Fagonia* and *Tetraena*, also *Fagonia indica* has been separated from the other *Fagonia* and the *Tribulus terrestris* var. *terrestris* was separated from the other studied taxa of *Tribulus*. The present study demonstrates the importance of seed coat sculpture and epidermal study for understanding the taxonomy of Zygophyllaceae species.

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