

Significance of Fruit and Seed Coat Morphology in Taxonomy and Identification for Some Species of Brassicaceae

Dalia G. Gabr^{1,2}

¹Department of Basic Science, Faculty of Education, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

²Botany and Microbiology Department, Faculty of Science (Girls Branch), AL Azhar University, Cairo, Egypt

Email: dggabr@iau.edu.sa

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Abstract

Fruit, seed morphology and seed coat sculpturing were recorded for 10 species belongs to 9 genera and five tribes of Brassicaceae in eastern region of Saudi Arabia by using light microscope (LM) and scanning electron microscope (SEM). Four seed patterns were recognized based on surface sculpturing pattern; Reticulate, with four subtypes, undulate, verrucate and ocellate. The results of numerical analysis showed that species were grouped into two major clusters and each cluster divided into two groups. The cluster analysis indicates that the characters of fruit and seed are very important characters for identification and taxonomical classification of the tribes. The results indicate that the most tribes of Brassicaceae are polyphyletic, and some are related to each other's as Lepidieae, Alyssieae and Sisymbrieae.

Keywords

Fruit, Seed Coat, Morphology, Scanning Electron Microscope

1. Introduction

The Brassicaceae (Cruciferae), or mustard family, is one of the largest angiosperm families, can be recognized easily by its floral and fruiting characters. It is cosmopolitan but chiefly distributed in the temperate and Mediterranean region, it represented by 338 genera and 3709 species [1]. In Saudi Arabia, it is represented by 53 genera and 74 species [2]. In eastern region of Saudi Arabia, [3] recorded 46 species and 30 genera.

The morphological characters of the family Brassicaceae especial fruit seed and cotyledons are used in the tribal classification of the family [4] recognized

10 tribes based on fruit and cotyledon characters. [5] recognized 4 tribes and 19 sub tribe, then [6] put the members of the family in 10 tribes by using nectar glands and myrosin cells characters. [7] divided the family into three groups according to fruit characters into Siliquosae, Siliquulosae and Lomentaeae, each composed of number of tribes. [8] classifies the genera of the family in 19 tribes. [9] reduced the total number of Schulz's tribes from 19 to 15 tribes depending on very large numbers of features of flowers and fruit. [10] recognized 6 tribes under the family Cruciferae according to fruit characters, but [11] divided the family into 6 groups and 10 tribes. [12] [13] reduced Schulz's tribes to three tribes: The lypodieae, Brassiceae and Sisymbrieae. [14] classified the family Brassicaceae of the southeastern United States into 7 tribes; then [15] recognized 25 tribes for the family Brassicaceae. Recently there are some researches based on molecular and phylogenetic studies for the family as [16]-[29].

The morphology of seed coat structure is considered to be stable characters and is little influenced by external environmental conditions whilst the seeds develop and ripen within the fruit [30] [31] [32] [33]. The seed coat characters have been used successfully to solve systematic and phylogenetic problems between different taxa [34] [35]. In the Brassicaceae, several studies have been carried out on the seed coat and its taxonomic significance. [36] [37] [38] [39] studied the external morphology and anatomy of seeds of 90 genera and 200 species of the Cruciferae. [40] [41] [42] investigated the seed coats and development of epidermal slime bodies of *Malcolmia*. [43] studied the seed coat of *Lepidium* in East tropical Africa. [44] investigated the structure of seed coat and development in certain species of Brassicaceae. [45] [46] studied some taxa of *Brassica* seeds by using scanning electron microscope (SEM). [47] used SEM on the seed surface to distinguish *Matthiola*, *Morettia*, and *Diceratella*. [48] studied the seed coat morphology, particularly the epidermal slime structure of *Morettia*. [49] studied the seed coat of genus *Farsetia* by using SEM to distinguish between species. [50] investigated the seed coat sculpture in species of the tribe Brassiceae in Egypt by using SEM. [51] investigated the seed morphology and taxonomy with SEM in the tribe Lepidieae in Egypt. More recently, [52] gave detailed descriptions of seed morphology in 44 species of the subtribes Brassicinae, Raphaninae and Moricandiinae, and elucidated the phylogenetic relationships between taxa. [53] study seed morphology of 45 taxa belonging to 23 genera from tribes Arabideae, Euclidieae, Hesperideae, Lunariae, Matthioleae, and Sisymbrieae, of Brassicaceae were examined using light and scanning electron microscopy. [54] studied the macro and micromorphological characters of approximately 22 genera, 30 species of Brassicaceae. [55] study seed coat sculpture of 93 taxa belonging to 45 genera of the family Brassicaceae from Egypt by using light and scanning electron microscope. [56] investigated seed morphology and seed coat sculpturing of 32 Taxa of Family Brassicaceae by using light microscope and scanning electron microscope. [57] study the relation between 26 taxa of family Brassicaceae from Egypt by using morphological characters including fruit and

seed characters, [58] study the seed exomorphic characteristics of 14 taxa of *Lepidium* L. in Brassicaceae from Turkey by light microscope and scanning electron microscope (SEM). [59] study macro and micromorphological characters of 9 species and 30 accessions seeds in *Ricotia* L. by light (LM) and scanning electron microscopy (SEM) to evaluate the taxonomic relevance of these traits. [60] studies the morphological Characteristics of some species of Brassicaceae (Cruciferous) in Setifian High Plateau, Algeria.

The fruit and seed coat morphological feature in Brassicaceae species growing in eastern region of Saudi Arabia is not yet correctly projected. Therefore, our knowledge about the fruit and seed coat morphology of these plants is only measly. Therefore the main objective of this study is to examine and describe the fruit and seed coat of some species of Brassicaceae growing in eastern region of Saudi Arabia by using light and scanning electron microscope to indicate the important of these characters for taxonomy and identification of different species.

2. Material and Methods

Ten species belong to nine genera were collected fresh in spring season from different area in eastern region of Saudi Arabia (Table 1). The materials studied were identified according to plant key of [2] [3]. Foliar details for fruit and seed were examined with the aid of binocular stereo microscope (Olympus Bx53). The details of seed morphology were investigated in electron scanning microscope (SEM) with the use of FEI, ISPECT S50 (Czech Republic). 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 seeds were

Table 1. Collection data of the studied species and its tribal classification. All species from eastern region of Saudi Arabia.

Species	Locality and date	Tribes		
		Prantl (1891)	Schulz (1936)	Al-Shehbaz, <i>et al.</i> , (2006)
<i>Brassica juncea</i> (L.) Czern	Rawda—Dammam, 3/2017	Sinapeae	Brassicaceae	Brassicaceae
<i>Brassica tournefortii</i> Gouan	Rayan—Dammam, 3/2017	Sinapeae	Brassicaceae	Brassicaceae
	Rawda—Dammam, 3/2017			
<i>Eruca sativa</i> Mill	Rawda—Dammam, 4/2017	Sinapeae	Brassicaceae	Brassicaceae
<i>Raphanus sativus</i> L.	Rawda—Dammam, 4/2017	Sinapeae	Brassicaceae	Brassicaceae
<i>Cakile arabica</i> vel. et Bornm	Rayan—Dammam, 3/2017	Sinapeae	Brassicaceae	Brassicaceae
	Rawda—Dammam, 3/2017			
<i>Savignya parviflora</i> (del.) Webb	Rayan—Dammam, 3/2016	Sinapeae	Brassicaceae	Brassicaceae
<i>Coronopus didymus</i> (L.) Sm.	Rayan—Dammam, 3/2017	Sinapeae	Lepidieae	Lepidieae
<i>Farsetia burtonae</i> Oliv.	Salasal—Dammam road, 4/2017	Hesperideae	Alysseae	Alysseae
<i>Cardamine hirsuta</i> L.	Rayan—Dammam, 3/2017	Sinapeae	Arabideae	Cardamineae
<i>Sisymbrium irio</i> L.	Rayan—Dammam, 3/2017	Sinapeae	Sisymbrieae	Sisymbrieae

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 [32] [61] [62] was adopted to describe the SEM aspects of the seed coat. The morphological characters of the fruit and seed for the studied species were collected and creating data matrix used for numerical analysis. The relationships between the studied species have been demonstrated as dendrograms **Figure 4** by using PRIMER 6, version 6.1.6 analyses using agglomeration of Schedule measure Euclidean distance, using complete linkage between groups.

3. Results

The fruit and seed morphological characters for ten species of the family Brassicaceae are summarized in **Tables 2-6** and the image by and SEM are represented in **Figures 1-3**.

3.1. Fruit Characters

3.1.1. Fruit Pedicel

The texture of pedicel is glabrous in most studied species except in *Coronopus didymus* and *Farsetia burtonae* are hairy. The pedicel length has greeted variation in the studied species; it ranged from short, long and very long pedicel. Short (2 - 5 mm) recorded in *Eruca sativa*, *Cakile arabica*, *Coronopus didymus*, *Farsetia burtonae*, and *Cardamine hirsute*. Long (6 - 10 mm) present in *Brassica juncea*, *Brassica tournefortii* and *Sisymbrium irio*. Very long (more than 10 mm) present in *Raphanus sativus* and *Savignya parviflora*.

3.1.2. Fruit Types

The family Brassicaceae is characterized by two types of fruit; siliqua and silicula, in the present study the silicula fruit noted in *Savignya parviflora* and *Coronopus didymus*. The reminder studied species have the type of siliqua.

3.1.3. Fruit Segmentation

Most studied species are non-segmented, segmented fruit are present in *Raphanus sativus*, *Cakile Arabica* and *Farsetia burtonae*.

3.1.4. Fruit Shape

The shape of fruit showed great variation. In the studied species the fruit shape recorded three main types; linear or terete in *Brassica juncea*, *Brassica tournefortii*, *Farsetia burtonae*, *Cardamine hirsute* and *Sisymbrium irio*. Oblong in *Eruca sativa*, *Raphanus sativus*, *Cakile arabica* and *Savignya parviflora*. Subreniform in *Coronopus didymus*.

3.1.5. Fruit Surface & Texture

The texture in half studied species is glabrous with non-ribbed surface. Glabrous and ribbed in *Raphanus sativus*, *Cakile arabica* and *Sisymbrium irio*. Tuberculated and not-ribbed in *Coronopus didymus* and hairy in *Farsetia burtonae*.

Table 2. Fruit morphological characters of the studied species.

Species	Characters		pedicel		Fruit										Beak				
	Texture	Pedicel length mm.		Segm ⁿ entation	Shape	Surface	Texture	Direction	Opening	Fruit length				Fruit width				Seeding	Shape
		Stander	Average							Cm.	mm.	mm.	mm.	mm.	mm.				
<i>Brassica juncea</i>	Glabrous	±2.09	7	Siliqua	Non	Linear or terete	Not ribbed	Glabrous	Erect-slightly curved	Dehiscent	±0.72	3	±0.18	1.75	±0.58	7.14	Seedless	Conical	
<i>Brassica tournefortii</i>	Glabrous	±1.5	9.5	Siliqua	Non	Linear or terete	Not ribbed	Glabrous	Erect-slightly curved	Dehiscent	±1.06	4.5	±0.54	1.85	±1.09	13	One-two seed	Conical	
<i>Eruca sativa</i>	Glabrous	±1.12	3.5	Siliqua	Non	Oblong	Not ribbed	Glabrous	Erect	Dehiscent	±0.60	2.2	±0.75	3.1	±1	7.8	Seedless	Conical	
<i>Raphanus sativus</i>	Glabrous	±4.12	13	Siliqua	Segmented	Oblong ovate	Ribbed	Glabrous	Curved	Indehiscent	±0.36	2.7	±0.35	4.5	±3.40	16	Seedless	Conical	
<i>Cakile arabica</i>	Glabrous	±0.79	3.2	Siliqua	Segmented	Oblong ovate	Ribbed	Glabrous	Erect	Indehiscent	±0.19	1.5	±0.23	2.13	±0.08	0.4	Seedless	Flat	
<i>Savignya parviflora</i>	Glabrous	±4.02	13.75	Silicula	Non	Broad oblong	Not ribbed	Glabrous	Erect	Dehiscent	±0.13	0.8	±0.96	4.88	±0.37	0.88	Seedless	Flat	
<i>Coronopus didymus</i>	Hairy	±0.19	2.23	Silicula	Non	Sub reniform	Not ribbed	Tuberculate	Erect	Indehiscent	±0.01	0.2	±0.36	2.4	±0.07	0.2	Seedless	Cylindrical	
<i>Farsetia burtonae</i>	Hairy	±0.20	2.85	Siliqua	Segmented	Linear or terete	Not ribbed	Hairy	Slightly curved	Dehiscent	±0.31	1.1	±0.08	1.58	±0.45	1.12	Seedless	Cylindrical	
<i>Cardamine hirsuta</i>	Glabrous	±0.98	2.68	Siliqua	Non	Linear or terete	Not ribbed	Glabrous	Erect-slightly curved	Dehiscent	±0.18	1.5	±0.05	0.95	±0.18	0.76	Seedless	Cylindrical	
<i>Sisymbrium irio</i>	Glabrous	±1.39	7.63	Siliqua	Non	Linear or terete	Ribbed	Glabrous	Erect	Dehiscent	±0.22	3.9	±0.18	1.25	±0.06	1	Seedless	Cylindrical	

Table 3. Macro morphological characters of seed of studied species.

Species	Characters		Hilum	Seed length (mm.)			Seed width (mm.)		Ratio L/W	Surface	Winged margin
	Color	Shape		Stander	Average	Stander	Stander	Average			
<i>Brassica juncea</i>	Dark brown	Globose	Terminal	± 0.15	1.33	± 0.11	1.18	1.13	1.13	Reticulate	Absent
<i>Brassica tournefortii</i>	Brown	Globose	Terminal	± 0.08	1.08	± 0.08	1.08	1	1	Warty	Absent
<i>Eruca sativa</i>	Brownish-yellow	Sub-globose	Terminal	± 0.29	1.68	± 0.27	1.40	1.19	1.19	Glabrous	Absent
<i>Raphanus sativus</i>	Brown	Oblong	Terminal	± 0.19	2.78	± 0.19	1.78	1.56	1.56	Reticulate	Absent
<i>Cakile arabica</i>	Yellowish-brown	Oblong	Sub terminal	± 0.16	3.10	± 0.07	1	3.1	3.1	Glabrous	Absent
<i>Savignya parviflora</i>	Yellowish-brown	Oblong ovate	Sub terminal	± 0.38	2.58	± 2.32	3.83	0.67	0.67	Glabrous	Present
<i>Coronopus didymus</i>	Yellowish-brown	Kidney shape	Terminal	± 0.15	1.18	± 0.07	0.80	1.47	1.47	Glabrous	Absent
<i>Farsetia burtonae</i>	Yellowish-brown	Oblong ovate	Sub terminal	± 0.38	1.70	± 0.05	0.85	2	2	Glabrous	Present
<i>Cardamine hirsuta</i>	Yellow	Oblong ovate	Terminal	± 0.08	0.88	± 0.15	0.73	1.21	1.21	Glabrous	Absent
<i>Sisymbrium irio</i>	Yellow	Oblong	Sub terminal	± 0.08	0.88	± 0.04	0.48	1.84	1.84	Glabrous	Absent

Table 4. Seed coat micromorphology of the studied species.

Species	Characters			Anticinal wall			Pericinal wall			Seed wing					
	Seed coat pattern sculpture	Outer epidermal cell shape	Shape	Texture	Thick	Level	Level	Texture	Coat pattern sculpture	Outer epidermal cell shape	Shape	Texture	Thick	Level	Texture
<i>Brassica juncea</i>	Reticulate	Polygonal to elongated cell	Straight	Reticulate	Thin	Raised	Flat	Micro-reticulate	-	-	-	-	-	-	-
<i>Brassica tournefortii</i>	Undulate	Irregular polygonal cell	Undulate	Smooth to folded	Thick	Raised	Concave	Fine folded	-	-	-	-	-	-	-
<i>Eruca sativa</i>	Reticulate foveolate	Isodiametric polygonal cell	Straight to sinuous	Smooth to warty	Thick	Raised	Concave	Smooth	-	-	-	-	-	-	-
<i>Raphanus sativus</i>	Reticulate	Polygonal cell	Straight to sinuous	Striated	Thick	Raised	Concave	Micro-reticulate	-	-	-	-	-	-	-
<i>Cakile arabica</i>	Irregular reticulate	Polygonal to elongated cell	Straight to sinuous	Smooth to striated	Thin	Raised	Flat	Striate	-	-	-	-	-	-	-
<i>Savignya parviflora</i>	Verrucate	Irregular polygonal cell	Undulate	Striate	Thin	Grooved	Convex	Striate	Tuberculate	Isodiametric to irregular polygonal cell	Straight to undulate	Striate	Thin	Grooved	Convex
<i>Coronopus didymus</i>	Reticulate	Polygonal to elongated cell	Straight to sinuous	Smooth	Thick	Raised	Concave	Smooth	-	-	-	-	-	-	-
<i>Farsetia burtonae</i>	Reticulate	Polygonal to elongated cell	Straight to sinuous	Smooth	Thin	Raised	Flat to concave	Smooth to fine striate	Scalariform	Elongated cell	Straight to sinuous	Smooth	Thin	Grooved	Convex
<i>Cardamine hirsuta</i>	Reticulate with globular central body	Polygonal cell	Undulate	Smooth	Thin	Raised	Flat and convex in center	Smooth to striate	-	-	-	-	-	-	-
<i>Sisymbrium irio</i>	Ocellate	Polygonal cell	Undulate	Smooth	Thin	Grooved	Convex	Smooth	-	-	-	-	-	-	-

Table 5. Characters list for the numerical analysis of the studied species of Brassicaceae.

Pedicel	Texture	1- Glabrous [1]/Hairy [2].
	Length	2- 2 - 5 mm. [1]/6 - 10 mm. [2]/more than 10 mm. [3].
Fruit	Type	3- Siliqua [1]/Silicula [2].
	Segmentation	4- Segmented [1]/Non-segmented [2].
	Shape	5- Linear or terete [1]/Oblong [2]/Subreniform [3].
	Surface	6- Ribbed [1]/Not ribbed [2].
	texture	7- Glabrous [1]/Tuberculate [2]/Hairy [3].
	Direction	8- Erect [1]/Erect to slightly curved [2]/Curved [3].
	Opening	9- Dehiscent [1]/Indehiscent [2].
	Length	10- 0.1 - 0.9 cm. [1]/1 - 2 cm. [2]/2.1 - 3 cm. [3]/3.1 - 4 cm [4]/4.1 - 5 cm [5].
	Width	11- 0.1 - 0.9 cm. [1]/1 - 2 cm. [2]/2.1 - 3 cm. [3]/3.1 - 4 cm [4]/4.1 - 5 cm [5].
	Beak length	12- Less than 1 mm. [1]/1 - 4 mm. [2]/5 - 7 mm. [3]/More than 7 mm. [4].
	Beak seeding	13- Seedless [1]/one-two seed [2].
	Beak shape	14- Conical [1]/Cylindrical [2]/Flate [3].
Seed	Color	15- Brown [1]/Dark brown [2]/Yellowish brown [3]/Yellow [4].
	Shape	16- Globose [1]/Sub-globose [2]/Oblong [3]/Kidney shape [4].
	Hilum	17- Terminal [1]/Sub terminal [2].
	Length	18- Less than 1 mm [1]/1 - 2 mm [2]/more than 2 mm [3].
	Width	19- 18-Less than 1 mm [1]/1 - 2 mm [2]/more than 2 mm [3].
	Surface	20- Reticulate [1]/Warty [2]/Glabrous [3].
	Margin	21- Winged [1]/Not winged [2].
	Seed coat pattern scalpture	22- Reticulate [1]/Undulate [2]/Verrucate [3]/Ocellate [4].
	Outer epidermal cell shape	23- Polygonal to elongated cell [1]/Irregular polygonal cell [2]/Isodiametric polygonal cell [3]/polygonal cell [4].
	Anticinal wall shape	24- Straight [1]/Undulate [2]/Straight to sinuous [3].
	Anticinal wall texture	25- Reticulate [1]/Smooth [2]/Striate [3]/Smooth to folded [4]/Smooth to warty [5]/Smooth to striated [6].
	Anticinal wall thickening	26- Thick [1]/Thin [2].
	Anticinal wall level	27- Raised [1]/Grooved [2].
	Periclinal wall level	28- Flat [1]/Concave [2]/Convex [3]/Flat to concave [4]/Flat to convex [5].
	Periclinal wall texture	29- Micro reticulate [1]/Fin folded [2]/Smooth [3]/Striate [4]/Smooth to striate [5].
Seed wing	coat pattern scalpture	30- Tuberculate [1]/Scalariform [2].
	Outer epidermal cell shape	31- Isodiametric to irregular polygonal [1]/Elongated [2].
	Anticinal wall shape	32- Straight to undulate [1]/Straight to sinuous [2].
	Anticinal wall texture	33- Striate [1]/Smooth [2].
	Periclinal wall texture	34- Striate [1]/Smooth to fold [2].

Table 6. Data matrix of morphological characters listed in **Table 5**.

Species Characters	<i>Brassica juncea</i>	<i>Brassica tournefortii</i>	<i>Eruca sativa</i>	<i>Raphanus sativus</i>	<i>Cakile arabica</i>	<i>Savignya parviflora</i>	<i>Coronopus didymus</i>	<i>Farsetia burtonae</i>	<i>Cardamine hirsuta</i>	<i>Sisymbrium irio</i>
a1	1	1	1	1	1	1	2	2	1	1
a2	2	2	1	3	1	3	1	1	1	2
a3	1	1	1	1	1	2	2	1	1	1
a4	2	2	2	1	1	2	2	1	2	2
a5	1	1	2	2	2	2	3	1	1	1
a6	2	2	2	1	1	2	2	2	2	1
a7	1	1	1	1	1	1	2	3	1	1
a8	2	2	1	3	1	1	1	2	2	1
a9	1	1	1	2	2	1	2	1	1	1
a10	3	5	3	3	2	1	1	2	2	4
a11	2	2	4	5	3	5	3	2	1	2
a12	4	4	3	4	1	1	1	2	1	2
a13	1	2	1	1	1	1	1	1	1	1
a14	1	1	1	1	3	3	2	2	2	2
a15	1	2	3	1	3	3	3	3	4	4
a16	1	1	2	3	3	3	4	3	3	3
a17	1	1	1	1	2	2	1	2	1	2
a18	2	2	2	3	3	3	2	2	1	1
a19	2	2	2	2	2	3	1	1	1	1
a20	1	2	3	1	3	3	3	3	3	3
a21	2	2	2	2	2	1	2	1	2	2
a22	1	2	1	1	1	3	1	1	1	4
a23	1	2	3	4	1	2	1	1	4	4
a24	1	2	3	3	3	2	3	3	2	2
a25	1	4	5	3	6	3	2	2	2	2
a26	2	1	1	1	2	2	1	2	2	2
a27	1	1	1	1	1	2	1	1	1	2
a28	1	2	2	2	1	3	2	4	5	3
a29	1	2	3	1	4	4	3	5	5	3
a30	0	0	0	0	0	1	0	2	0	0
a31	0	0	0	0	0	1	0	2	0	0
a32	0	0	0	0	0	1	0	2	0	0
a33	0	0	0	0	0	1	0	2	0	0
a34	0	0	0	0	0	1	0	2	0	0



Figure 1. Fruit morphology of 10 species of Brassicaceae as revealed by light microscope: 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 hirsute*; 10. *Sisymbrium irio*.

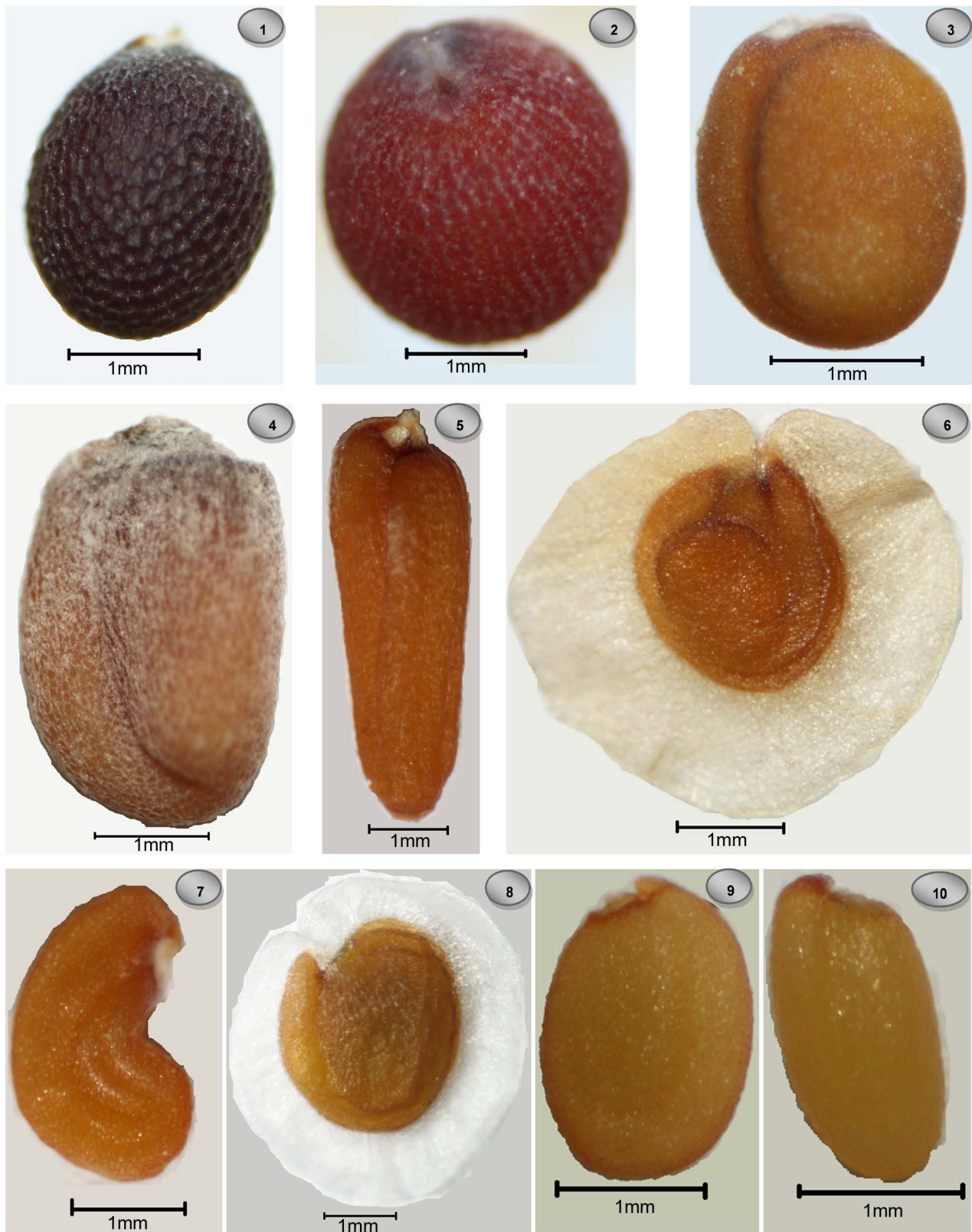


Figure 2. Seed morphology of 10 species of Brassicaceae as revealed by light microscope: 1. *Brassica juncea*; 2. *Brassica tournefortii*; 3. *Eruca sativa*; 4. *Raphanus sativus*; 5. *Cakile Arabica*; 6. *Savignya parviflora*; 7. *Coronopus didymus*; 8. *Farsetia burtonae*; 9. *Cardamine hirsute*; 10. *Sisymbrium irio*.

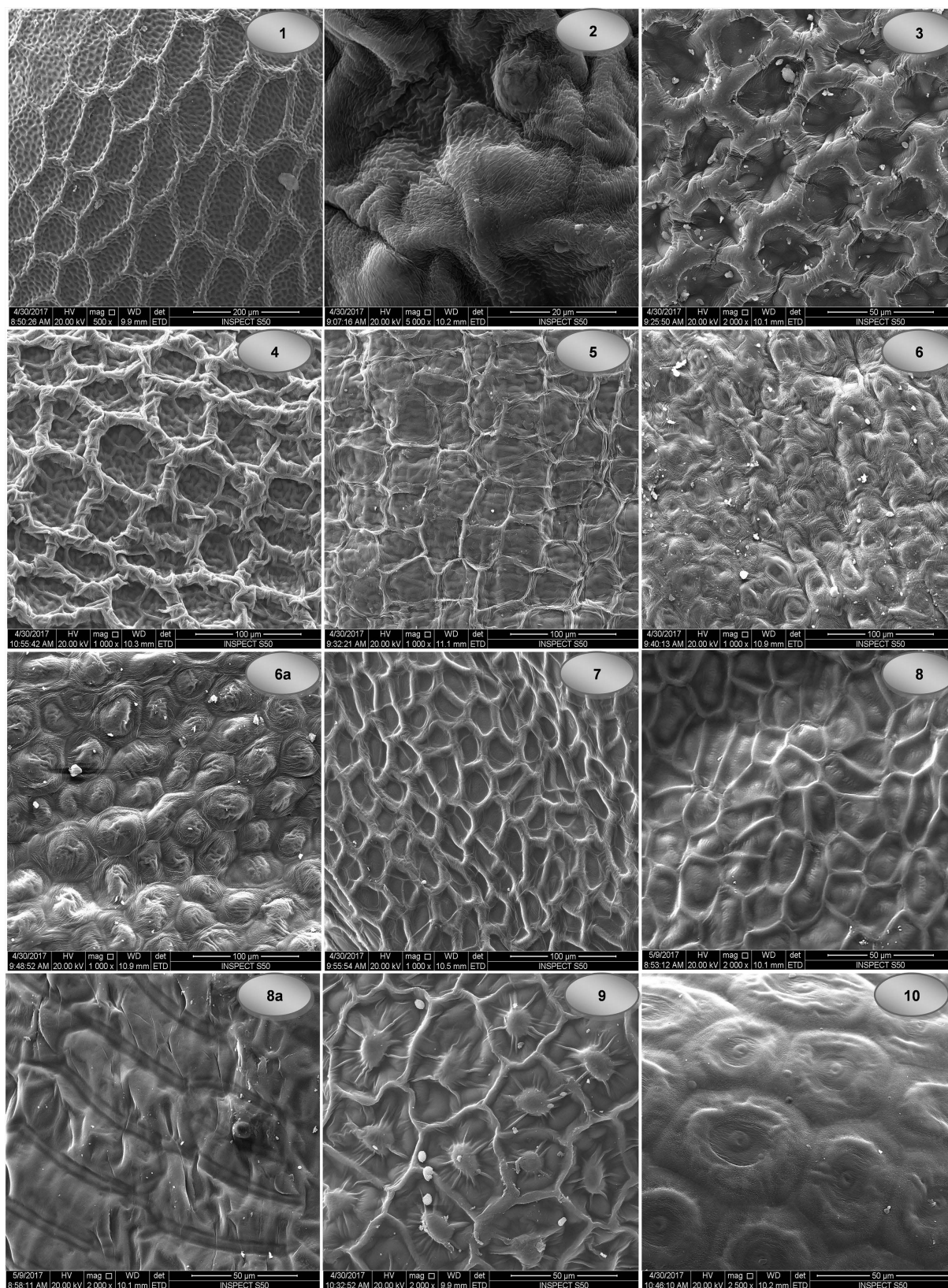


Figure 3. Scanning electron microscope micrographs of seeds (a), Surface sculpture of wing margin; 1. *Brassica juncea*; 2. *Brassica tournefortii*; 3. *Eruca sativa*; 4. *Raphanus sativus*; 5. *Cakile Arabica*; 6. *Savignya parviflora*; 7. *Coronopus didymus*; 8. *Farsetia burtonae*; 9. *Cardamine hirsute*; 10. *Sisymbrium irio*.

3.1.6. Fruit Direction and Opening

The most species of family Brassicaceae are characterized by erect and dehiscent fruit. In the studied species four species [*Eruca sativa*, *Savignya parviflora*, *Cardamine hirsute* and *Sisymbrium irio*] are erect and dehiscent, while erect and indehiscent in *Cakile arabica* and *Coronopus didymus*. Erect to slightly curved and dehiscent in *Brassica juncea*, *Brassica tournefortii* and *Farsetia burtonae*. Curved and indehiscent in *Raphanus sativus* only.

3.1.7. Fruit Size

The size of fruit in the studied species are different from very long (more than 3 cm.), long (1 - 3 cm.) and short (less than 1 cm.) in length or narrow (less than 2 mm.) or wide (more than 2 mm.) in width. Very long and narrow in *Brassica tournefortii* and *Sisymbrium irio*; long and narrow in *Brassica juncea*, *Farsetia burtonae* and *Cardamine hirsute* while long and wide in *Eruca sativa*, *Raphanus sativus* and *Cakile arabica*. Short and wide in *Savignya parviflora* and *Coronopus didymus*.

3.1.8. Beak Characters

The size of the beak ranged from 0.2 mm - 16 mm in length. The smallest size 0.2 mm present in *Coronopus didymus*, while the longest 16 mm present in *Raphanus sativus*. The beak is seedless in all studied species except in *Brassica tournefortii* have one or two seed. The shape of the beak in the studied species recorded three types, conical in *Brassica juncea*, *Brassica tournefortii*, *Eruca sativa* and *Raphanus sativus*, flat in *Cakile Arabica* and *Savignya parviflora*, cylindrical in the remainder.

3.2. Seed Characters

3.2.1. Seed Color

The color of seeds is highly important for distinguish between different species. The seed color in studied species varied from yellow in *Cardamine hirsute* and *Sisymbrium irio*; yellowish-brown in *Cakile arabica*, *Savignya parviflora*, *Coronopus didymus* and *Farsetia burtonae*; brownish-yellow in *Eruca sativa* and brown in the remainder species.

3.2.2. Seed Shape

Seed shape of the studied species recorded four types; globose in *Brassica juncea* and *Brassica tournefortii*; sub-globose in *Eruca sativa*; kidney shape in *Coronopus didymus*; oblong ovate in *Savignya parviflora*, *Farsetia burtonae* and *Cardamine hirsute*; oblong in the remainder.

3.2.3. Seed Hilum

The hilum of seed in most studied species is terminal except in *Cakile arabica*, *Savignya parviflora*, *Farsetia burtonae*, and *Sisymbrium irio* are sub terminal.

3.2.4. Seed Size

Seed length and width varied among the studied species; it ranged from 0.8 mm -

3.1 mm length \times 3.8mm - 0.48 mm width. The longest seed have size 3.1 mm \times 1 mm present in *Cakile arabica*, while the smallest seed (0.88 mm \times 0.48 mm) present in *Sisymbrium irio*.

3.2.5. Seed Surface

The seed surface of the studied species recorded four types; reticulate in *Brassica juncea* and *Raphanus sativus*; warty in *Brassica tournefortii*; glabrous in the reminder.

3.2.6. Seed Margin

The seed are winged in two species only [*Savignya parviflora* and *Farsetia burtonae*], and not winged in the rest studied species.

3.2.7. Seed Coat Pattern Sculpture: Table 4 & Figure 3

Examination by scanning electron microscope on the studied species shows four different types of seed coat pattern; the reticulate is the main types in which seven species belong to it:

- 1) Reticulate, with four subtypes:
 - a) Regular reticulate recorded in *Brassica juncea*, *Raphanus sativus*, *Coronopus didymus* and *Farsetia burtonae*.
 - b) Reticulate-foveolate in *Eruca sativa*.
 - c) Irregular reticulate present in *Cakile arabica*.
 - d) Reticulatewith globular central body in *Cardamine hirsute*.
- 2) Undulate in *Brassica tournefortii*.
- 3) Verrucate in *Savignya parviflora*.
- 4) Ocellate in *Sisymbrium irio*.

3.2.8. Shape of Outer Epidermal Cell

Outer epidermal cells can be of considerable important value for systematics. The outer epidermal cells varied from polygonal to elongated cell in *Brassica juncea*, *Cakile arabica*, *Coronopus didymus* and *Farsetia burtonae*; irregular polygonal cell in *Brassica tournefortii* and *Savignya parviflora*; isodiametric polygonal cells in *Eruca sativa*; polygonal cell in the reminder species.

3.2.9. Anticlinal Wall

The characters of anticlinal wall can be used for identification and differentiate the different species. The shape of anticlinal wall recorded three types in the studied species; undulate in *Brassica tournefortii*, *Savignya parviflora*, *Cardamine hirsute* and *Sisymbrium irio*; straight in *Brassica juncea* only and straight to sinuous in the reminder species. The main types for the texture of the anticlinal wall is smooth, but it smooth to fold in *Brassica tournefortii*; smooth to warty in *Eruca sativa* and smooth to striated in *Cakile arabica*. The texture is reticulate in *Brassica juncea* and striated in *Raphanus sativus*. The anticlinal wall varied from thin to thick, it is thick in *Brassica tournefortii*, *Eruca sativa*, *Raphanus sativus* and *Coronopus didymus*; while thin in the reminder species. All the studied

species with raised anticlinal wall except in *Savignya parviflora* and *Sisymbrium irio* are grooved.

3.2.10. Periclinal Wall

The characters of the outer periclinal cell wall can be good diagnostic characters. The present study recorded five types for the shape of Periclinal wall; flat in *Brassica juncea* and *Cakile arabica*; flate to concave in *Farsetia burtonae*; flat and convex in center in *Cardamine hirsute*; convex in *Savignya parviflora* and *Sisymbrium irio*; concave in the rest of species. The texture of Periclinal wall have five types; micro-reticulate in *Brassica juncea* and *Raphanus sativus*; fine folded in *Brassica tournefortii*; striate in *Cakile arabica* and *Savignya parviflora*; smooth to fine striate in *Farsetia burtonae*; smooth in the rest studied species.

3.2.11. Seed Wing

There are two seeds from the studied species are winged, the coat pattern sculpture for these seeds are tuberculate in *Savignya parviflora* and scalariform in *Farsetia burtonae*. The outer epidermal cell shape is isodiametric to irregular polygonal in *Savignya parviflora* while elongated in *Farsetia burtonae*. The shape of anticlinal wall is straight to undulate in *Savignya parviflora* while straight to sinuous in *Farsetia burtonae*. The texture of anticlinal wall is striate in *Savignya parviflora* and smooth in *Farsetia burtonae*. The thickening and level of anticlinal wall for the two species are thin and grooved. The shape of Periclinal wall is convex in the two winged species, but the texture is striate in *Savignya parviflora* while smooth to folded in *Farsetia burtonae*.

4. Numerical Analysis

The fruit and seed morphology characters of the studied species obtained by light and scanning electron microscope 34 characters (14 characters for fruit and 20 characters for seed) were used for numerical analysis.

The cluster analysis obtained from fruit characters only (**Figure 4(a)**) showed that species were grouped into two major clusters and each cluster divided into two groups, the first cluster I consisted of five species: *Coronopus didymus* and *Farsetia burtonae* recorded in group I while *Cardamine hirsute*, *Cakile arabica* and *Sisymbrium irio* present in group II. The cluster II comprised five species: *Raphanus sativus* (group I) and *Brassica juncea*, *Eruca sativa*, *Brassica tournefortii* and *Savignya parviflora* (group II).

The results obtained from seed characters only (**Figure 4(b)**) also divided the studied species into two main clusters and two groups. Cluster I contained *Savignya parviflora* and *Farsetia burtonae*. While cluster II consisted of eight species present in two groups; *Cardamine hirsute*, *Raphanus sativus*, *Coronopus didymus*, *Eruca sativa* and *Cakile arabica* in group I and *Brassica juncea*, *Brassica tournefortii* and *Sisymbrium irio* in group II.

The results obtained from combined of fruit and seed characters (**Figure 4(c)**) divided the species into two main clusters and two groups. Cluster I contained

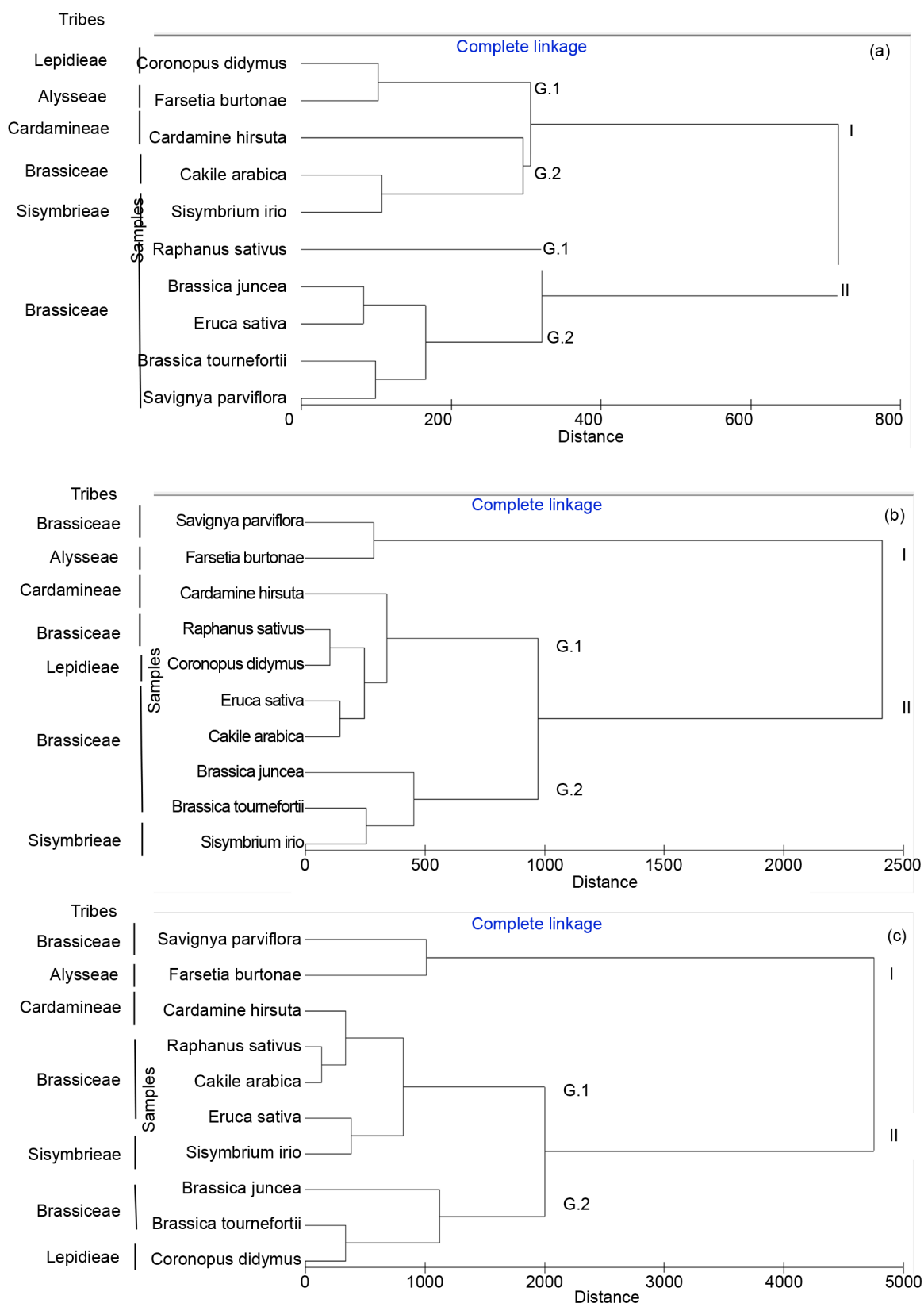


Figure 4. Dendrograms showing the interrelationships between ten species of Brassicaceae based on 34 characters, (I) cluster one, (II) cluster two, (G1) group one, (G2) group two, (a) fruit characters, (b) seed characters, (c) combined fruit and seed characters by using PRIMER Program.

Savignya parviflora and *Farsetia burtonae*. While cluster II consisted of eight species present in two groups; *Cardamine hirsute*, *Raphanus sativus*, *Cakile arabica*, *Eruca sativa* and *Sisymbrium irio* in group I and *Brassica juncea*, *Brassica tournefortii* and *Coronopus didymus* in group II.

5. Discussion

The tribal classification of Brassicaceae has long been problematic and not well understood phylogenetically because of the traditionally used few characters as cotyledons in the embryo, fruit characters, number of rows of seeds in each locule, trichome type, and features of the nectarines etc. to delimit different tribes of the family Brassicaceae [23] [63]. In the present study, the species are recorded in five tribes (Alysseae, Lepideae, Sisymbrieae, Cardamineae, Brassiceae) [15].

The tribe Alysseae DC. As delimited by [15] [64] are polyphyletic. It consists of approximately 280 species, the *Farsetia* species put in different tribes in different studies; [5] put it in tribe Hesperideae under subtribe Malcolmiinae while [7] placed the *Farsetia* species with *Lobularia* in tribe Alyssineae, [10] put it in tribe Arabideae. Recently [15] put the species *Farsetia* in tribe Alysseae as [8]. The tribe has recently been studied phylogenetically as [65] [66]. The position of Alysseae relative to the other tribes varied in different studies; [20] put the tribe with tribe Brassiceae in lineage II, while [63] say the tribe appeared either related to Erysimum or unrelated to any tribe. [67] found the tribe related to the Noccaeeae, while [68] it grouped together with the tribes Chorisporeae, Hesperideae, and Malcolmieae. In the present study tribe Alysseae (*Farsetia burtonae*) are placed with tribe Lepideae (*Coronopus didymus*) in the cluster result from fruit characters only, this differ from the traditional classification which separate them into different tribes or different groups, but it agree with recent molecular studies as [27] which put the two tribes in the same lineage. While in the clusters results from seed or seed and fruit characters the tribe Alysseae (*Farsetia burtonae*) are placed with species of tribe Brassiceae (*Savignya parviflora*), this agree with some molecular studies as [27] [69].

The tribe Lepideae consists of three to five genera and over 240 species, [15] [70]. It is polyphyletic. Some genetic studies as done on the tribes, [27] [69] [71] [72] [73]. In the present study the tribe Lepideae (*Coronopus didymus*) are placed with some species of tribe Brassiceae, this agree with some traditional studies as [5] which put them in the same tribe but in different sub-tribes, or placed with species of tribe Alysseae (*Farsetia burtonae*) in the same group.

The tribe Sisymbrieae was recognized with 70 genera and 400 species according to [8] [74] but molecular data as, [18] [75] [76] [77], supported the removal of many of its component genera to other tribes. The tribe is monophyletic and is sister to the Schizopetaleae [20] or the Brassiceae [18] [75]. In the present study the tribe Sisymbrieae (*Sisymbrium irio*) found together with species from tribe Brassiceae, this agrees with [5] [18] [27] [69] [75].

[9] presented a phylogenetic network and indicated close relationships of

Sisymbrieae with Arabideae and Lepidieae. [78] [79] have shown that the boundaries between the tribes Lepidieae, Lunarieae, Sisymbrieae, Euclidieae, and Alysseae are highly artificial. The results from previous studies of phylogenetic relationships [71] [72] [73] demonstrated a close relationship among the tribes Sisymbrieae, Lepidieae and Arabideae. [70] indicated that the tribes Arabideae, Lepidieae and Sisymbrieae are polyphyletic.

The tribe Cardamineae DC is monophyletic. The tribe has been subjected to several molecular studies as; [80] [81] [82] [83] [84] that support its monophyly. The position of Cardamineae relative to the other tribes is done by some studies as, [27] [69]. The study put the tribe Cardamineae with tribe Lepidieae and tribe Alysseae in the same lineage. In the present study the tribe Cardamineae (*Cardamine hirsute*) placed together in the same group with some species of tribe Brassiceae and tribe Sisymbrieae in all clusters, this agree with [5].

The tribe Brassiceae DC is consider monophyletic according to some molecular studies as [19] [85] [86]. This tribe was subjected to extensive molecular studies, [20] [63] [67] [69] [87] [88]. In the present study the most species of tribe Brassieae are placed together but some species present with different tribes. This results support the Brassiceae to be heterogenous.

6. Conclusion

The fruit and seed coat characters are very important characters that can be used for identification and differentiate between the different tribes and also between species belong to the same genera. Most tribes of Brassicaceae are polyphyletic, and some are related to each other's as Lepidieae, Alyssieae and Sisymbrieae. The results indicate the tribe Brassiceae to be heterogenous.

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