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Research Article
Taxonomic Importance of Pollen Morphology for Some Species of Brassicaceae

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Abstract

Background and Objective: Pollen morphology is one of the significant tools in solving some taxonomic problems on the family, generic or specific level and has become part of the multidisciplinary and collaborative approach in plant systematic and evolution. Therefore, this study aimed to investigate and describe the pollen morphology of 10 species belongs to 9 genera and five tribes of Brassicaceae from eastern region of Saudi Arabia by using light and scanning electron microscope. **Materials and Methods:** To study the pollen morphology for ten species representing 9 genera and 5 tribes of Brassicaceae the Light Microscope (LM) and Scanning Electron Microscope (SEM). For the SEM the pollen was placed directly on brass stubs without treatment and mounted onto a metallic stub with a double-sided adhesive tape. Gold coating of few nanometers was applied using sputter coating machine (Quorum, Q150R ES, UK) to avoid charging and capture high quality images. Two statistical program; PRIMER 6, version 6.1.6 and SPSS version 16are used to fine the relationships among the studied species. **Results:** The shape of pollen grains recorded three types; prolate, subprolate and prolate-spheroidal, the main types were prolate. The apertures were tricolpate in all studied species. Exine ornamentation of studied species recorded two types; reticulate and coarsely reticulate. The results of numerical analysis showed that species were grouped into two major clusters and each cluster divided into two groups. **Conclusion:** The results show that the morphology of pollen grains cannot be useful for taxonomical classification of the tribes but can be useful for differentiate between species belong to the same genera.

Key words: Pollen grains, pollen morphology, light microscope, brassicaceae, scanning electron microscope

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Competing Interest: The author have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Brassicaceae (Cruciferae) is one of the largest angiosperm families comprising approximately 350 genera and about 3660 species[1]. It is cosmopolitan but chiefly distributed in the temperate and mediterranean regions. The major centers of distribution of the family are in the Irano-Turanian, Mediterranean and Saharo-Sindian regions[2,3]. In Saudi Arabia, Brassicaceae is represented by 74 species belonging to 53 genera according to Chaudhary[4]. In the eastern region of Saudi Arabia there are 46 species and 30 genera[5].

The morphology of pollen grain is one of the significant tools in solving some taxonomic problems on the family, generic or specific level and has become part of the multidisciplinary and collaborative approach in plant systematic and evolution[6]. The first study to use the morphological characters of pollen grains concerned with the classification of plants was done by Brown[7]. Later a greater number of pollen features including shape and ornamentation as well as aperture number are used to define a range of pollen types[8].

The characters of pollen morphology of the family Brassicaceae has provided an approach to the systematic relationships among the genera and species[9,10]. Many studies on the morphological characters of pollen grains for species and taxa of Brassicaceae growing elsewhere other than Saudi Arabia were done, by Erdtman *et al.*[11,] Chiguriaeva[12], Carter *et al.*[13], Perveen *et al.*[14], Pinar *et al.*[15], Arora and Modi[16], Keshavarzi *et al.*[17], Mutlu and Erik[18] and Sharma and Singh[19].

The pollen morphological characters of Brassicaceae species growing in eastern region of Saudi Arabia has not been thoroughly investigated. Therefore, our knowledge about the pollen morphology of these plants is may be insufficient. The main objective of this study is to examine and describe the pollen grains of some species of Brassicaceae growing in eastern region of Saudi Arabia in order to increase the number of studied species of the family at the global level and indicate the taxonomical importance of pollen characters.

MATERIALS AND METHODS

Ten species representing 9 genera and 5 tribes of Brassicaceae were collected fresh from different areas in eastern region of Saudi Arabia (Table 1) according to Al-Shehbaz *et al.*[20]. The materials studied were identified according to plant keys of Chaudhary [4] and Mandaville[5].

The observations in Light Microscope (LM) were carried out with Olympus Bx53 without any prior treatment of the pollen grains. The details of pollen morphology were investigated in electron scanning microscope (SEM) with the use of FEI, ISPECT S50 (Czech Republic). Pollen was placed directly on brass stubs without treatment and used to record the surface morphological features of the studied species. The SEM was operated at 20 kV with working distance 10 mm. Long and high magnification was performed to capture the recognized features of the specimen. The pollen grains were mounted onto a metallic stub with a double-sided adhesive tape. Gold coating of few nanometers was applied using sputter coating machine (Quorum, Q150R ES, UK) to avoid charging and capture high quality images. The terminology of Erdtman[21] was adopted to describe the pollen shape and the terminology of Abdel Khalik *et al.*[22] for exine ornamentation.

The morphological characters of the pollen grains studied species were collected and creating data matrix used for numerical analysis (Table 3, 4). The relationships among the studied species was demonstrated as dendrograms (Fig. 3) by using two statistical program; PRIMER 6, version 6.1.6 analyses using agglomeration of Schedule measure Euclidean distance, using complete linkage between groups and SPSS version 16,

Table 1: List of studied species and its collection data (species from eastern region of Saudi Arab)

Tribes[a]	Species	Locality and date
Brassicaceae	*Brassica juncea* (L.) Czern	Rawda-Dammam, 3/2017
		Rayan-Dammam, 3/2017
Brassicaceae	*Brassica tournefortii* Gouan	Rawda-Dammam, 3/2017
Brassicaceae	*Eruca sativa* Mill	Rawda-Dammam, 4/2017
Brassicaceae	**Raphanus sativus* L.	Rawda-Dammam, 4/2017
		Rayan-Dammam, 3/2017
Brassicaceae	*Cakile arabica* vel.et Bornm	Rawda-Dammam, 3/2017
Brassicaceae	*Savignya parviflora* (del.) Webb	Rayan-Dammam, 3/2016
Lepidiea	*Coronopus didymus* (L.) Sm.	Rayan-Dammam, 3/2017
Alysseae	*Farsetia burtonae* Oliv.	Salasal-Dammam road, 4/2017
Cardamineae	*Cardamine hirsuta* L.	Rayan-Dammam, 3/2017
Sisymbrieae	*Sisymbrium irio* L.	Rayan-Dammam, 3/2017

*Cultivated, [a]Source: Al-Shehbaz *et al.*[20]

by using methods of clustering particularly analyzed by average linkage between groups and measure distance cluster combine.

RESULTS

Morphology of pollen grains as, pollen size, shape, exine thickness and ornamentation characters are very useful in plant identification. The pollen morphology of the studied species obtained by light and scanning electron microscope are summarized in Table 2 and Fig. 1 and 2.

***Brassica juncea* (L.) Czern.:** Pollen grains 33.46 μm (polar axis)×22.62 μm (equatorial axis), subprolate, tricolpate with coarsely reticulate exine ornamentation, exine thickness is 1.00 μm, colpus 7.17 μm length×1.81 μm width, lumina irregular, 2.08 μm length×1.25 μm width and murus thickness is 0.69 μm.

***Brassica tournefortii* Gouan.:** Pollen grains 38.73 μm (polar axis)×18.36 μm (equatorial axis), prolate, tricolpate with reticulate exine ornamentation, exine thickness is 0.91 μm, colpus 10.80 μm length×1.69 μm width, lumina nearly rounded, 1.54 μm length×0.93 μm width and murus thickness is 0.37 μm.

***Eruca sativa* Mill.:** Pollen grains 27.34 μm (polar axis)×13.88 μm (equatorial axis), prolate, tricolpate with reticulate exine ornamentation, exine thickness is 0.68 μm, colpus 8.92 μm length×1.43 μm width, lumina irregular, 1.18 μm length×0.58 μm width and murus thickness is 0.47 μm.

***Raphanus sativus* L.:** Pollen grains 29.64 μm (polar axis)×15.98 μm (equatorial axis), prolate, tricolpate with reticulate exine ornamentation, exine thickness is 1.20 μm, colpus 7.11 μm length×2.11 μm width, lumina irregular-nearly rounded, 1.89 μm length×1.06 μm width and murus thickness is 0.34 μm.

***Cakile arabica* vel.et Bornm.:** Pollen grains 36.53 μm (polar axis)×20.63 μm (equatorial axis), prolate, tricolpate with reticulate exine ornamentation, exine thickness is 1.08 μm, colpus 11.56 μm length×2.91 μm width, lumina polygonal, 1.85 μm length×1.30 μm width and murus thickness is 0.54 μm.

Table 2: Pollen characters of studied species

	Species								
Characters (SD±Mean)	*Brassica juncea*	*Brassica tournefortii*	*Eruca sativa*	*Raphanus sativus*	*Cakile arabica*	*Savignya parviflora*	*Coronopus didymus*	*Farsetia burtonae*	*Cardamine hirsuta*	*Sisymbrium irio*
Polar axis (μm) (P)	0.47±33.46	0.75±38.73	1.03±27.34	6.76±29.64	2.76±36.53	3.57±35.81	1.51±25.68	0.67±15.48	1.54±37.47	1.02±30.28
Equaterial axis (μm) (E)	0.28±22.62	0.73±18.36	0.21±13.88	1.37±15.98	0.68±20.63	1.29±23.20	0.52±13.50	0.40±15.26	0.70±21.66	0.85±14.25
P/E ratio	1.48	2.11	1.97	1.85	1.77	1.54	1.90	1.01	1.73	2.12
Shape	I	II	II	II	II	II	II	III	II	II
Exine thickness (μm)	0.14±1.00	0.15±0.91	0.13±0.68	0.29±1.20	0.21±1.08	0.15±1.22	0.19±1.04	0.12±0.96	0.09±1.22	0.15±0.83
Exine ornamentation	I	II	II	II	II	I	II	II	I	II
Colpus length (μm)	0.47±7.17	1.35±10.80	0.75±8.92	0.91±7.11	0.70±11.56	1.33±9.62	1.20±7.15	0.22±6.05	0.60±9.17	0.64±6.90
Colpus width (μm)	0.24±1.81	0.84±1.69	0.24±1.43	0.46±2.11	0.49±2.91	0.1±1.91	0.34±1.72	0.27±2.41	0.88±2.36	0.31±1.38
Colpus length/width ratio	3.96	6.38	6.21	3.37	3.96	5.04	4.14	2.51	3.88	5.00
Lumen length (μm)	0.47±2.08	0.30±1.54	0.17±1.18	0.34±1.89	0.41±1.85	0.32±2.01	0.13±1.23	0.15±1.20	0.34±2.24	0.11±1.25
Lumen width (μm)	0.17±1.25	0.18±0.93	0.14±0.58	0.37±1.06	0.16±1.30	0.13±1.40	0.15±0.68	0.17±0.86	0.30±1.18	0.08±0.53
Lumen length/width ratio	1.66	1.65	2.04	1.79	1.42	1.44	1.81	1.40	1.90	2.36
Murus thickness (μm)	0.05±0.69	0.04±0.37	0.06±0.47	0.18±0.34	0.09±0.54	0.05±0.36	0.05±0.36	0.10±0.57	0.05±0.36	0.17±0.73

Shape: I: Subprolate, II: Prolate, III: Prolate spheroidal, Exine ornamentation: I: Coarsely reticulate, II-Reticulate, Std: Standart deviation

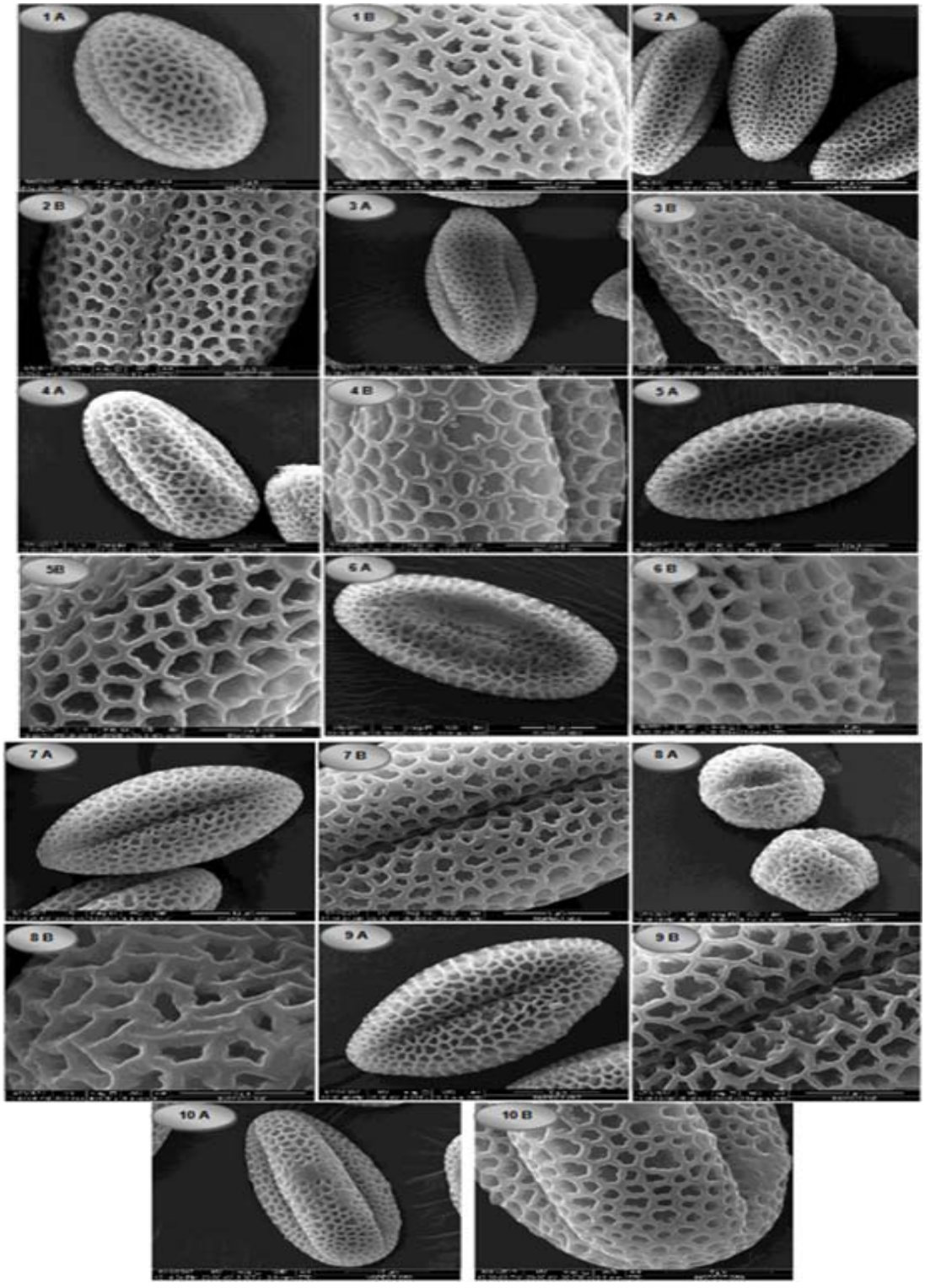

Fig. 1: Scanning electron microscope micrographs of pollen shapes, (a) Lumina shapes and (b) Exine ornamentation of 1: *Brassica juncea*, 2: *Brassica tournefortii*, 3: *Eruca sativa*, 4: *Raphanus sativus*, 5: *Cakile arabica*, 6: *Savignya parviflora*, 7: *Coronopus didymus*, 8: *Farsetia burtonae*, 9: *Cardamine hirsuta*, 10: *Sisymbrium irio*

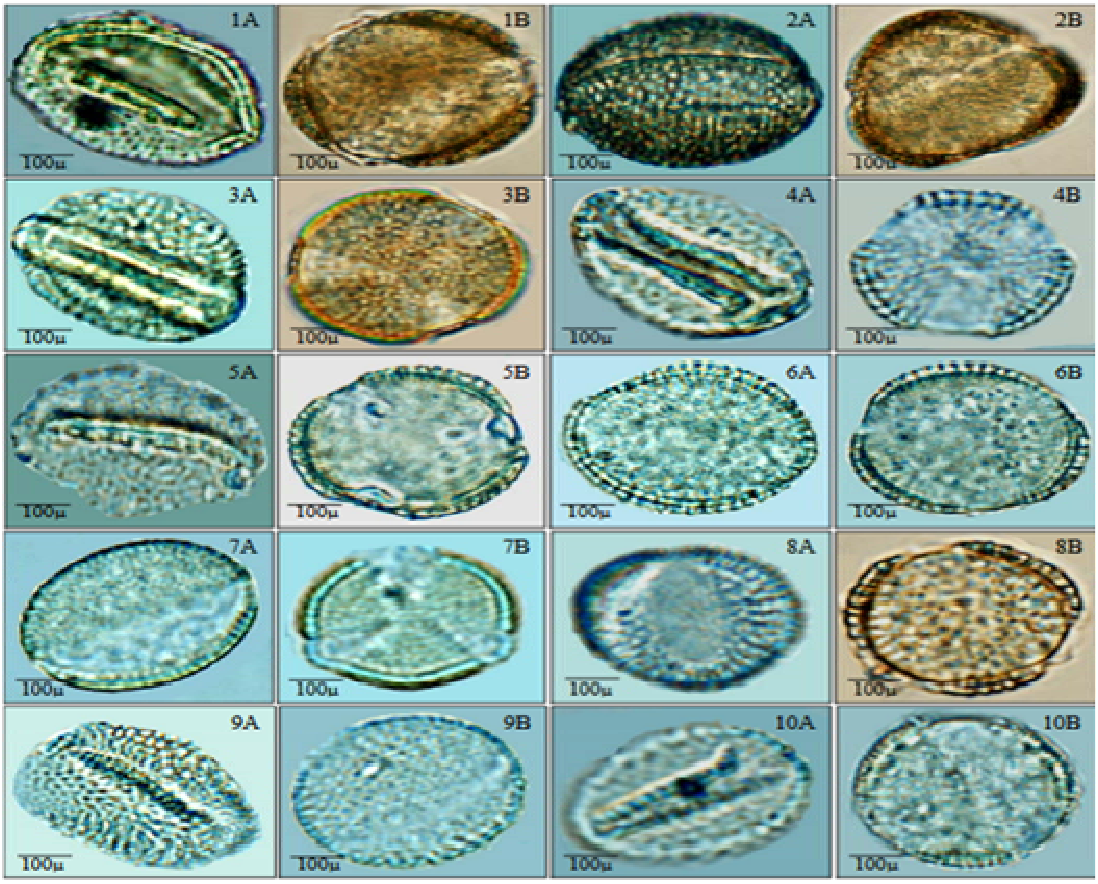

Fig. 2: Pollen morphology of 10 species of Brassicaceae as detected by LM. 1: *Brassica juncea*, 2: *Brassica tournefortii*, 3: *Eruca sativa*, 4: *Raphanus sativus*, 5: *Cakile arabica*, 6: *Savignya parviflora*, 7: *Coronopus didymus*, 8: *Farsetia burtonae*, 9: *Cardamine hirsuta*, 10: *Sisymbrium irio*, (a) Equatorial view and (b) Polar view

Table 3: Characters and character states used for numerical analysis of the studied species of Brassicaceae

Characters	Character state(µm) and code
Polar axis	15-20[1]/25-30 [2]/ 30.1-35[3]/ 35.1-40 [4]
Equatorial axis	13-14 [1]/14.1-15 [2]/ 15.1-17 [3]/17.1-19 [4]/ 20-22[5]/22.1-24[6]
Exine thickness	0.5-0.7 [1]/ 0.8-9 [2]/ 0.91-1 [3]/ 1.1-1.3 [4]
Colpus length	6-7 [1]/ 7.1-8 [2]/8.1-9 [3]/ 9.1-10 [4]/ 10.1-11 [5]/11.1-12 [6]
Colpus width	1-1.5 [1]/ 1.6-2 [2]/ 2.1-2.5 [3]/ 2.6-3 [4]
Lumen length	1-1.5 [1]/ 1.6-2 [2]/ 2.1-2.5 [3]
Lumen width	0.5-0.7 [1]/ 0.8-1 [2]/ 1.1-1.3 [3]/ 1.4-1.6 [4]
Murus thickness	0.3-0.4 [1]/ 0.41-0.5 [2]/ 0.51-0.6 [3]/ 0.61-7 [4]/ 0.71-0.8 [5]
Pollen shape	Subprolate [1]/ Prolate [2]/ Prolate spheroidal [3]
Apertures	Tricolpate [1]
Exine ornamentation	Coarsely reticulate [1]/ Reticulate [2]
Lumen shape	Polygonal [1]/ Irregular [2]/ Nearly rounded [3]/ Irregular-polygonal [4]/ Irregular-nearly rounded [5]

***Savignya parviflora* (del.) Webb.:** Pollen grains 35.81 µm (polar axis)×23.20 µm (equatorial axis), prolate, tricolpate with coarsely reticulate exine ornamentation, exine thickness is 1.22 µm, colpus 9.62 µm length×1.91 µm width, lumina polygonal, 2.01 µm length×1.40 µm width and murus thickness is 0.36 µm.

***Coronopus didymus* (L.) Sm.:** Pollen grains 25.68 µm (polar axis)×13.50 µm (equatorial axis), prolate, tricolpate with reticulate exine ornamentation, exine thickness is 1.04 µm, colpus 7.15 µm length×1.72 µm width, lumina polygonal, 1.23 µm length×0.68 µm width and murus thickness is 0.36 µm.

Table 4: Data matrix of morphological characters listed in Table 3

Species	*Brassica juncea*	*Brassica tournefortii*	*Eruca sativa*	*Raphanus sativus*	*Cakile arabica*	*Savignya parviflora*	*Coronopus didymus*	*Farsetia burtonae*	*Cardamine hirsuta*	*Sisymbrium irio*
Polar axis	3	4	2	2	4	4	2	1	4	3
Equaterial axis	6	4	1	3	5	6	1	3	5	2
Exine thickness	3	3	1	4	4	4	3	3	4	2
Colpus length	2	5	3	2	6	4	2	1	4	1
Colpus width	2	2	1	3	4	2	2	3	3	1
Lumen length	3	1	1	2	2	2	1	1	3	1
Lumen width	3	2	1	3	3	4	1	2	3	1
Murus thickness	4	1	2	1	3	1	1	3	1	5
Pollen shape	1	2	2	2	2	2	2	3	2	2
Apertures	1	1	1	1	1	1	1	1	1	1
Exine ornamentation	1	2	2	2	2	1	2	2	1	2
Lumen shape	2	3	2	5	1	1	4	2	1	5

***Farsetia burtonae* Oliv.:** Pollen grains 15.48 µm (polar axis)×15.26 µm (equatorial axis), prolate-spheroidal, tricolpate with reticulate exine ornamentation, exine thickness is 0.96 µm, colpus 6.05 µm length×2.41 µm width, lumina irregular, 1.20 µm length×0.86 µm width and murus thickness is 0.57 µm.

***Cardamine hirsuta* L.:** Pollen grains 37.47 µm (polar axis)×21.66 µm (equatorial axis), prolate, tricolpate with coarsely reticulate exine ornamentation, exine thickness is 1.22 µm, colpus 9.17 µm length×2.36 µm width, lumina polygonal, 2.24 µm length×1.18 µm width and murus thickness is 0.36 µm.

***Sisymbrium irio* L.:** Pollen grains 30.28 µm (polar axis)×14.25 µm (equatorial axis), prolate, tricolpate with reticulate exine ornamentation, exine thickness is 0.83 µm, colpus 6.90 µm length×1.38 µm width, lumina irregular-nearly rounded, 1.25 µm length×0.53 µm width and murus thickness is 0.73 µm.

Numerical analysis: The characters rather of pollen grains of the studied species obtained by light and scanning electron microscope (12 characters) are collected in Table 3 as characters and character states, then used to creating data matrix (Table 4) which used for numerical analysis by using the method of clustering as a tool to determine relationships and the value of pollen characters in the taxonomy of Brassicaceae. The cluster analysis (Fig. 3a) showed that species were grouped into two major clusters and each cluster divided into two groups, the first cluster I consisted of five species: *Brassica juncea* (group I) and *Brassica tournefortii*, *Cakile arabica*, *Coronopus didymus* and *Cardamine hirsute* (group II). The cluster II comprised five species; *Raphanus sativus* and *Sisymbrium irio* recorded in group I while *Eruca sativa*, *Savignya parviflora* and *Farsetia burtonae* present in group II.

The results obtained from anther program (Fig. 3b) also divided the studied species into two main clusters and four groups. Cluster I contained *Sisymbrium irio* (group I) and *Savignya parviflora* and *Cardamine hirsute* (group II). While cluster II consisted of *Eruca sativa*, *Raphanus sativus* and *Coronopus didymus* in group I and *Farsetia burtonae*, *Cakile arabica, Brassica juncea* and *Brassica tournefortii* in group II.

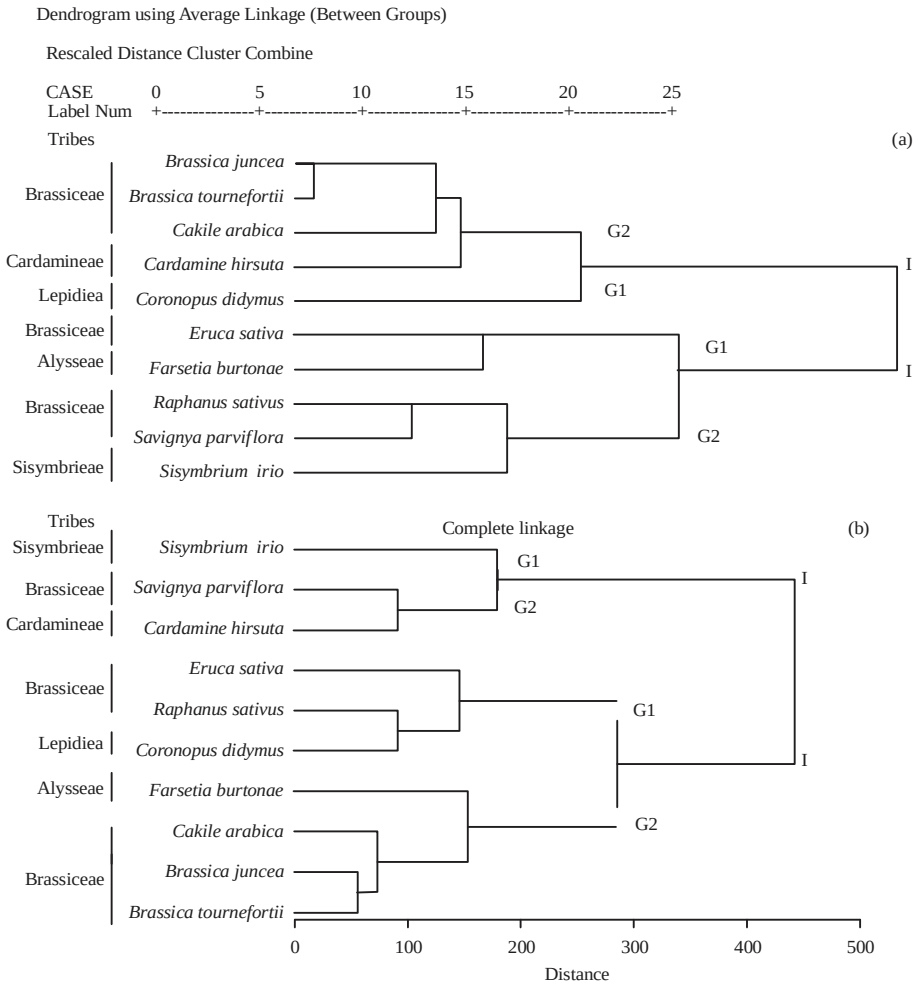

Fig. 3(a-b): Dendrograms showing the interrelationships between 10 species of Brassicaceae based on 12 characters of pollen morphological characters, (a) SPSS program and (b) PRIMER program

DISCUSSION

The Brassicaceae is a stenopalynous family[23]. In this study, the size of pollen grains varies significantly; the largest were found in *Brassica tournefortii* with a polar axis 38.73 µm and an equatorial axis of 18.36 µm. The smallest grains were observed in *Farsetia burtonae* with a polar axis 15.48 µm and an equatorial diameter 15.26 µm. The shape of pollen grains in present study recorded three types[21]: Subprolate in *Brassica juncea*, prolate-spheroidal in *Farsetia burtonae* and prolate in the reminder, it was consistent with literature data[10,16,18,22].

The exine thickness in the studied species ranges from 0.68 µm in *Eruca sativa* to 1.22 µm in *Cardamine hirsuta*. The apertures pollen grains in all studied species are tricolpate and this agrees with Abdel Khalik *et al*.[22] and Apple and Al-Shehbaz[24]. The colpi are usually widest at the equator and gradually narrowing towards the poles. The colpus length ranged from 6.05 µm in *Farsetia burtonae* to 11.56 µm in *Cakile arabica*.

Exine ornamentation of pollen grains has important role within the significance between the tribes or species within the same genus[22]. The pollen ornamentation of the studied species of Brassicaceae is reticulate with simplibacculate, straight or slightly sinuous muri. Three types of exine ornamentation based on variation in diameter of lumina[22]. The studied species recorded two types of exine ornamentation according to Abdel Khalik *et al*.[22]. Exine is reticulate, lumina between 1-2 µm. This type is present in all most studied species. The exine is coarsely reticulate, with lumina more than 2 µm. This type is present in *Brassica juncea*, *Savignya parviflora* and *Cardamine hirsuta*.

The characters of lumina and muri also plays a significant role in differentiation between the tribes of Brassicaceae[22,25].

The thickening of muri ranged from 0.36-0.89 µm and the length of lumina ranged from 1.18-2.24 µm. Lumina vary from polygonal, nearly rounded to irregular in shape. All studied species have lumina size more than muri, this agrees with[25]. They indicated two types: Reticulate with lumina equal to or exceeding muri in size. These types are found in all tribes or perforated reticulate with lumina equal or smaller than muri size, this existing in *Erysimum*.

The results of numerical analysis indicate most species from tribe Brassicaceae are grouped together in the same cluster but associated with another species from different tribes in the groups, as *Savignya parviflora* from tribe Brassicaceae and *Cardamine hirsute* from tribe Cardamineae. Also *Coronopus didymus* from tribe Lepidiea present with species from tribe Brassicaceae. The obtained results in current work indicate the tribe Brassicaceae to be heterogenous. The Program SPSS separate the two species of *Brassica juncea* and *Brassica tournefortii* in two different groups and the program Primer put them in the same groups. From all of this the present study indicated that the morphology of pollen grains cannot be useful in differentiation of genera from different tribes. However, it can be helpful for differentiate between species belong to the same genera and this agree with[26-29]. They revealed that most pollen types contain members of more than one section, also some study described more than one pollen type found in the same section[22,30].

CONCLUSION

The morphology of pollen grains as, size, shape, exine thickness, its ornamentation, especially lumen and murus characters and colpi are very important characters of pollen grains and can be used for identification and differentiate between the species belong to the same genera but this characters cannot be used as a tool for taxonomical clusters for the different tribes.

SIGNIFICANCE STATEMENT

This study give important morphological characters of pollen grain for some species for the first time because some species are endemic for the study area and others are examined for the first time, this increase the number of studied species of the family at the global level. Also the comparison between the common species with other studies gives more detail and indicates the taxonomical importance of pollen characters.

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