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Seed and pollen morphology and numerical analysis of *Tephrosia* Pers. (Fabaceae) and their taxonomic significance

Ahmed Elkordy[1*], Ahmed K. Osman[2], Mohamed O. Badry[2]

[1] Sohag University, Faculty of Science, Botany and Microbiology Department, 82524, Sohag, Egypt

[2] South Valley University, Faculty of Science, Botany and Microbiology Department, Qena 83523, Egypt

Abstract – The seed and pollen grain morphology of the genus *Tephrosia* Pers. in Egypt was studied using light and scanning electron microscopy. Qualitative and quantitative characters of seeds and pollen grains are presented. The data suggest that several seed and pollen morphological characters can be used to distinguish the taxa of *Tephrosia*. Based on UPGMA clustering analysis and PCA, three main clades were recognized: Clade A comprising *T. kassasii*, clade B comprising *T. apollinea*, *T. purpurea*, *T. quartiniana*, and *T. uniflora*, and clade C comprising *T. nubica*. The seed and pollen morphological data obtained in this study provide additional characters that assist in the classification of the genus. Dichotomous artificial keys based on seed and pollen data of the investigated taxa are presented.

Keywords: pollen, principal component analysis, scanning electron microscopy, seeds, taxonomy, *Tephrosia*

Introduction

Tephrosia Pers. is one of the larger genera of the tribe Millettieae, subfamily Papilionoideae, of Fabaceae. The genus comprises ca. 350 species and infraspecific taxa with a pantropical distribution, concentrated in Africa-Madagascar (170 taxa), Asia (40), Central and tropical N. America (45), and Australia (90) (Lewis et al. 2005, Zhi and Pedley 2010, POWO 2021). Taxonomically, the genus *Tephrosia* is a very problematic complex as a distinction between some taxa of the genus is still subject to discussion and needs much further investigation (Schrire 2005, Lakshmi et al. 2008).

Many taxonomists classified the genus *Tephrosia* into subgenera or sections based mainly on morphological traits. de Candolle (1825) suggested classifying the genus *Tephrosia* into four sections: *Brissonia*, *Craccoides*, *Mundulea*, and *Reineria*. Bentham (1862) classified *Tephrosia* into two sections: sect. *Brissonia* (Neck.) DC. and sect. *Recueria* Benth. Later, three subgenera of *Tephrosia* were proposed by Bentham (1865) and Baker (1876) as *Macronyx*, which comprises *T. tenuis*, *Brissonia,* which comprises *T. candida,* and *Reineria*, which comprises the remaining species of *Tephrosia*. The most recent infrageneric taxonomy was suggested by Brummitt (1980), who classified *Tephrosia* into two subgenera: *T.* subgenus *Tephrosia* and *T.* subgenus *Barbistyla* based on the presence or absence of trichomes on styles and stigmas (de Queiroz et al. 2015). Although this ranking has been largely agreed upon, there is some overlap in diagnostic characteristics among the taxa of the two suggested subgenera (Lakshmi et al. 2008). Based on a few analyzed taxa, *Tephrosia* was considered monophyletic (Hu et al. 2002), but the subgenera of Brummitt (1980) were not supported by the genetic and other molecular studies conducted on the genus (Hu et al. 2002, Acharya et al. 2004, Lakshmi et al. 2008). Instead, three species complexes in *T.* subgenus *Tephrosia*, represented by *T. purpurea*, *T. cinerea*, and *T. adunca* were distinguished based on density and coloration of the indumentum, flower location, and size (de Queiroz 2012).

The seed surface micromorphology of angiosperms and gymnosperms can exhibit significant structural details (Barthlott 1981). Seed micromorphology has proven to be a good taxonomic tool, giving several characters significant for classifying some genera of Faboideae (Papilionoideae), particularly for the genus *Tephrosia* (Rao and Rao 2008, Al-Ghamdi and Al-Zahrani 2010, de Queiroz et al. 2013). The seed surface of *Tephrosia* subgenus *Tephrosia* has a simple reticulate pattern, while in *Tephrosia* subgenus

* Corresponding author e-mail: aelkordy@science.sohag.edu.eg

Barbistyla, the surface has a crested ornamentation pattern (de Queiroz et al. 2013). Moreover, the variations in the morphology of pollen grains are significant for the identification delimitation of taxa in *Tephrosia* (Buril et al. 2011, Antonio-Domingues et al. 2019). Combining the macro- and microstructure of seeds and pollen grains is important for the taxonomy of many angiosperm taxa (Abdelkhalik et al. 2008).

In Egypt, the genus *Tephrosia* is represented by six species and two subspecies, mainly distributed in Gebel Elba, desert wadis, the Nile valley, and oases (Täckholm 1974, Boulos 1999). The current study aims to investigate the macro-and micromorphology of seeds and pollens of the genus *Tephrosia* in Egypt using light microscope and scanning electron microscope (SEM) to assess their taxonomic significance. Provision of identification keys to the studied *Tephrosia* species based on seed and pollen macro- and micromorphological characters is aimed at further applications in other taxonomic studies of the genus *Tephrosia*.

Materials and methods

Plant material

The current study was mainly based on specimens kept in the following Egyptian herbaria: CAI Herbarium at Cairo and South Valley University herbarium at Qena (herbarium acronyms follow Thiers (2020) (On-line Suppl. Tab. 1). The nomenclature of the studied taxa was updated according to POWO (2021).

Samples and techniques

To study seeds' macro and micro-morphological characters, 15-30 seeds per species taken from five healthy and fully mature plants and seed specimens were studied by a conventional method using a stereomicroscope (SM) and scanning electron microscope (SEM). Seed samples were examined with an Olympus SZX7 stereo dissecting microscope.

For SEM, dried seed specimens were mounted on metallic stubs utilizing double adhesive tape and coated with gold for 4-6 minutes (150-200 Angstrom) in a sputtering chamber employing a JEOL JFC1100E ion-sputtering device. All quantitative measurements of seed characters from the SM and SEM images were obtained utilizing the program ImageJ v1.45 (Schneider et al. 2012). Mean quantitative measurements were obtained from 35 readings from each specimen.

Pollen grains of the studied taxa were extracted from 5 to 10 flowers per species, taken from five healthy plant specimens, to study macro and micro-morphological characters of *Tephrosia* pollen grains. Examination of pollen grains using Light Microscopy (LM) was achieved employing an Olympus BH-2 research microscope. The pollen grain samples were acetolyzed and mounted on a metallic stub in some drops of ethanol based on the methods summarized in Moore et al. (1991). The mean quantitative measurements of pollen grains were conducted based on a minimum of 40 pollen grains per specimen. Pollen grain specimens were studied employing scanning electron microscopy (JEOL JSM 5400 LV), under an accelerated voltage of 15 kV (150-200 Angstrom). All photomicrographs were captured at the Unit of Electron Microscope, University of Assiut, Egypt. All quantitative measurements were compiled using the ImageJ v1.45 program (Schneider et al. 2012).

The main morphological characters, terminology, and concepts of pollen grains and seeds used here follow previous studies (Huysmans et al. 2003, Punt et al. 2007, de Queiroz et al. 2013, Lawrence 2017) with some modifications by the authors.

Data analyses

Aspects of plant, seed, and pollen micro-and macromorphology based on 62 characters were recorded and scored for the OTUs (operational taxonomic units) in a data matrix: 30 qualitative (nine binary and 21 multi-state characters) and 32 quantitative continuous characters. Two analyses were performed with PAST (Paleontological Statistics software, ver. 4.03) (Hammer et al. 2001). A hierarchical cluster analysis (HCA) based on Euclidean distance similarity index and using the unweighted pair group method using the arithmetic averages (UPGMA) clustering method (Sokal 1958, Seberg et al. 1991) was generated to classify the studied taxa into clusters based on overall character similarity. Then, a principal component analysis (PCA) was conducted to reveal whether the analyzed characters could cluster taxa and to recognize the most distinctive morphological character(s) for the studied taxa, as in other studies (e.g., Coutinho et al. 2011, Lopes et al. 2013, Badry et al. 2020). Eigenvalues were plotted in a two-dimensional scatter plot along the first two principal component axes (PCA1, PCA2), accounting for the highest character variation.

Results

Seed

Seed shape among the studied taxa showed large variation, from reniform, elliptical to oblong, oblong reniform, oval, rectangular, and quadrate (Tab. 1). However, seed shape is reniform in *T. apollinea* (Fig. 1A), elliptical to oblong in *T. nubica* (Fig. 1D), oblong reniform in *T. purpurea* (Fig. 1G), oval in *T. quartiniana* (Fig. 1J), rectangular in *T. uniflora* (Fig. 1M) and quadrate in *T. kassasii* (Fig. 1P).

The seed size of the investigated taxa ranged from 1.96 × 1.34 mm in *T. quartiniana* to 4.35 × 2.77 mm in *T. apollinea*. The rest of the taxa have intermediate-sized seeds. However, it is approximately 4.00 × 2.45 mm in *T. nubica,* 3.34 × 2.30 mm in *T. purpurea,* 2.54 × 1.34 mm in *T. uniflora,* and 2.09 × 1.80 mm in *T. kassasii* (Tab. 1). Seeds with the lowest length-width ratio occur in *T. quartiniana* (1.12) and the highest in *T. uniflora* (1.90) (Tab. 1).

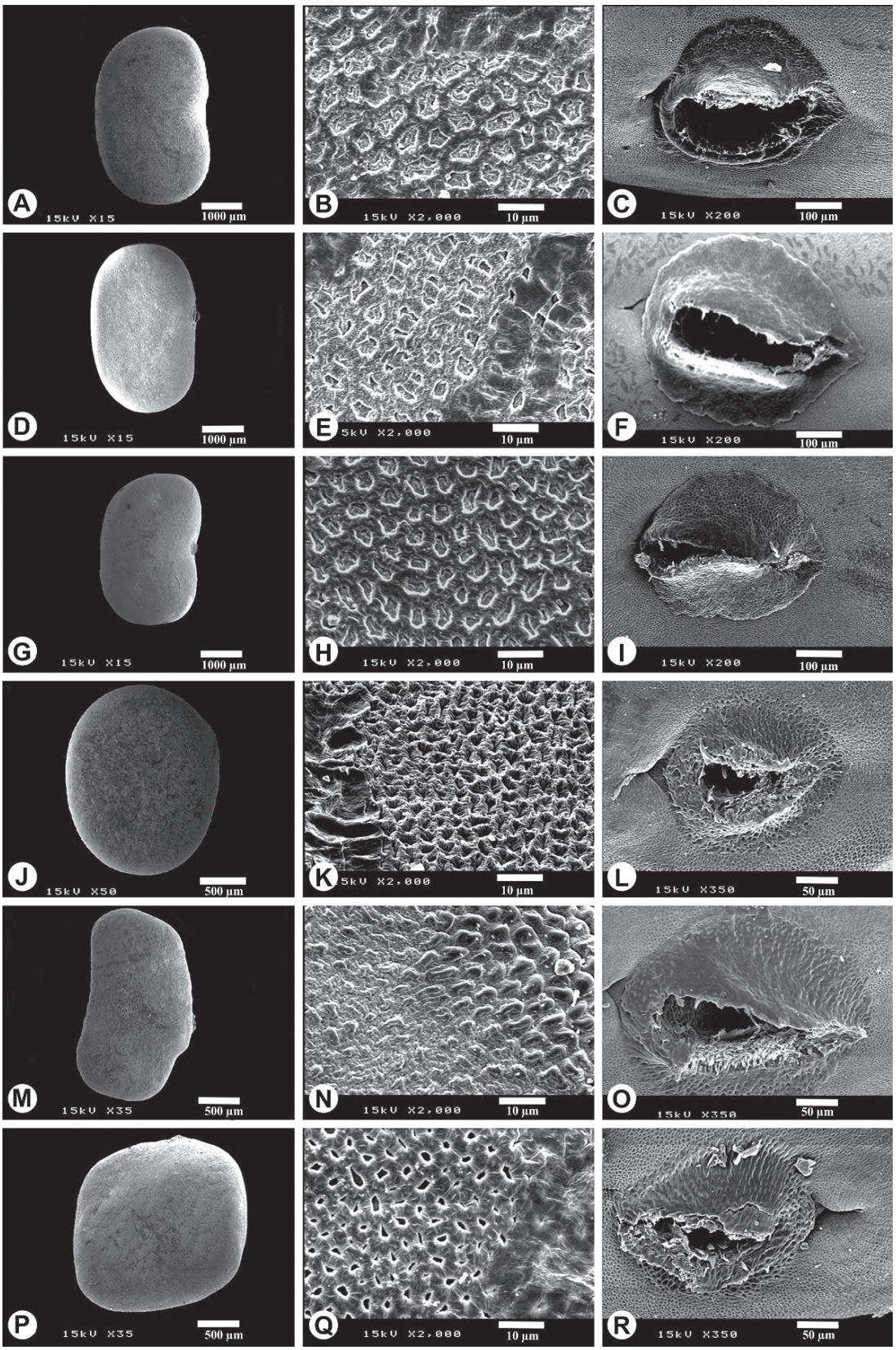

Fig. 1. Scanning electron microscope micrographs of *Tephrosia* seeds. Side view of whole seed (A, D, G, J, M, P), testa ornamentation (B, E, H, K, N, Q) and hilum shape (C, F, I, L, O, R). *T. apollinea* (A–C), *T. nubica* (D–F), *T. purpurea* (G–I), *T. quartiniana* (J–L), *T. uniflora* (M–O), *T. kassasii* (P–R).

Three different patterns of testa texture have been reported: reticulated, multi reticulated, and multi crested. However, it is reticulated in *T. apollinea* and *T. purpurea* (Fig. 1B, H) respectively; multi reticulated in *T. nubica, T. quartiniana*, and *T. kassasii* (Fig. 1E, K, Q) respectively; multi crested in *T. uniflora* (Fig. 1N).

Tab. 1. Morphological characteristics of the studied *Tephrosia* taxa. Min – minimum, Max – maximum, L – length, W – width. The number of measurements for each taxon N = 35.

Taxon	Shape	Seed dimensions			Testa texture	Epidermal cell	
		L (mm) (Min–Max)	W (mm) (Min–Max)	L/W (Min–Max)		Shape	Area (μm²) (Min–Max)
Tephrosia apollinea (Delile) DC.	Reniform	4.35 (4.27–4.43)	2.77 (2.71–2.93)	1.57 (1.58–1.76)	Reticulate	5,6 gonals	19.02 (6.79–32.03)
Tephrosia nubica (Boiss.) Baker	Elliptical to Oblong	4.00 (3.91–4.29)	2.45 (2.23–2.66)	1.63 (1.68–1.74)	Multi Reticulate	4,5,6 gonals	7.73 (2.14–10.19)
Tephrosia purpurea (L.) Pers.	Oblong reniform	3.34 (2.97–3.91)	2.30 (2.01–2.62)	1.45 (1.43–1.58)	Reticulate	5,6 gonals	8.67 (3.41–15.68)
Tephrosia quartiniana Cufod.	Oval	1.96 (1.82–2.29)	1.75 (1.71–1.89)	1.12 (1.06–1.27)	Multi Reticulate	Polygonal	5.38 (2.89–8.24)
Tephrosia uniflora Pers.	Rectangular	2.54 (2.43–2.84)	1.34 (1.28–1.49)	1.90 (1.44–1.97)	Multi Crested	Polygonal	2.45 (1.19–6.41)
Tephrosia kassasii Boulos	Quadrate	2.09 (2.05–2.32)	1.80 (1.54–2.16)	1.16 (1.33–1.03)	Multi Reticulate	4,5,6 gonals	3.80 (0.29–5.51)

The cellular shapes can be of considerable diagnostic and systematic value among the studied taxa. They vary from polygonal; 5, 6 gonals; 4, 5, 6 gonals. However, it is polygonal in *T. quartiniana* (Fig. 1K); 5, 6 gonals in *T. apollinea* and *T. purpurea* (Fig. 1B, H) respectively; 4, 5, 6 gonals in *T. nubica, and T. kassasii* (Fig. 1E, Q) respectively, while it was crested in *T. uniflora* (Fig. 1N).

The area of the epidermal cells shows a highly significant variation among the studied taxa. However, it varies from 2.45 μm² in *T. kassasii* to 19.02 μm² in *T. apollinea* (Tab. 1, Fig. 1Q, B) respectively, while the rest of the studied taxa are characterized by intermediate epidermal cell areas: 5.38 μm² in *T. quartiniana*, 7.73 μm² in *T. nubica*, 8.67 μm² in *T. purpurea* (Fig. 1K, E, H) respectively.

The form of the anticlinal boundaries is of less taxonomic and systematic value among the studied taxa. However, all investigated taxa are characterized by raised and straight anticlinal boundaries. The surface of the anticlinal boundaries of the studied taxa is smooth to fine folded in *T. kassasii* (Fig. 1Q), coarse folded in *T. purpurea* (Fig. 1H), wavy in *T. uniflora* (Fig. 1N), and fine folded in the rest of the taxa.

The thickness of the anticlinal boundaries varies from 2.34 μm in *T. quartiniana* to 3.92 μm in *T. nubica* (Tab. 2).

The form of the periclinal cell wall is concave in *T. quartiniana* (Fig. 1K), Convex in *T. uniflora* (Fig. 1N), and flat in the rest of the studied taxa. There are three different shapes for the surface of the periclinal cell wall: rugulate in *T. apollinea* (Fig. 1B), favulariate in *T. nubica* and *T. purpurea* (Fig. 1E, H) and smooth in *T. quartiniana, T. uniflora and T. kassasii* (Fig. 1K, N, Q).

Three different shapes of hilum have been recognized among the studied taxa: ovate in *T. apollinea, T. nubica, T.*

Tab. 2. Morphological characteristics of the studied *Tephrosia* taxa. Min – minimum, Max – maximum, L – length, W – width. The number of measurements for each taxon N = 35.

Taxon	Anticlinal boundaries			Periclinal cell wall		Hilum				
	Form	Thickness (μm) (Min–Max)	Surface	Form	Surface	Shape	Position	L (mm)	W (mm)	Area (mm²)
T. apollinea	Raised, straight	3.24 (2.68–3.61)	Fine folded	Flat	Rugulate	Ovate	Central	0.44	0.33	0.10
T. nubica	Raised, straight	3.92 (3.27–4.46)	Fine folded	Flat	Favulariate	Ovate	Sub–central	0.50	0.42	0.15
T. purpurea	Raised, straight	3.55 (2.47–4.00)	Fine folded	Flat	Favulariate	Ovate to elliptic	Central	0.43	0.32	0.10
T. quartiniana	Raised, straight	2.34 (1.14–3.20)	Course folds	Concave	Smooth	Ovate	Sub–central	0.24	0.20	0.030
T. uniflora	Raised, wavey	3.96 (3.29–4.48)	Smooth	Convex	Smooth	Circular	Sub–central	0.33	0.25	0.06
T. kassasii	Raised, straight	3.30 (2.67–3.78)	Smooth to fine folded	Flat	Smooth	Ovate	Sub–central	0.25	0.20	0.04

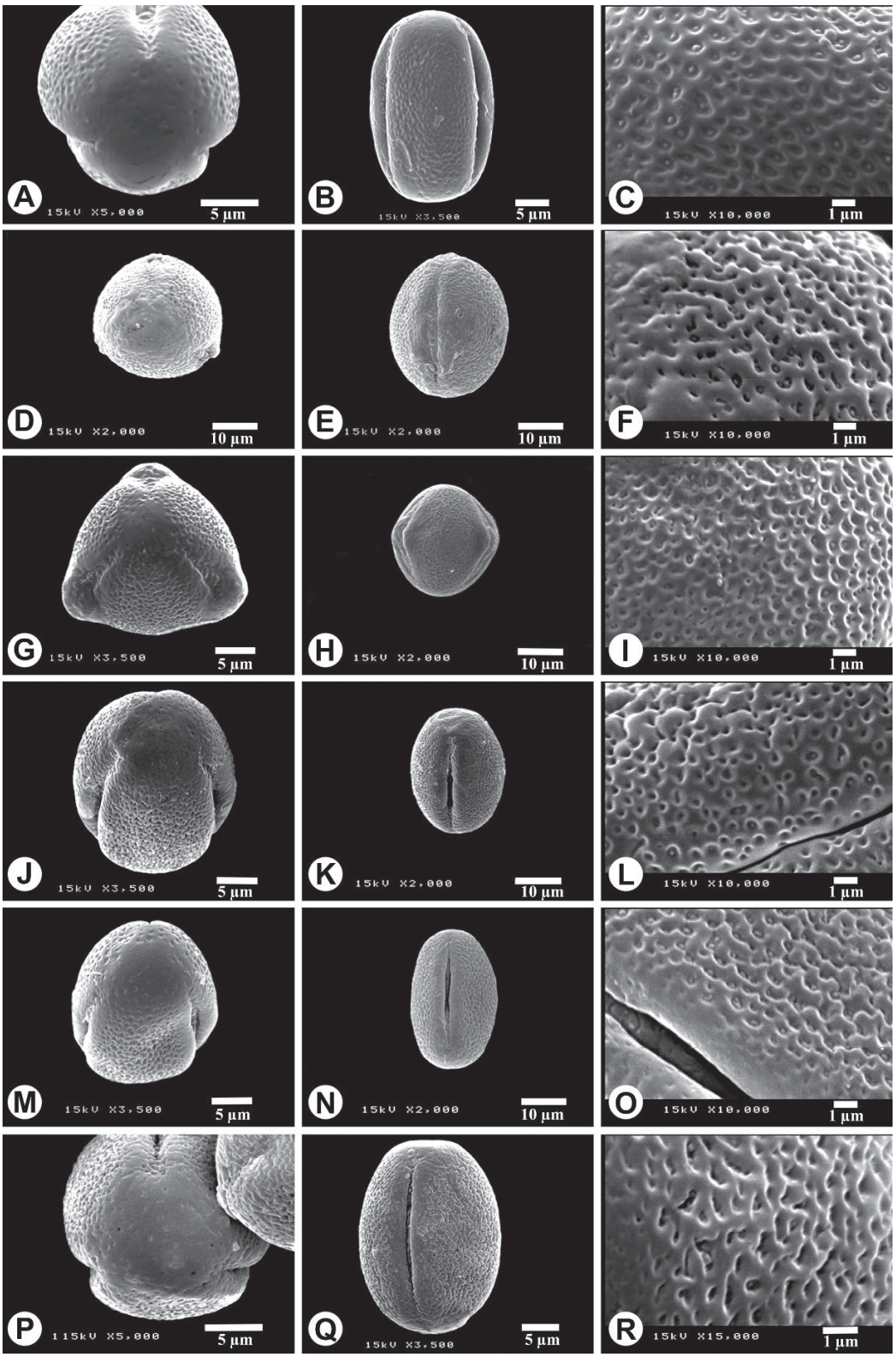

Fig. 2. Scanning electron microscope micrographs of *Tephrosia* pollen grains. Polar view (A, D, G, J, M, P), equatorial view (B, E, H, K, N, Q) and exine ornamentation (C, F, I, L, O, R). *T. apollinea* (A–C), *T. nubica* (D–F), *T. purpurea* (G–I), *T. quartiniana* (J–L), *T. uniflora* (M–O), *T. kassasii* (P–R).

quartiniana, and *T. kassasii* (Fig. 1C, F, L, R) respectively, ovate to elliptic in *T. purpurea* (Fig. 1I) and circular in *T. uniflora* (Fig. 1O). The position of the hilum is either central in *T. apollinea* and *T. purpurea* (Fig. 1C, I), or subcentral in the rest of the investigated taxa.

The diameter of the hilum varies from 0.25 × 0.20 mm in *T. kassasii* to 0.50 × 0.42 mm in *T. nubica* (Tab. 2, Fig. 1R, F), respectively. The hilum area varies from 0.03 mm^2 in *T. quartiniana* to 0.15 mm^2 in *T. nubica* (Tab. 2, Fig. 1L, F) respectively.

Artificial key to taxa of *Tephrosia* based on the seed morphological characteristics:

1.a. Seed testa texture multicrested ..... *T. uniflora*
1.b. Seed testa texture reticulate to multireticulate 2
2.a. Seed epidermal cell shape polygonal ..... *T. quartiniana*
2.b. Seed epidermal cell shape 4,5,6 gonals or 5,6 gonals .. 3
3.a. Seed width 1.8 (1.54-2.16) mm ..... *T. kassasii*
3.b. Seed width 2.00-3.00 4
4.a. Seed length 3.34 (2.97-3.91) ..... *T. purpurea*
4.b. Seed length 3.90-4.5 mm 5
5.a. Seed epidermal cell area 19.02 (6.79-32.03) mm ..... *T. apollinea*
5.b. Seed epidermal cell area 7.73 (2.14-10.19) mm ..... *T. nubica*

Pollen

Pollen dispersed as monads, isopolar, radially symmetric, 3-zonocolporate. Colpi with pointed apices, with granulate, scabrate to granulate–scabrate membrane; endoaperture lolongate with endexine protrusions (Main characteristics of the analyzed pollen grains of the studied taxa are summarized in detail in (Fig. 2, Tabs. 3-5).

The shape in the equatorial view varies from the prolate to the subprolate (Tab. 5), while they are circular, either fossaperturate (Fig. 2A, P) or planaperturate (Fig. 2D) or triangular planaperturate (Fig. 2G), in polar view.

Pollen grains of the studied taxa are relatively small to medium size, the Polar axis length (*P*) exceeds 30.0 µm in only one species (*T. nubica*) at 31.11 µm (Fig. 2E). The average polar axis (*P*) ranges from 25.02 µm in *T. purpurea* to 31.11 µm in *T. nubica*, while the average equatorial axis length (*E*) ranges from 18.43 µm in *T. apollinea* to 26.62 in *T.* nubica.

All the investigated taxa have tectate exine with either punctuate ectexine with small, rounded granules in the luminae (Fig. 2C, O) or foveolate to microreticulate in the rest of the studied taxa (Fig. 2C-R).

Artificial key to taxa of *Tephrosia* based on the palynological results of this study

1a. Pollen grains have punctuated ectexine, small, rounded granules in the luminae 2
1b. Pollen grains have foveolate to microreticulate ectexine 3
2a. Colpus length ranges from 10.68 to 19.67 µm ..... *T. uniflora*
2b. Colpus length ranges from 20.25 to 26.78 µm ..... *T. apollinea*
3a. Pollen grains prolate-spheroidal ..... *T. purpurea*
3b. Pollen grains prolate or sub prolate 4
4a. Pollen grains sub prolate with microreticulate ectexine ..... *T. nubica*
4b. Pollen grains prolate with rugulate to rugulate-reticulate ectexine 5
5a. Polar axis ranges from 23.81 to 26.75 µm ..... *T. kassasii*
5b. Polar axis ranges from 27.00 to 33.92 µm .. *T. quartiniana*

Statistical analyses

The phenetic relationships of the studied taxa of *Tephrosia* reflected in the morphological diversity of the seeds and pollens are presented through two statistical analyses. The dendrogram of all OTUs studied, clustered by the UPGMA method, is shown in (Fig. 3). The cophenetic correlation of the distance matrix and tree matrix was 0.9883, indicating a good fit of the dendrogram to the distance matrix (see Rohlf 1990). Three main clades were recognized: Clade A comprising *T. kassasii*, Clade B comprising *T. apollinea*, *T. purpurea*, *T. quartiniana*, and *T. uniflora*, and clade C comprising *T. nubica*. Generally, Clade B is divided into two subgroups: subgroup (I) comprising *T. apollinea*, *T. purpurea*, and subgroup (II) comprising *T. quartiniana* and *T. uniflora*. The scatter plot of six OTUs on the first two principal component axes is shown in (On-line Suppl. Fig. 1), interpreting 99.81% of the total observed variation. On the first component axis, 74.81% of the total variation, segregation is veri-

Tab. 3. Morphological characteristics of the studied *Tephrosia* taxa. Min – minimum, Max – maximum, L – length, W – width, P – polar axis, E – equatorial diameter. The number of measurements for each taxon N = 40.

Taxon	*P*			*E*		(*P*/*E*) (Min–Max)
	Axis (µm) (Min–Max)	Distance (µm) (Min–Max)	View area (µm²) (Min–Max)	Diameter (µm) (Min–Max)	View area (µm²) (Min–Max)	
T. apollinea	28.31 (26.29–30.14)	14.22 (13.54–14.60)	302.71 (231.64–332.03)	18.43 (16.59–19.83)	420.98 (336.06–448.09)	1.54 (1.46–1.69)
T. nubica	31.11 (27.89–32.54)	15.56 (13.94–16.27)	604.24 (555.73–624.13)	26.62 (26.00–27.08)	639.13 (583.99–669.25)	1.18 (1.06–1.23)
T. purpurea	25.02 (24.98–25.06)	20.04 (12.49–25.20)	397.06 (360.16–437.04)	24.22 (23.95–24.96)	433.35 (430.56–435.54)	1.02 (1.00–1.04)
T. quartiniana	30.28 (27.00–33.92)	15.14 (13.50–16.96)	355.15 (351.26–358.30)	19.85 (17.49–22.64)	470.57 (420.22–521.70)	1.56 (1.22–1.92)
T. uniflora	29.23 (23.64–30.96)	14.61 (11.82–15.48)	258.55 (238.31–286.20)	18.80 (18.35–19.37)	428.68 (340.64–481.55)	1.55 (1.29–1.69)
T. kassasii	25.52 (23.81–26.75)	12.76 (11.91–13.37)	240.99 (230.74–255.84)	18.65 (16.99–20.21)	380.33 (343.92–409.88)	1.37 (1.20–1.57)

Tab. 4. Morphological characteristics of the studied *Tephrosia* taxa. Min – minimum, Max – maximum, L – colpus length, W – colpus width. The number of measurements for each taxon N = 40.

Taxon	Lumen		Murus thickness (μm) (Min–Max)	Colpus			Apocolpium		Mesocolpium Diameter (μm) (Min–Max)
	Diameter (μm) (Min–Max)	Area (μm²) (Min–Max)		L (μm) (Min–Max)	W (μm) (Min–Max)	Area (μm²) (Min–Max)	Diameter (μm) (Min–Max)	Index (Min–Max)	
T. apollinea	0.81 (0.32–1.29)	0.17 (0.03–0.37)	0.49 (0.16–0.85)	24.04 (20.25–26.78)	4.48 (3.58–5.55)	87.58 (72.07–99.70)	5.95 (4.97–6.55)	0.49 (0.45–0.53)	13.98 (12.37–15.25)
T. nubica	0.56 (0.28–1.06)	0.16 (0.06–0.25)	0.58 (0.36–0.84)	19.26 (16.17–21.23)	6.12 (4.60–8.13)	99.30 (66.03–119.80)	12.86 (12.59–13.68)	0.86 (0.80–0.94)	20.35 (19.21–21.15)
T. purpurea	0.52 (0.26–0.98)	0.12 (0.03–0.32)	0.31 (0.16–0.55)	23.88 (20.90–24.90)	3.97 (2.21–8.15)	61.37 (43.68–86.95)	7.88 (3.79–10.92)	0.40 (0.33–0.43)	19.32 (19.31–19.33)
T. quartiniana	0.26 (0.11–0.62)	0.03 (0.01–0.10)	0.52 (0.32–0.82)	15.78 (10.68–19.67)	4.08 (2.22–5.96)	56.84 (35.59–76.60)	6.37 (3.99–7.67)	0.52 (0.46–0.59)	14.29 (13.82–14.52)
T. uniflora	0.50 (0.17–0.81)	0.12 (0.03–0.26)	0.45 (0.15–0.89)	15.78 (10.68–19.67)	4.25 (2.90–5.12)	74.96 (48.95–93.96)	6.44 (4.80–8.44)	0.60 (0.56–0.67)	12.11 (10.61–13.88)
T. kassasii	0.48 (0.10–1.05)	0.13 (0.03–0.72)	0.40 (0.18–0.62)	15.78 (10.68–19.67)	4.67 (2.58–6.13)	47.07 (38.85–52.46)	6.22 (5.14–7.69)	0.58 (0.54–0.64)	23.48 (22.91–24.07)

fied between two groups: 1) *T. kassasii,* 2) *T. apollinea*, *T. purpurea*, *T. quartiniana,* and *T. uniflora*. The main characters explaining this segregation (characters with factor loading ≥ ± 0.6) (On-line Suppl. Tab. 2) were stem type, leaflet shape, abaxial leaflet surface, pod indumentum, epidermal cell shape, intercolpium area, mesocolpium diameter, equatorial view surface sculpture, and endoaperture. On the second component axis, 24.13% of the total variation in (On-line Suppl. Fig. 1) segregation is verified between two groups: 1) *T. nubica,* 2) *T. apollinea*, *T. purpurea*, *T. quartiniana,* and *T. uniflora*. The main characters explaining this segregation (characters with factor loading ≥ ± 0.6) were stem type, stem indumentum, stem branching, pod length, number of seeds per pod, hilum width, hilum area, polar view area, equatorial diameter, equatorial view area, apocolpium diameter, apocolpium index, apocolpium field, colpus width, and endoaperture.

Generally, the results show unity between the principal component analysis (PCA) and UPGMA clustering, suggesting three groups.

Discussion

The sculpture and structure of seed coats are conservative and have stable characters, which have been used effectively in the taxonomy and phylogeny of different plant groups (El-Naggar 2005, Gabr 2018).

Seed size and shape among the studied taxa of *Tephrosia* are quite consistent. Still, some characters are diagnostic, e.g., seeds in *T. nubica* and *T. apollinea* equal or exceed 4.0 mm in length (Fig. 1d, a), respectively, while seeds in *T. apollinea*, *T. nubica,* and *T. purpurea* exceed 2.0 mm in width. The seed shape of the studied taxa is of a high taxonomic significance, and it could be used as a tool to distinguish taxa

Tab. 5. Morphological characteristics of the studied *Tephrosia* taxa.

Taxon	Pollen Shape	Sculpture type				Endoaperture	Lumina shape
		Surface		Aperture	Ectoaperture		
		Equatorial view	Polar view				
T. apollinea	Prolate	Punctuate	Psilate	Psilate	Granulate	Lalongate	Regular, with small, rounded granules
T. nubica	Subprolate	Foveolate to microreticulate	Scabrate-perforate	Scabrate	Granulate	Lolongate	Irregular
T. purpurea	Prolate-spheroidal	Foveolate to microreticulate	Psilate	Psilate-scabrate	Granulate	Lalongate	Regular
T. quartiniana	Prolate	Foveolate to microreticulate	Scabrate-perforate	Perforate	Granulate-scabrate	Lalongate	Irregular
T. uniflora	Prolate	Punctuate	Psilate-perforate	Psilate	Scabrate	Lalongate	Regular, with small, rounded granules
T. kassasii	Prolate	Foveolate to microreticulate	Scabrate-perforate	Scabrate	Granulate-scabrate	Lolongate	Irregular

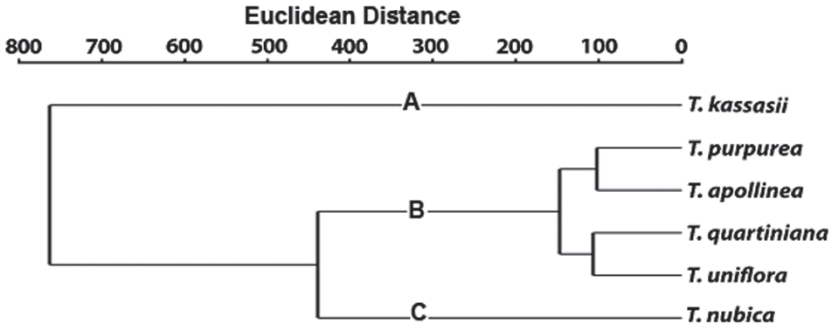

Fig. 3. Cluster analysis of the studied *Tephrosia* taxa based on 62 measured variables using the unweighted pair group method with arithmetic averages (UPGMA) and Euclidean similarity index showing three Clades: A – subgenus Macronyx, B – subgenus Reineria, C – subgenus Brissonia.

at the interspecific level. These results align with those of Al-Ghamdi and Al-Zahrani (2010).

The epidermal cell area is polygonal in *T. uniflora*, as the testa has multi crested texture. In *Tephrosia*, the epidermal cell shape is a less diagnostic character at the interspecific level. However, it could aid in the classification at the infrageneric level. The epidermal cell area is highly taxonomic, particularly in distinguishing some studied taxa, with the largest area in *T. apollina*. In contrast, the smallest is found in *T. kassasii*.

The form, thickness, and surface of anticlinal boundaries of the studied taxa are of less taxonomic significance, with the form of anticlinal boundaries being raised and straight in all studied taxa (Fig. 1B, E, H, K, Q) excluding *T. uniflora*, in which it is raised and wavy (Fig. 1N). Moreover, the surface of anticlinal boundaries is less of a diagnostic feature among the studied taxa (Tab. 2, Fig. 1B-Q).

Periclinal cell wall form is useful in distinguishing *T. quartiniana* from other studied taxa, being concave in *T. quartiniana* (Fig. 1K). At the same time, it is convex in *T. uniflora* (Fig. 1N) and flat in the rest of the studied taxa (Fig. 1B, E, H, Q). The surface of the periclinal cell wall is a good taxonomic character in segregation of the studied taxa. Moreover, the hilum shape, position, size, and area are of high taxonomic value among the studied taxa. These results agree with the findings of Al-Ghamdi and Al-Zahrani (2010).

The morphology of pollen grains was an important taxonomic tool in *Tephrosia* as formerly observed in other palynological studies (Perveen and Qaiser 1998, Buril et al. 2011). The genus *Tephrosia* is commonly stenopalynous. The pollen morphology is more or less similar, especially the size and surface sculpture (Antonio-Domingues et al. 2019). Although pollen grains of the studied taxa of *Tephrosia* are very similar morphologically, the analyses executed here highlight the importance of both quantitative and qualitative characters in differentiating the studied taxa. The most common shape of pollen is prolate. The pollen grain size of the examined taxa is of less taxonomic significance. According to measurements of the polar axis, the largest pollen grains are those of *T. nubica* 31.11 (27.89-32.54) μm, and the smallest ones are those of *T. purpurea* 25.02 (24.98-25.06) μm. The equatorial diameter measurements showed that the largest pollen grains are *T. nubica* 26.62 (26.00-27.08) μm, and the smallest ones are *T. apollinea* 18.43 (16.59-19.83) μm. Venkateswarlu and Kameswara Rao (1967) performed only size measurements on *Tephrosia purpurea* pollen grains, and their data agree with the specimen analyzed in our study.

Moreover, the length of ectocolpus and apocolpium can also distinguish the studied taxa. The longest colpus is found in *T. apollinea*, and the shortest in *T. quartiniana*, *T. uniflora*, and *T. kassasii*. The longest apocolpium is found in *T. nubica* and the shortest in *T. apollinea*.

The ectexine of the studied taxa showed a high taxonomic value for segregation of closely related species. It is punctuated with small, rounded granules in the luminae in *T. apollinea* and *T. uniflora,* while it is foveolate to microreticulate in the rest of the studied taxa. These results agree with those of Buril et al. (2011) and, Antonio-Domingues et al. (2019) but disagree with the findings of Perveen and Qaiser (1998).

Generally, the seed and pollen morphological characters observed in this study along with the UPGMA and PCA analyses confirm the subgenera classification suggested by Bentham (1865), Baker (1876), and de Queiroz et al. (2013), but did not prove the two subgenera proposed by Brummitt (1980).

Conclusion

The seed and pollen grain morphologies were significant differentiating tools for the studied taxa of *Tephrosia*. This work contributes to the understanding of the diversity of seeds and pollen grains in *Tephrosia* and the considerable variation through a range of morphological characters. Some characters, which include seed shape and size, testa texture, epidermal cell shape and area, the sculpture of ectexine and ectoaperture membranes, length of the ectocolpi, apocolpium diameter, *P/E* ratio, and lumina area, bring new taxonomic clarification to the genus *Tephrosia*. These results are congruent with other seed and pollen studies on different genera within the tribe Milletieae. The quantita-

tive and qualitative seed and pollen characters offer a helpful taxonomic key for segregating closely related species. These data support morphological knowledge and can be used in future phylogenetic approaches to help develop the systematic and morphological study of the Fabaceae-Papilionoideae subfamily.

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