

Seed Morphology of Some *Trigonella* L. Species (Fabaceae) and its Taxonomic Significance

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Abstract: Examination of seeds morphology of nineteen species of the genus *Trigonella* L. with light microscope and scanning electron microscope showed considerable variations in the seeds among the studied species. The different seed types are described compared based on seed characters such as shape, colour, hilum shape, position and coat pattern, then their taxonomic significance is discussed. Five groups of seeds were recognized. The studied characters indicated importance in systematic discrimination and identification of *Trigonella* species.

Keywords: *Trigonella*, Fabaceae, Seed Morphology, Scanning Electron Microscopy (SEM).

1. Introduction

Trigonella L. is a large genus with about 135 species belonging to the family Leguminosae (Syn: Fabaceae). *Trigonella* is distributed in the dry regions around Mediterranean, W. Asia, Europe, North and South Africa, N. America and S. Australia [1], [2]. Several investigators have attempted to employ the taxonomy of the genus *Trigonella*. 35 species of the genus *Trigonella* were classified on the base of pod shape, thickness, and number of seeds into two subgenus; *Eutrigonella* and *Pocockia*, The subgenus *Eutrigonella* was divided into eight sections on the base of flower, pod, and seed surface [3]. [4], [5] and [6] have noticed the great variation in seed shape, colours and testa ornamentation within *Trigonella*.

[7] examined seeds morphology of 37 taxa of *Trigonella* L. belonging to twelve sections based on seed features such as shape, colour and surface ornamentation pattern, they recognized seven morphological types revealing the presence of great variations.

It was found that there was no previous complete work on macro- and micro seed morphology of the studied taxa. The objective of the present work is to study and revise critically the taxonomic relationships and importance of seed characteristics in systematic discrimination and identification of the studied *Trigonella* species.

2. Materials and Methods

Seeds of *Trigonella* species used in this study were collected from plants in their natural habitats in Egypt. In addition to seeds obtained from seed banks (Institute of Plant Genetics and Crop Plant Research (IPK) in Germany and National Plant Germplasm System (NPGS) in United States). For light microscopic studies, about 10 mature dry seeds were thoroughly cleaned with alcohol to avoid any alteration in the micro morphological features and examined for diagnostic features of shape, colour and size. SEM study of the investigated material carried out by mounting seeds on brass stubs, coated with a thin layer of gold, and examined using JEOL JSM 530P SEM at Electron Microscopic Unit, Faculty

of Science, Alexandria University, Egypt.

Features of seed surface were coded and used for numerical analysis; thirty two characters were recorded for each taxon. Phenogram illustrating the relationship between the studied taxa were constructed by calculating the average taxonomic distance (dissimilarity) by using the NTSys.

3. Results

Observations based on light and scanning electron microscopy showed variation of the examined *Trigonella* taxa in both qualitative and quantitative seed characteristics as indicated in the following:

Trigonella seeds indicated variability in size. According to the values of the seed length and width, the seeds are classified into two groups, Table 2 and Figure 1.

- Small seeds with length up to 1.1 mm represented in *T. balansae*, *T. corniculata*, *T. stellata*, *T. caerulea*, *T. strangulate*, *T. grandiflora*, *T. suavissima*, *T. rechingeri*, *T. procumbans*, *T. calliceras*, *T. anguina*, *T. Arabica*, *T. maritime*, *T. hamosa*, and *T. coerulescens*.
- Large seeds with length from 1.3 - 2.1 mm represented in *T. cretica*, and *T. foenum-graecum*.

Variations have been noticed in the shape and the colour of species of *Trigonella*. Four shapes of seed were recognized: **Elliptic** in *T. balansae*, *T. corniculata*, *T. stellata*, *T. caerulea*, *T. strangulate*, *T. rechingeri*, *T. suavissima*, *T. cretica*, *T. procumbans*, *T. anguina*, *T. maritime*, and *T. hamosa*, **Rhomboid** in *T. monspeliaca* var. *eigii*, *T. monspeliaca* var. *petiolata*, and *T. foenum-graecum*, **Ovoid** in *T. calliceras*, *T. coerulescens*, and *T. Arabica* and **Rectangular** in *T. grandiflora* and *T. foenum-graecum*. Four colours of seeds were recognized: **Yellow** in *T. stellata*, *T. coerulescens*, *T. cretica*, *T. foenum-graecum*, *T. maritime* and *T. hamosa*, **Orange** in *T. balansae*, *T. corniculata*, *T. strangulate* and *T. calliceras*, **Pale brown** in *T. rechingeri*, *T. suavissima*, *T. anguina*, *T. foenum-graecum*, *T. arabica*, *T. caerulea* and *T. procumbans* and **dark brown** in *T. monspeliaca* var. *eigii*, *T. monspeliaca* var. *petiolata* and

T. grandiflora (Table 2 and figure 1). Two positions of hilum were recognized in the studied species: **Apical** in *T. arabica*, *T. anguina*, *T. calliceras*, *T. cretica*, *T. coerulescens*, *T. suavissima* and *T. stellata* and **subapical** in all other species. Three shapes of hilum were recognized: **Longitudinally elliptic** in *T. corniculata*, *T. strangulate*, *T. rechingeri*, *T. suavissima*, *T. cretica*, *T. calliceras*, *T. foenum-graecum* and *T. Arabica*, **transversally elliptic** in *T. balansae*, *T. monspeliaca* var. *eigii*, *T. monspeliaca* var. *petiolata*, *T. grandiflora* and *T. caerulea* and **circular** in *T. stellata*, *T. coerulescens*, *T. anguina*, *T. procumbans*, *T. maritima*, *T. hamosa*. Two types of Hilum poles were also recognized: **round** in *T. corniculata*, *T. rechingeri*, *T. suavissima*, and *T. foenum-graecum*, and **Acute** in all other species. Two seed coat patterns were recognized: **Papillate** in *T. suavissima*, *T. monspeliaca* var. *eigii*, *T. monspeliaca* var. *petiolata*, *T. maritima*, and *T. hamosa* and **mounded with papillae** in all other studied species. Four outlines of seed coat cells were recognized: **Isodiametric** in *T. balansae*, *T. corniculata*, *T. stellata*, *T. caerulea*, *T. strangulate*, *T. grandiflora*, *T. suavissima*, *T. coerulescens*, *T. procumbans*, *T. calliceras*, *T. anguina*, *T. hamosa*, *T. foenum-graecum*, *T. monspeliaca* var. *eigii*, **Tangentially elongated** in *T. maritima*, and *T. monspeliaca* var. *petiolata*, **Irregular** in *T. rechingeri*, and *T. cretica* and **Irregular folded** in *T. arabica*. Anticlinal wall **straight** in *T. balansae*, *T. corniculata*, *T. stellata*, *T. caerulea*, *T. strangulate*, *T. grandiflora*, *T. monspeliaca* var. *eigii*, *T. monspeliaca* var. *petiolata*, *T. cretica*, *T. procumbans*, *T. calliceras*, *T. suavissima*, *T. anguina*, *T. maritima*, *T. foenum-graecum*, *T. hamosa*, *T. coerulescens* and **wavy** in *T. rechingeri*, *T. arabica*. Outer periclinal wall **convex** in *T. balansae*, *T. corniculata*, *T. stellata*, *T. caerulea*, *T. strangulate*, *T. monspeliaca* var. *eigii*, *T. monspeliaca* var. *petiolata*, *T. procumbans*, *T. calliceras*, *T. anguina*, *T. maritima*, *T. suavissima*, *T. foenum-graecum*, *T. hamosa*, *T. coerulescens*, **flat** in *T. grandiflora* and *T. Arabica* and **concave** in *T. rechingeri*, *T. cretica* (Table 3 and Figure 4).

Features of seed surface according to numerical analysis

T. foenum-graecum was early separated at the bottom of the phenogram at 1.86 dissimilation distance. This species characterized by seed length from 2-2.2mm. All other species characterized by seed length up to 1.4mm. These species are grouped into two series which split off at distance level 1.73. The first series represented by *T. arabica* characterized by irregular reticulate cells of seed coat. The second series characterized by isodiametric or tangentially elongated cells of seed coat. This series split off at distance level 1.59 into two subseries. The first subseries is characterized by irregular cells of seed coat, concave periclinal walls. This subseries is represented by two species viz. *T. cretica* which is characterized by yellow seeds, hilum apical with acute poles, anticlinal walls straight. *T. rechingeri* is characterized by pale brown seeds, subapical hilum with rounded poles, anticlinal walls wavy. The second subseries is characterized by isodiametric to tangentially elongated cells of seed coat, flat to convex periclinal walls. This subseries split off at distance level 1.43 into two clusters. The first cluster is characterized by dark brown seeds. This cluster split off at

distance 1.27 into two groups. The first group is characterized by rhomboid seeds, papillate seed coat and convex periclinal walls. This group split off at distance level 0.68, it includes two varieties of species *monspeliaca*: var. *petiolata* characterized by tangentially elongated cells of seed coat and var. *eigii* characterized by isodiametric cells of seed coat. The second group is represented by *T. grandiflora* which is characterized by elliptic seeds with seed coat mounded with papillae, with flat periclinal walls. The second cluster is characterized by yellow, orange to pale brown seeds. This cluster split off at distance level 1.09 into two two groups. The first group characterized by papillate seed coat. This group split off at distance level 1.11 into two sub groups. The first subgroup represented by *T. suavissima* which is characterized by pale brown seeds with apical elliptic hilum with rounded poles. The second sub group is characterized by yellow seeds with subapical circular hilum with acute poles. This subgroup split off at distance level 0.7 and includes two species: *T. hamosa* which is characterized by isodiametric cells of seed coat and *T. maritima* which is characterized by tangentially elongated cell of seed coat. The second group is characterized by seed coat mounded with papillae, this group split off at distance level 1 into two subgroups. The first subgroup is characterized by pale brown seeds. This subgroup split off at distance level 0.93 into two classes. The first class is represented by *T. caerulea* which is characterized by transeversely elliptic hilum. The second class is characterized by circular hilum, this class split off at distance level 0.77 and include two species: *T. procumbans* which is characterized by subapical hilum and *T. anguina* which is characterized by apical hilum. The second subgroup is characterized by yellow to orange seeds. This subgroup split off at distance level 0.85 into two classes. The first class is characterized by apical hilum, this class split off at distance level 0.67 into two subclasses. The first subclass is represented by *T. stellata* which is characterized by elliptic seeds. The second subclass is characterized by ovoid seeds; this class split off at distance level 0.53 and include two species: *T. coerulescens* which is characterized by yellow seeds with circular hilum and *T. calliceras* which is characterized by orange seeds with elliptic hilum. The second class is characterized by supraical hilum, this class split off at distance level 0.65 into two subclasses. The first subclass is represented by *T. corniculata* which is characterized by round hilum poles. The second subclass is characterized by acute hilum poles; this class split off at distance level 0.53 and includes two species: *T. strangulate* which is characterized by longitudinally elliptic hilum and *T. balansae* which is characterized by transversally elliptic hilum.

4. Discussion

The present study indicated that the seed characteristics of the studied taxa have a taxonomic significance. Variation in *Trigonella* seed morphology is manifested mainly in seed size, shape, colour, seed coat ornamentation and hilum morphology. Most of the seeds vary from **elliptic**, **rhomboid**, **ovoid** to **rectangular**. The seed shape as observed in the present study seems to be diagnostic at the specific level. Four colours of seeds were recognized: **Yellow**, **Orange**, **Pale**

brown and dark brown. Seeds are classified on the base of size into two groups; small seeds with length up to 1.1 mm and large seeds with length from 1.3 - 2.1 mm. Two positions of hilum were recognized in the studied species: **Apical** in *T. arabica*, *T. anguina*, *T. calliceras*, *T. cretica*, *T. coerulescens*, *T. suavissima* and *T. stellata* and **subapical** in the other studied species.

Seed coat pattern or the micro-ornamentation on the surface of the outer cell wall can be considered of taxonomic value in the identification of the species: seed coat patterns were recognized: **Papillate** in *T. suavissima*, *T. monospeliaca* var. *eigii*, *T. monospeliaca* var. *petiolata*, *T. maritima*, and *T. hamosa* and **mounded with papillae** in all other studied species.

Outlines of seed coat cells ranged between: **Isodiametric, Tangentially elongated, irregular and irregular reticulate. Anticlinal walls wavy in T. rechingeri, T. arabica** and **straight** in all other studied species. Outer periclinal wall **concave** in *T. rechingeri*, *T. cretica*, **flat** in *T. grandiflora* and **convex** in all other studied species. The results of the present study agree with those of [4], [5], [6] and [7].

Artificial key to *Trigonella* species:

1a- seed length up to 1.1 mm	2
1b- seed length up to 2.1 mm	17
2a- seed poles round	3
2b- seed poles truncate	16
3a- seed coat papillate.....	4
3b- seed coat mounded with papillae.....	7
4a- hilum circular.....	5
4b- hilum elliptic.....	<i>T. suavissima</i>
5a- outline of cells isodiametric.....	6
5b- outline of cells tangentially elongated	<i>T. maritima</i>
6a- hilum apical, seed yellow.....	<i>T. stellata</i>
6b- hilum subapical, seed light brown	<i>T. hamosa</i>
7a- anticlinal walls wavy.....	8
7b- anticlinal walls straight.....	9
8a- hilum apical, periclinal wall flat	<i>T. arabica</i>
8b- hilum sub apical, periclinal wall convex ...	<i>T. rechingeri</i>
9a- hilum apical.....	10
9b- hilum sub apical	11
10a- hilum elliptic.....	<i>T. cretica</i>
10b- hilum circular.....	<i>T. coerulescens</i>
11a- hilum elliptic	<i>T. anguina</i>
11b- hilum circular	12
12a- seed orange.....	13
12b- seed light brown.....	15
13a- hilum elliptic.....	14
13b- hilum transversally elliptic	<i>T. balansae</i>
14a- hilum poles round.....	<i>T. strangulate</i>
14b- hilum poles acute	<i>T. corniculata</i>
15a- hilum transversally elliptic	<i>T. caerulea</i>
15b- hilum circular.....	<i>T. procumbans</i>
16a- seed coat papillate.....	<i>T. monospeliaca</i>
16b- seed coat mounded with papillae.....	<i>T. grandiflora</i>
17a- seed poles round, hilum apical.....	<i>T. cretica</i>

17b- seed poles truncate, hilum sub apical.....*T. foenum-graecum*

Key to *T. monospeliaca* subspecies:

1a- outline of cells isodiametric...*T. monospeliaca* var. *eigii*

1b- outline of cells tangentially elongated ...*T. monospeliaca* var. *petiolata*

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Table 1: Names, collection details and sources of seeds included in the present study

No.	Localities	Source Of Seeds	Date Of Collection	Collector
<i>T. anguina</i>		Morocco (NPGS)		
<i>T. arabica</i>	Al Arish –Rafah Road	Egypt	3-2008	Turki et al
<i>T. balansae</i>		Griechenland (IPK)		
<i>T. caerulea</i>		Georgien (IPK)		
<i>T. calliceras</i>		Indien (IPK)		
<i>T. coerulescens</i>		Turkey(IPK)		
<i>T. corniculata</i>		Italian(IPK)		
<i>T. cretica</i>		Unknown (IPK)		
<i>T. foenum-graecum</i>		Italien(IPK)		
<i>T. foenum-graecum</i>		turkey(NPGS)		
<i>T. grandiflora</i>		Kasachstan		
<i>T. hamosa</i>	55km west Alex, Zomorda	Egypt	4-2009	Turki et al
<i>T. maritima</i>	55km west Alex, Zomorda	Egypt	4-2009	Turki et al
<i>T. monspeliaca</i>		Frankreich (IPK)		
<i>T. monspeliaca</i>		Egypt		
<i>T. procumbans</i>		Ungarn (IPK)		
<i>T. rechingeri</i>		Griechenland (IPK)		
<i>T. stellata</i>	3km south Matrouh	Iran (NPGS)	4-2008	Turki et al
<i>T. strangulata</i>		United Kingdom (NPGS)		
<i>T. suavissima</i>		Australlia (NPGS)		

Table 2: The maco-morphological aspect of the spermoderm of studied trigonella species

NO.	Outline	Length (mm)			Width (mm)			Size (mm ²)	L\W (mm ²)	Colour
		min	max	mean	min	max	mean			
<i>T. anguina</i>	Elliptic	0.7	0.9	0.8	0.5	0.6	0.55	0.44	1.45	pale brown
<i>T. Arabica</i>	Ovoid	0.8	1	0.9	0.5	0.7	0.6	0.54	1.5	pale brown
<i>T. balansae</i>	Elliptic	0.8	0.9	0.85	0.5	0.6	0.55	0.47	1.55	Orange
<i>T. caerulea</i>	Elliptic	0.9	1	0.95	0.6	0.8	0.7	0.67	1.36	pale brown
<i>T. calliceras</i>	Ovoid	0.8	1	0.9	0.7	0.9	0.8	0.72	1.13	Orange
<i>T. coerulescens</i>	Ovoid	0.7	0.8	0.75	0.6	0.8	0.7	0.53	1.07	Yellow
<i>T. corniculata</i>	Elliptic	1	1.1	1.05	0.7	0.8	0.75	0.79	1.4	Orange
<i>T. cretica</i>	Elliptic	1.7	1.9	1.8	1.5	1.7	1.6	2.88	1.13	Yellow
<i>T. foenum-graecum</i>	Rectangle Rhomboid	2	2.2	2.1	1.3	1.4	1.35	2.84	1.55	Yellow to pale brown
<i>T. grandiflora</i>	Rectangle	0.9	1.1	1	0.5	0.6	0.55	0.55	1.82	Dark brown
<i>T. hamosa</i>	Elliptic	0.7	0.9	0.8	0.5	0.7	0.6	0.48	1.33	pale brown
<i>T. maritime</i>	Elliptic	0.9	1.1	1	0.5	0.6	0.55	0.55	1.82	Yellow
<i>T. monspeliaca</i>	Rhomboid	0.6	0.7	0.65	0.4	0.5	0.45	0.29	1.44	dark brown
<i>T. monspeliaca</i>	Rhomboid	0.8	0.9	0.85	0.4	0.6	0.5	0.43	1.7	Dark brown
<i>T. procumbans</i>	Elliptic	0.8	1	0.9	0.7	0.8	0.6	0.54	1.5	pale brown
<i>T. rechingeri</i>	Elliptic	1.1	1.2	1.15	0.7	0.8	0.75	0.86	1.53	pale brown
<i>T. stellata</i>	Elliptic	0.5	0.6	0.55	0.4	0.5	0.45	0.25	1.2	Yellow
<i>T. strangulate</i>	Elliptic	0.8	0.9	0.85	0.5	0.6	0.55	0.47	1.55	Orange
<i>T. suavissima</i>	Elliptic	0.6	0.7	0.65	0.4	0.5	0.45	0.29	1.44	pale brown

Table 3: The micro-morphological aspect of the spermoderm of studied trigonella species

NO.	Seed poles	Seed coat pattern	hilum				Outline of cells	Anticlinal wall	Curvature of periclinal wall
			Position	shape	poles	Size(µm)			
<i>T. anguina</i>	truncate	Mounded with papillae	subapical	elliptic	acute	149x116	isodiametric	straight	Convex
<i>T. Arabica</i>	rounded	Mounded with papillae	apical	elliptic	round	125x56	Irregular	wavy	Flate
<i>T. balansae</i>	round	Mounded with papillae	subapical	Transversally elliptic	round	50x65	isodiametric	straight	Convex
<i>T. caerulea</i>	Round	Mounded with papillae	subapical	Transversally elliptic	round	73x82	isodiametric	straight	Convex
<i>T. calliceras</i>	Round	Mounded with papillae	apical	circular	round	30x28	isodiametric	straight	Convex
<i>T. coerulescens</i>	Round	Mounded with papillae	apical	circular	round	55x61	isodiametric	straight	Convex
<i>T. corniculata</i>	round	Mounded with papillae	subapical	elliptic	acute	65x37	isodiametric	straight	Convex
<i>T. cretica</i>	Round	Mounded with papillae	apical	elliptic	round	116x91	irrigular	straight	Concave
<i>T. foenum-graecum</i>	Round to truncate	Mounded with papillae	subapical	circular to elliptic	round to acute	152x118	isodiametric	straight	Convex
<i>T. grandiflora</i>	truncate	Mounded with papillae	subapical	Transversally elliptic	round	38x46	isodiametric	straight	Flate
<i>T. hamosa</i>	rounded	Papillae	subapical	circular	round	56x75	isodiametric	straight	Convex
<i>T. Maritima</i>	rounded	Papillae	subapical	circular	round	40x47	Tangentially elongated	straight	Convex
<i>T. monospeliaca var. eigii</i>	truncate	Papillae	subapical	Transversal elliptic	round	35x46	Tangentially elongated	straight	Convex
<i>T. monospeliaca var. petiolata</i>	truncate	Papillae	subapical	Transversally elliptic	round	35x46	isodiametric	straight	Convex
<i>T. procumbans</i>	Round	Mounded with papillae	apical	elliptic	round	72x54	isodiametric	straight	Convex
<i>T. rechingeri</i>	Round	Mounded with papillae	subapical	elliptic	acute	96x63	irrigular	wavy	Concave
<i>T. stellata</i>	round	Papillae	apical	circular	round	26x24	isodiametric	straight	Convex
<i>T. strangulata</i>	Round	Mounded with papillae	subapical	elliptic	round	59x50	isodiametric	straight	Convex
<i>T. suavissima</i>	Round	Papillae	apical	Elliptic	acute	60x49	isodiametric	straight	Convex

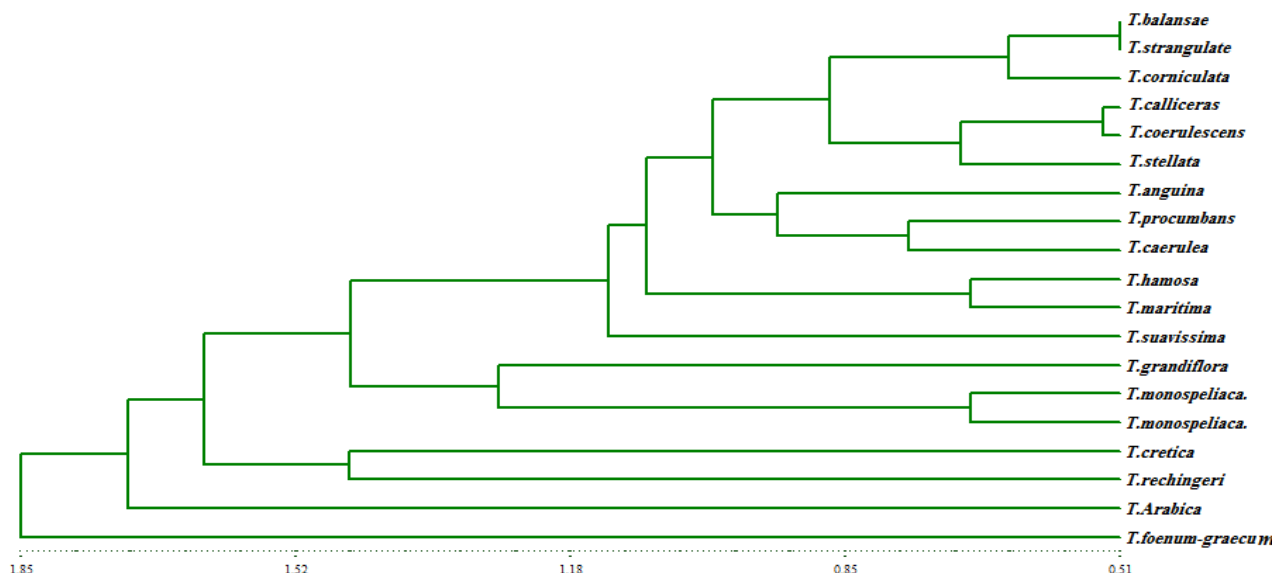


Figure 5: Phenogram illustrating the relationships between the Trigonella species on the bases of seeds characters

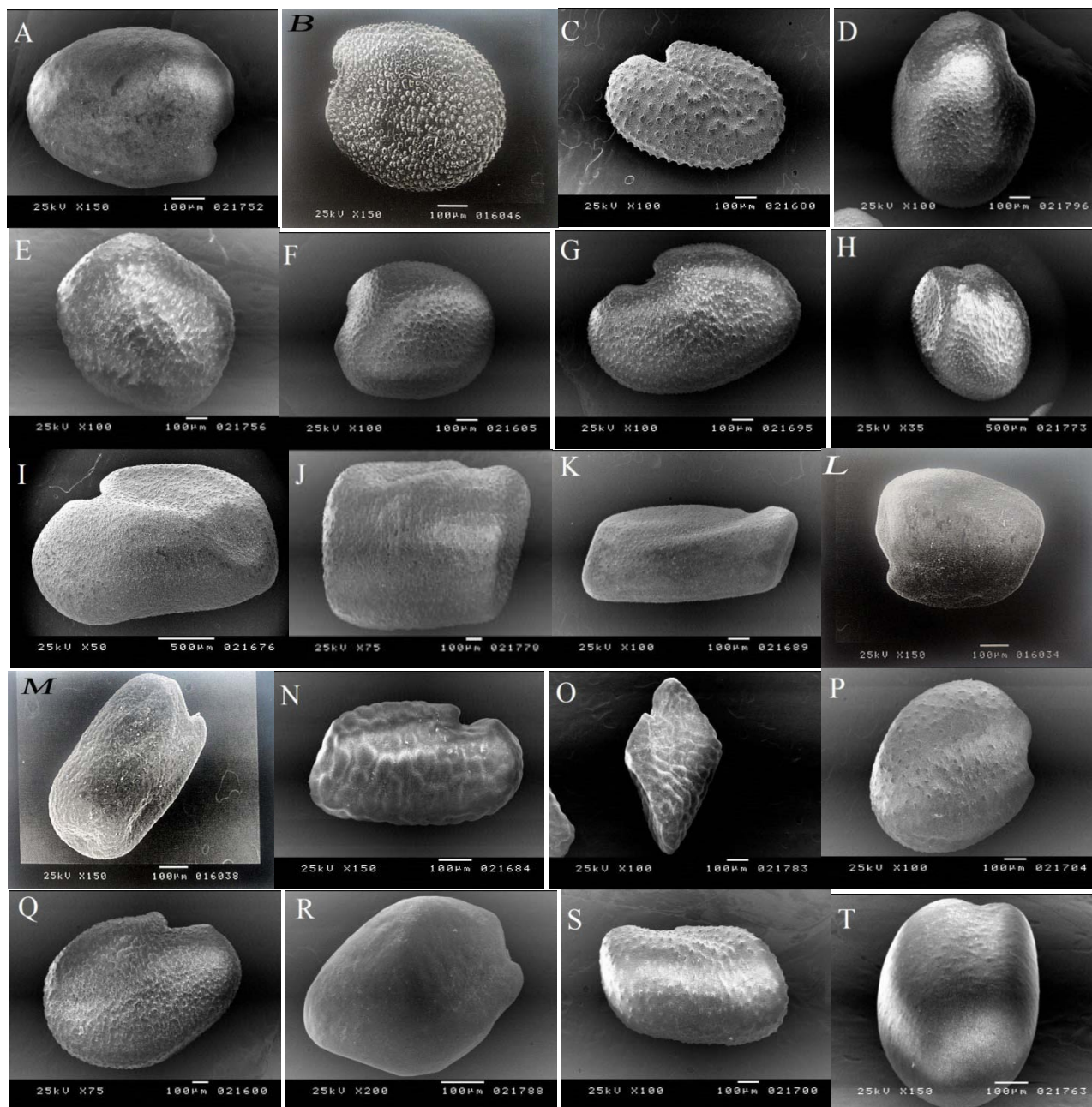


Figure 1: SEM of Seed morphology of *Trigonella* species: (A) *T. anguina*, (B) *T. Arabica*, (C) *T. balansae*, (D) *T. caerulea*, (E) *T. calliceras*, (F) *T. coerulescens*, (G) *T. corniculata*, (H) *T. cretica*, (I), (J) *T. foenum-graecum*, (K) *T. grandiflora*, (L) *T. hamosa*, (M) *T. maritime*, (N) *T. monspeliaca* var. *petiolata* (O) *T. monspeliaca* var. *eigii* (P) *T. procumbans*, (Q) *T. rechingeri*, (R) *T. stellata*, (S) *T. strangulate*, and (T) *T. suavissima*.

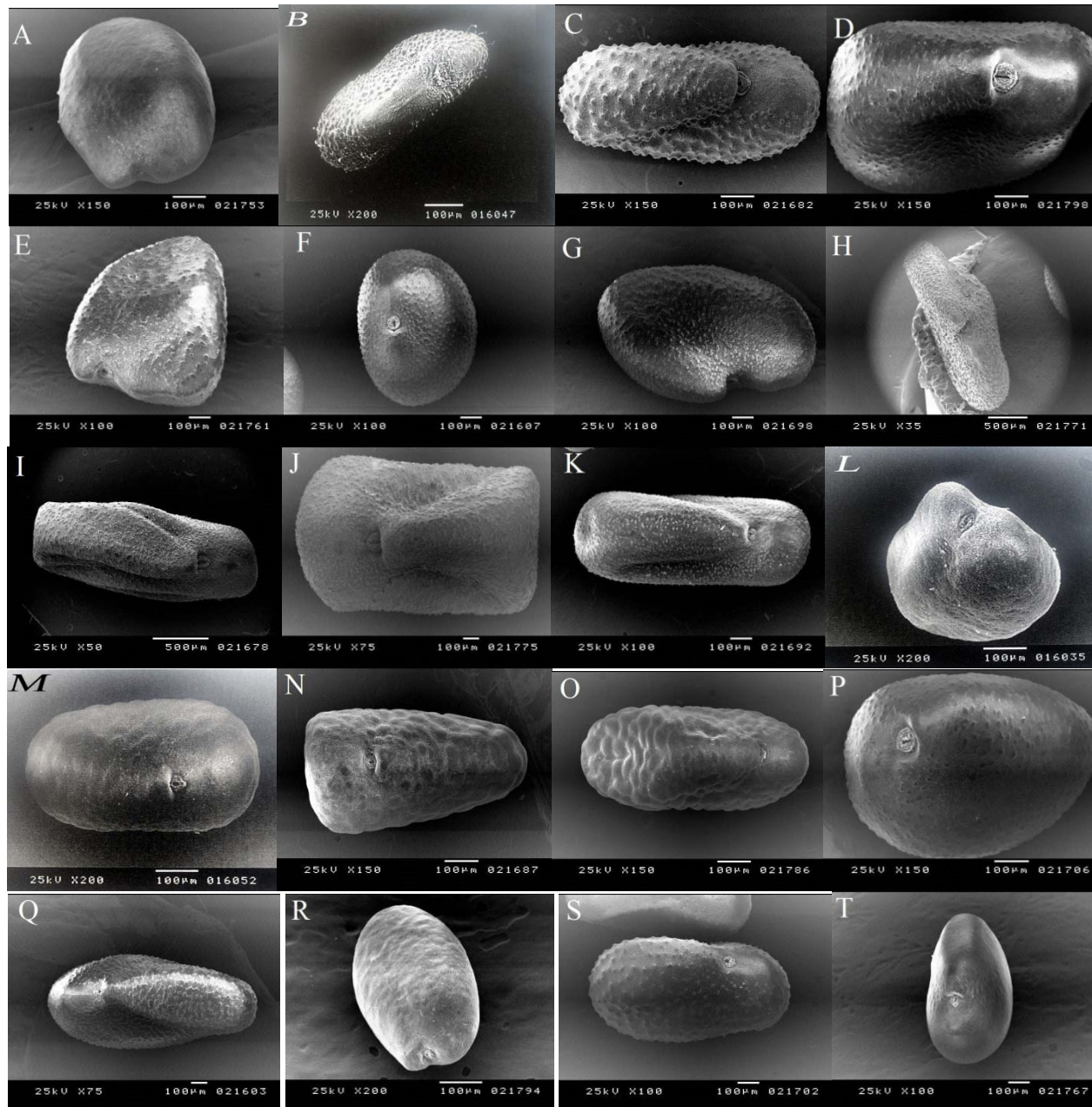


Figure 2: SEM of Hilum position of *Trigonella* species: (A) *T. anguina*, (B) *T. Arabica*, (C) *T. balansae*, (D) *T. caerulea*, (E) *T. calliceras*, (F) *T. coerulescens*, (G) *T. corniculata*, (H) *T. cretica*, (I), (J) *T. foenum-graecum*, (K) *T. grandiflora*, (L) *T. hamosa*, (M) *T. maritime*, (N) *T. monspeliaca* var. *petiolata* (O) *T. monspeliaca* var. *eigii* (P) *T. procumbans*, (Q) *T. rechingeri*, (R) *T. stellata*, (S) *T. strangulate*, and (T) *T. suavissima*.

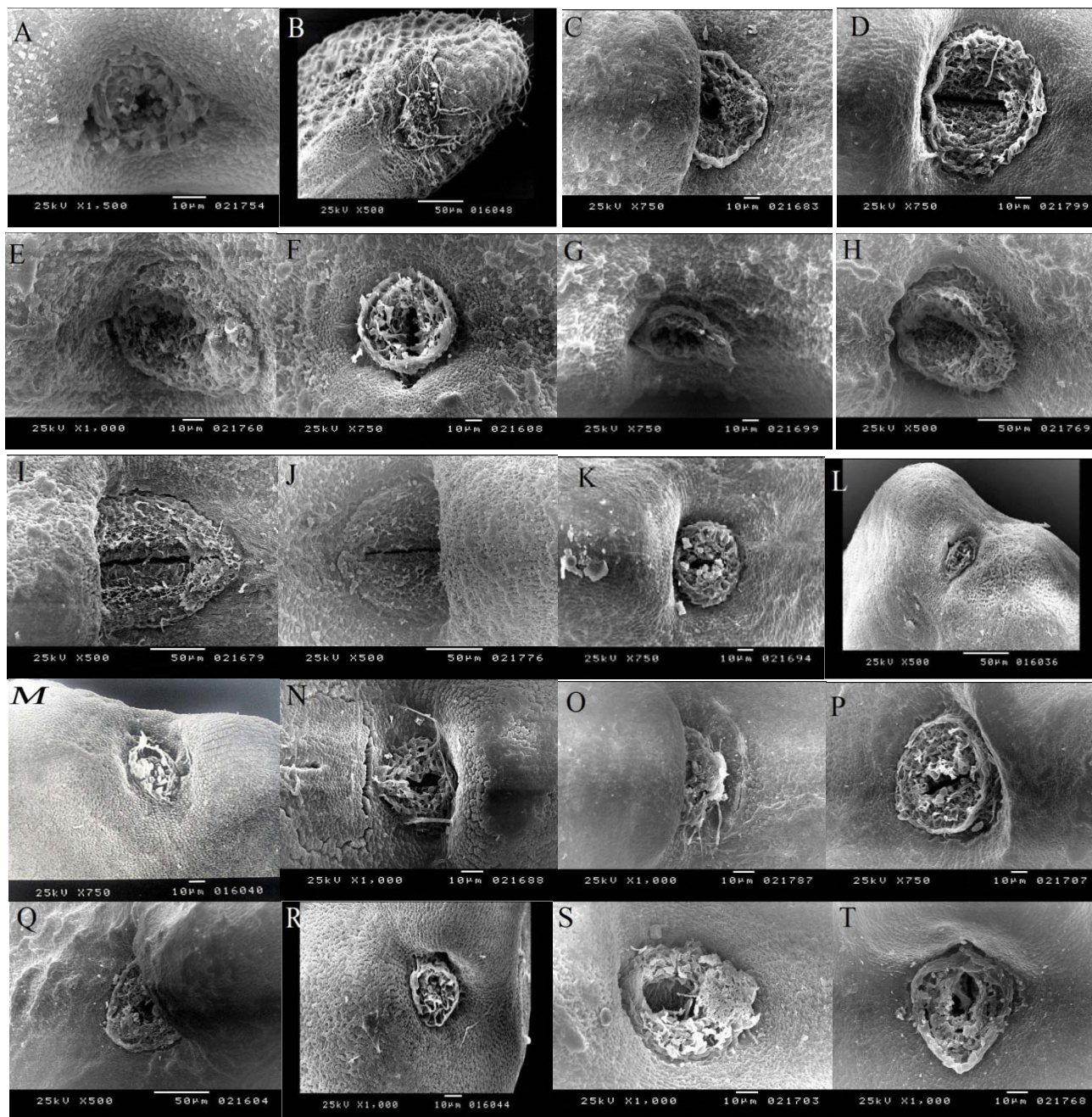


Figure 3: SEM of Hilum shapes of *Trigonella* species: (A) *T. anguina*, (B) *T. Arabica*, (C) *T. balansae*, (D) *T. caerulea*, (E) *T. calliceras*, (F) *T. coerulescens*, (G) *T. corniculata*, (H) *T. cretica*, (I), (J) *T. foenum-graecum*, (K) *T. grandiflora*, (L) *T. hamosa*, (M) *T. maritime*, (N) *T. monspeliaca* var. *petiolata* (O) *T. monspeliaca* var. *eigii* (P) *T. procumbans*, (Q) *T. rechingeri*, (R) *T. stellata*, (S) *T. strangulate*, and (T) *T. suavissima*.

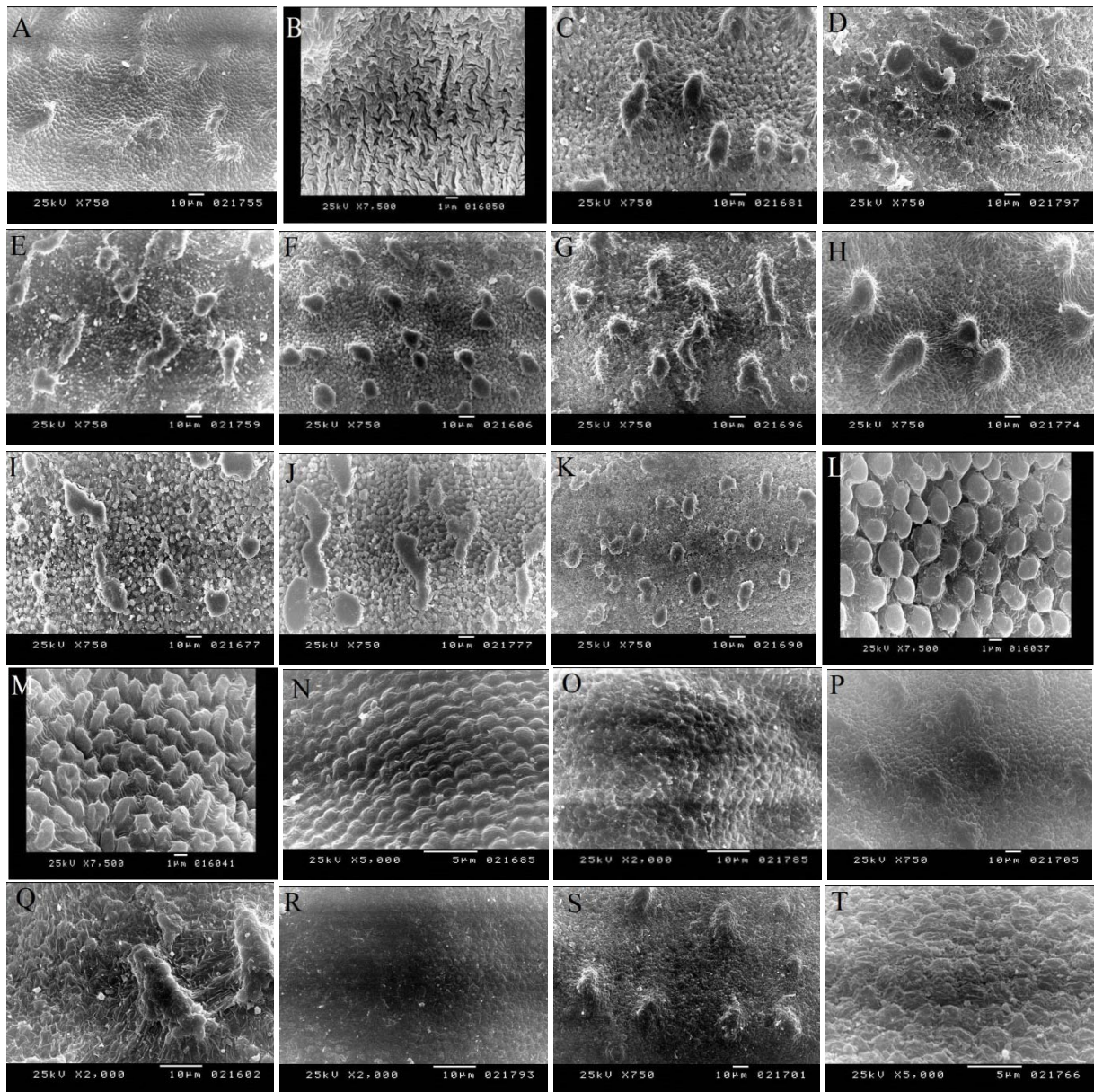


Figure 4: SEM of Seed coat pattern of Trigonella species: (A) *T. anguina*, (B) *T. Arabica*, (C) *T. balansae*, (D) *T. caerulea*, (E) *T. calliceras*, (F) *T. coerulescens*, (G) *T. corniculata*, (H) *T. cretica*, (I), (J) *T. foenum-graecum*, (K) *T. grandiflora*, (L) *T. hamosa*, (M) *T. maritime*, (N) *T. monspeliaca* var. *petiolata* (O) *T. monspeliaca* var. *eigii* (P) *T. procumbans*, (Q) *T. rechingeri*, (R) *T. stellata*, (S) *T. strangulate*, and (T) *T. suavissima*.