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Research Article

Comparative epidermal leaf anatomy of some Brassicaceae with emphasis on its systematic implication

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Abstract

Some epidermal leaf anatomy characteristics such as the absence/of presence trichomes and stomata and trichome types in adaxial and abaxial leaf surfaces within different species, subspecies, varieties, and populations are afforded valuable taxonomic characteristics in their determination. This study aimed to investigate the leaf epidermal characteristics in 72 populations belonging to 46 wild and native Brassicaceae taxa collected from central Iran. The adaxial and abaxial leaf epidermis was examined using light microscopy. Results showed that all studied samples lack crystals in their epidermis. They can be divided into three groups: 1) without trichomes in both adaxial and abaxial leaf surfaces, 2) having trichomes just on the abaxial side (*Lepidium sativum* and *Sisymbrium irio*), and 3) trichomes in both adaxial and abaxial surfaces. Two *Choriospora* species can be separated by their trichomes. *C. iberica* has glandular trichome, while *C. tenella* has no trichomes. This study revealed that just epidermal leaf anatomy characters are not sufficient to identify members of the Brassicaceae members, but can be helpful along with other features and may support the systematic value of taxa in this family.

Keywords: Cruciferae, leaf anatomy, epidermal cell, stomata, trichome

Introduction

The Brassicaceae (Cruciferae) with 321 genera and about 3660 species in the world is known as a huge dicot family (Al-Shehbaz 2012). The major distribution centers of the family are the Mediterranean, Irano-Turanian, and Saharo-Sindianregions (Hedge, 1976). Among the 127 genera and 469 species dealt with in Flora Iranica, 104 genera and 310 species have been reported from Iran (Rechinger, 1968) that ca. 47 taxa including 8 new species and 39 records were added. There have been some nomenclatural changes and reductionsino synonymy, which increased the total number of species to 358 and the number of genera to 120 for Iran (Akhani, 2003). Brassicaceae is an economically important family in agriculture, gardening, nutrition, molecular biology studies as model organisms, invasive and toxic plants (Guarino et al., 2000). It is known

that for a long time morphology was the only discipline contributing to the characters for systematics and phylogenetic reconstruction (Stuessy et al., 2003). Due to the insufficient morphological studies in plant diagnosis, leaf epidermal anatomy studies can be useful in separating the inferaspecies of plant classification (Jensen et al., 2002). Khalik (2005) found trichomes as being valuable characteristics for the identification of tribes, genera, species, subspecies, and varieties in Brassicaceae (Khalik, 2005). Freire et al. (2005) used leaf epidermal features such as anticlinal epidermal cell wall patterns, cuticular ornamentation, stomata, and hair types of toxic plants for cattle from the Salado river basin. Studies on epidermal characters revealed high diagnostic value for the identification of the toxic plants from the Salado River basin (Freire et al., 2005). Dennert (1884) was the first author to give attention to the different types of trichomes in Brassicaceae. He divided these hairs into simple, dichotomous, and stellate (Dennert, 1884). Prantl (1891) used the hair shape as a basic character for the classification of Brassicaceae and recognized four tribes (Prantl, 1891). The members of Brassicaceae were distinct. In *Brassica campestris* the epidermal cells were with slight undulations, polygonal to nearly linear, in *Lepidiumapitalum,* polygonal to nearly rounded and linear in *Raphanus sativus* and in *Sisymbrium irio* polygonal at some places, and polygonal to less undulating to clearly irregular at other spots (Ahmad et al., 2010). According to the observations of Pant and Kadwai (1967) the anticlinal walls of mature epidermal cells in *Lobularia maritima, B. compestris*var. *sarson, B. rapa, Coronopusdidymus, Iberis atnara,Nasturtium officinale, Raphanus sativus* are sinuous and in *B. oleracea* L. var. *capitata* L, *B. oleracea* L. var. *botrytis* L., and *Brassica caulorapa* Pasq are straight or arched (Pant & Kadwai, 1967). Doaigey et al. (2013) studied the stems and leaves epidermal characters of the 34 species in 26 genera of Brassicaceae for finding additional epidermal characteristics of these plants that grow under desert conditions in different areas of Saudi Arabia. Their results showed that epidermal cell dimensions (length and width), stomata number, stomata length, and width in both adaxial and abaxial leaf surfaces were significant (Doaigey et al., 2013). Here we report the epidermal leaf anatomy studies on 46 Brassicaceae (Cruciferae) taxa from central Iran and their taxonomic significance using light microscopy methods.

Materials and Methods

Plant material collection and determination

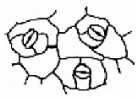
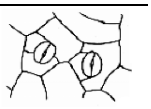
Plant material of 72 natural populations belonging to 46 wild and native Brassicaceae taxa was collected from the central Iran in 2018. Voucher specimens of each sample were prepared for reference as herbarium vouchers and deposited at the Arak University Herbarium (not listed in the herbarium index). Collected plants idenwere tified using valuable and available references (Davis, 1967; Rechinger, 1968; Jafri, 1973; Mobayen, 1979; Massoumi, 1980; Mozafarian, 2001;

Warwick et al., 2006; Ghahreman, 1976-2008; Asadi, 2017). Collection data of samples has been shown in Table 2.

Epidermal leaf anatomy studies

For examination segments of leaf from herbarium material, 5 mm in diameter, were soaked in water overnight and then dried at room temperature after blotting with filter paper. Then the leaves were soaked in 30% nitric acid at room temperature for 48 h and the epidermis of both abaxial and adaxial surfaces was removed with tweezers, washed in running water, and stained in 1% Safranine, and mounted in 10% glycerin for semi-permanent mounting (Noori 2002). Some characteristics of epidermal leaf anatomy such as crystal, stomata, and trichomes in adaxial and abaxial leaf surfaces were studied using a Leica Galen III monitoring light microscope. Then photomicrographs were taken from each sample (Fig. 1). Table 1 shows epidermal leaf anatomy characters of the studied Brassicaceae populations from Iran. The data from the study are presented in table 3.

Table 1. Examined epidermal leaf anatomy characters of the studied Brassicaceae from Iran.

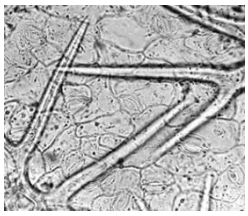
No.	Character	Type					
1	Epidermal leaf crystal	None					
2	Leaf ad/ab trichome	absence(1), presence (2)					
3	Leaf trichomes types	Absence=none	 1-armed-long	 1-armed-short	 Glandular	 2-armed basifixed	 Bifid multicellular
		 dendroid (3-armed)				 Stellate more than 3-armed	
		Stellate (dendroind)					
4	Leaf stomata types ad/ab	 Folded anisocytic		 anisotricytic	 Circular anisotricytic	 Angular anisotricytic	

Data analysis

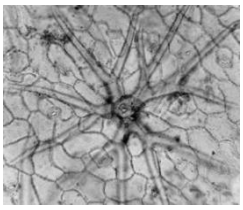
All of the studied qualitative characters (crystal, existence leaf ad/ab trichomes, Leaf trichomes types, and leaf stomata types ad/ab-in Table 1) except for crystal were coded as multistate characters. Crystal character was deleted in analysis the reason of similarity and lacking in all of the examined taxa. Data were analyzed using the SPSS (1997) for windows release 16.0

statistical package for social scientists by principal component analysis (PCA) test (Tables 4 and 5). Then cluster analysis using Ward, Average Linkage (between groups), and Median methods were performed on standardized epidermal leaf anatomy characters data. The cluster's correctness was checked with the obtained data using cophenetic correlation (Fig. 2).

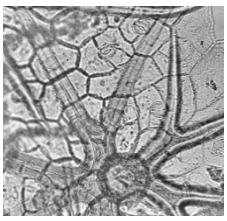
Aethinema carnemum



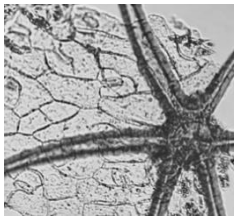
Alyssum alysoides



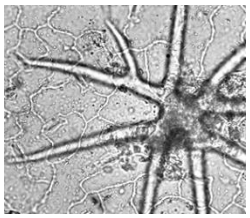
Alyssum dasycarpum



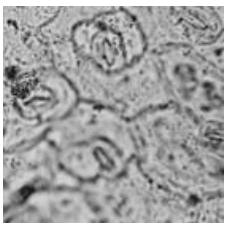
Alyssum desertorum



Alyssum longystylum

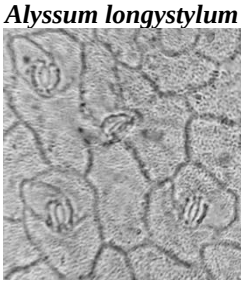


Alyssum linifolium

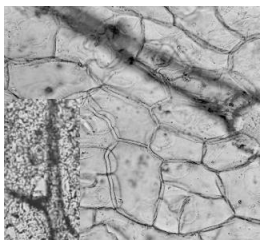


Brassica deflexa

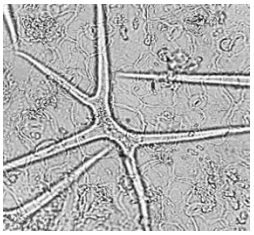
Brassica elongata



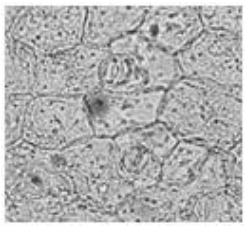
Brassica napus



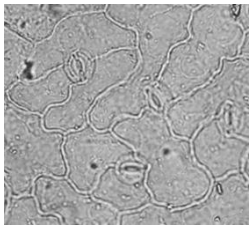
Camelina rumelica



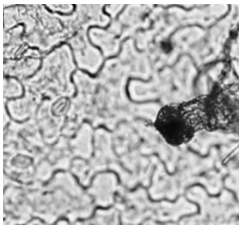
Capsella bursa pastoris



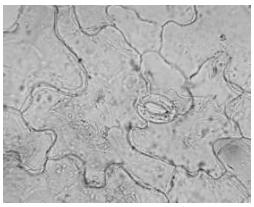
Cardamin hirsute



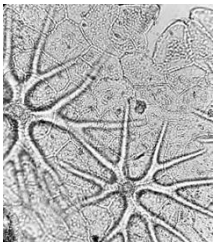
Cardamin impatience



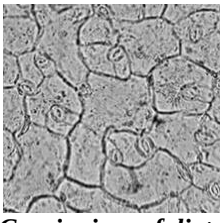
Choriospora iberica



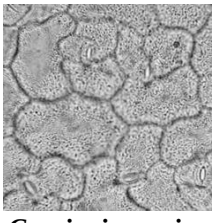
Choriospora tenella



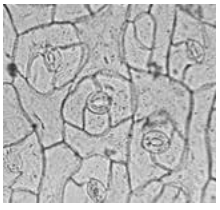
Clypeola jonthlaspi



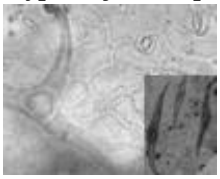
Conringia perfoliata



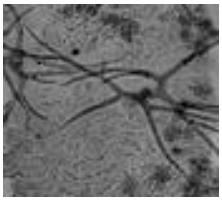
Conringia persica



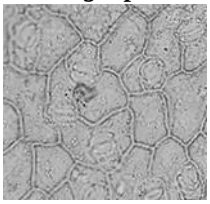
Crambe orientalis



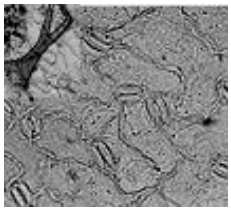
Cymatocarpus pilosissimus



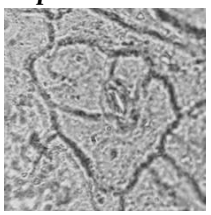
Descurainia sophia



Diplotaxis harra



Draba minima



Eruca sativa

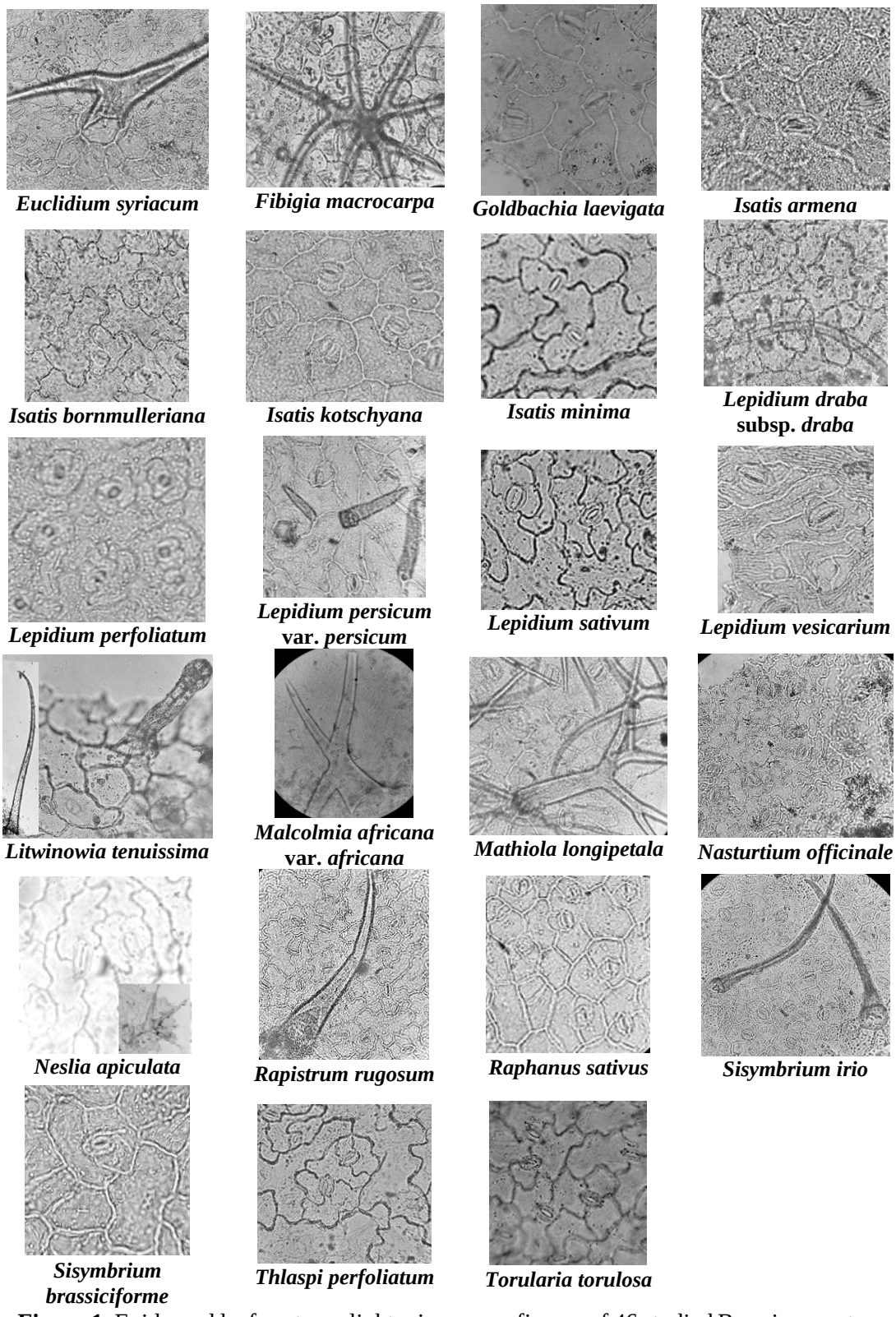


Figure 1. Epidermal leaf anatomy light microscopy figures of 46 studied Brassicaceae taxa from central Iran ×40

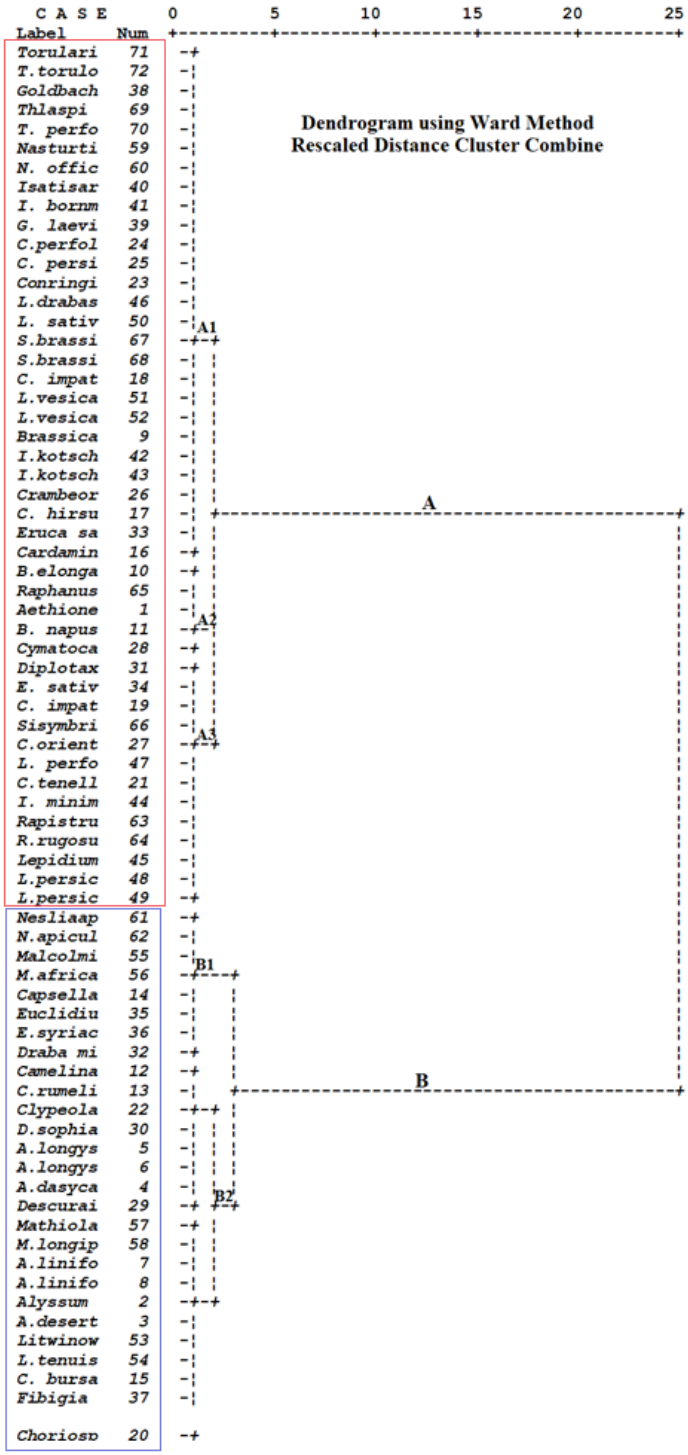


Figure 2. Cluster analysis (Ward) of epidermal leaf anatomy encoded qualitative characters for 72 Brassicaceae populations in central Iran. The red rectangle shows without trichomes and the blue one shows with trichomes populations. Scored characters for cluster analysis have been shown in table 1.

Results

All examined collections and herbarium specimens are listed in table 2. Data of studied epidermal leaf anatomy qualitative characters of Brassicaceae populations in Iran have presented in Table 3. Any trichomes were not observed in both adaxial and abaxial leaf surfaces of 37 examined populations. *Lepidium sativum* and *Sisymbrium irio* species had trichomes on their abaxial leaf

surface. While the rest samples had trichomes on both leaf surfaces. The most trichome types in the studied samples were 1-armed/conical and stellate and the most leaf stomata type ad/ab was anisocytic type (Table 3, Fig. 1). Table 4 is shown the total variance explained for principal component analysis for the studied fruit and seed morphological characters of Brassicaceae populations in central Iran. Table 5 shows components of PCA test and correlating epidermal leaf anatomy characters of the studied populations (P<0.05). Analyzing epidermal leaf anatomy quantitative characters using three different cluster analyzing methods showed that the Ward method provided the best data (Fig. 2). Table 6 shows an identification key based on the studied epidermal leaf anatomical characters.

Table 2. Collection information data of 72 studied Brassicaceae populations from Iran.

No.	Code	Taxon	Locality	Date	Latitude N	Longitude E	Height(m)
1	*CAN_1	*Aethionemacarnemum* (Bank & Soland) B. Fedtsch	Toreh	20.02.1397	34° 02′ 59′′	49° 17′ 40′′	1870
2	CAN_3	*Alyssum alysoides*(L.)	Arak	10.02.1397	34° 05′ 15′′	49° 42′ 09′′	1737
3	CAN_4	*Alyssum desertorum* Stapf	Arak-Khomein Road	28.02.1397	34° 01′ 06′′	49° 50′ 19′′	1751
4	CAN_5	*Alyssum dasycarpum* Steph. Ex Willd	Komeijan	12.02.1397	34° 48′ 36′′	49° 21′ 32′′	2344
5	CAN_6	*Alyssum longystylum* (Sommier & Levier) Grossh & Schischk	Khondab	01.02.1397	34° 23′ 32′′	49° 11′ 20′′	1699
6	CAN_7	*Alyssum longystylum* (Sommier & Levier) Grossh & Schischk	Tafresh-Moeenabad	14.02.1397	34° 41′ 13′′	49° 58′ 35′′	2000
7	CAN_8	*Alyssum linifolium* Steph. ex Willd (*Meniocus linifolius* **(**Stephan ex Willd.) DC.	Tafresh	01.03.1397	34° 38′ 55′′	49° 57′ 54′′	2393
8	CAN_9	*Alyssum linifolium* Steph. ex Willd (*Meniocus linifolius* (Stephan ex Willd.) DC.	Farmahin	18.02.1397	34° 30′ 16′′	49° 41′ 3′′	1782
9	CAN_{10}	*Brassica deflexa* Boiss	Hak-e-Bolagh	04.03.1397	33° 59′ 51′′	49° 21′ 47′′	1837
10	CAN_{11}	*Brassica elongate* Ehrh	Bagh Bar aftab	20.03.1397	33° 56′ 6′′	49° 30′ 10′′	1935
11	CAN_{12}	*Brassica napus* L.	Karahrod	12.02.1397	34° 03′ 45′′	49° 38′ 4′′	1832
12	CAN_{13}	*Camelina rumelica* Velen.	Hak-e-Bolagh	30.02.1397	33° 58′ 21′′	49° 22′ 41′′	1861
13	CAN_{14}	*Camelina rumelica* Velen.	Tafresh-Moeenabad	30.02.1397	34° 41′ 27′′	49° 58′ 01′′	2029
14	CAN_{15}	*Capsella bursa pastoris* (L.) Medicus	Arak-Park-e-Shahr	14.02.1397	34° 05′ 20′′	49° 41′ 58′′	1742
15	CAN_{16}	*Capsella bursa pastoris* (L.) Medicus	Astaneh	10.01.1397	33° 55′ 55′′	49° 24′ 02′′	1910
16	CAN_{17}	*Cardamin hirsute* L.	Nazmabad	08.01.1397	34° 03′ 8′′	49° 43′ 36′′	1838
17	CAN_{18}	*Cardamin hirsute* L.	Hosseinabad	29.01.1397	34° 01′ 38′′	49° 45′ 16′′	2028
18	CAN_{19}	*Cardamin impatience* L.	Khondab	10.02.1397	34° 23′ 32′′	49° 11′ 20′′	1699
19	CAN_{20}	*Cardamin impatience* L.	Arak-Khomein Road	05.02.1397	34° 02′ 35′′	49° 48′ 29′′	1713
20	CAN_{21}	*Choriosporaiberica* (M. B.) DC.	Arak-Tafresh-Moeenabad	30.02.1397	34° 41′ 13′′	49° 58′ 35′′	2010
21	CAN_{22}	*Choriosporatenella* (Pall) DC.	Arak-Bagh-e-Fadak	24.02.1397	34° 07′ 43′′	49° 57′ 21′′	1695
22	CAN_{23}	*Clypeola jonthlaspi* L.	Pang Ali	21.03.1397	34° 02′ 31′′	49° 18′ 08′′	2400
23	CAN_{24}	*Conringia perfoliata* (C. A. Mey) Busch.	Pang Ali	15.02.1397	34° 02′ 25′′	49° 18′ 03′′	1922
24	CAN_{25}	*Conringia perfoliata* (C. A. Mey) Busch.	Bagh Bar Aftab	15.02.1397	34° 02′ 59′′	49° 17′ 40′′	1870
25	CAN_{26}	*Conringia persica* Boiss	Tafresh	05.02.1397	34° 38′ 55′′	49° 57′ 54′′	2393
26	CAN_{27}	*Crambe orientalis* L.	Arak University	29.02.1397	34° 04′ 33′′	49° 38′ 14′′	1853

27	CAN_{28}	*Crambe orientalis* L.	Farmahin Road	23.03.1397	34° 32′ 33′′	49° 45′ 39′′	1876
28	CAN_{29}	*Cymatocarpus pilosissimus* (Trautv.) O. E. Schulz	Arak-Mobarakabad	30.03.1397	34° 08′ 03′′	49° 47′ 47′′	1666
29	CAN_{30}	*Descurainia sophia* (L.) Webb & Berth	Astaneh	10.01.1397	33° 53′ 40′′	49° 21′ 7′′	2000
30	CAN_{31}	*Descurainia sophia* (L.) Webb & Berth	Komeijan	20.01.1397	34° 40′ 10′′	49° 21′ 21′′	2140
31	CAN_{32}	*Diplotaxis harra* (Forssk.) Boiss	Saveh	27.03.1397	34° 53′ 50′′	50° 09′ 16′′	1155
32	CAN_{33}	*Draba minima* (C. A. Mey.) Steud.	Toreh	28.02.1397	34° 02′ 35′′	49° 17′ 28′′	1868
33	CAN_{34}	*Eruca sativa* Miller	Astaneh	10.01.1397	33° 53′ 53′′	49° 22′ 07′′	1981
34	CAN_{35}	*Eruca sativa* Miller	Karahrod	29.02.1397	34° 3′ 38′′	49° 37′ 47′′	1842
35	CAN_{39}	*Euclidium syriacum* (L.) R. Br. Aiton	Hosseinabad	28.02.1397	34° 01′ 38′′	49° 45′ 16′′	2028
36	CAN_{40}	*Euclidium syriacum* (L.) R. Br. Aiton	Zamenjan	05.03.1397	34° 01′ 55′′	49° 36′ 57′′	1868
37	CAN_{41}	*Fibigia macrocarpa* Boiss	Hak-e-Bolagh	12.02.1397	33° 59′ 15′′	49° 21′ 39′′	1858
38	CAN_{42}	*Goldbachia laevigata* (M. B.) DC.	Arak	13.02.1397	34° 05′ 15′′	49° 42′ 09′′	1737
39	CAN_{43}	*Goldbachia laevigata* (M. B.) DC.	Arak-Pang Ali	28.02.1397	34° 02′ 25′′	49° 18′ 3′′	1922
40	CAN_{44}	*Isatis armena* L. (Syn*: Sameraria armena* (L.) Desv)	Arak-Bagh-e-Fadak	28.01.1397	34° 07′ 38′′	49° 57′ 21′′	1702
41	CAN_{45}	*Isatis zarrei* Al-Shehbaz, Moazzeni & Mumm	Arak-Pang Ali	28.02.1397	34° 02′ 31′′	49° 18′ 08′′	2400
42	CAN_{46}	*Isatis kotschyana* Boiss. & Hohen	Arak-Bagh-e-Fadak	20.02.1397	34° 07′ 31′′	49° 57′ 28′′	1717
43	CAN_{47}	*Isatis kotschyana* Boiss. & Hohen.	Zamenjan	05.03.1397	34° 02′ 18′′	49° 36′ 48′′	1868
44	CAN_{48}	*Isatis minima* Bunge	Pang Ali	21.03.1397	34° 02′ 31′′	49° 18′ 08′′	2400
45	CAN_{50}	*Lepidium draba* L.subsp. *draba*	Zamenjan	28.02.1397	34° 01′ 55′′	49° 36′ 57′′	1868
46	CAN_{51}	*Lepidium draba* L. subsp. *draba*	Mashhad Meighan	21.01.1397	34° 11′ 45′′	49° 41′ 18′′	1673
47	CAN_{52}	*Lepidium perfoliatum* L.	Mashhad Meighan	25.01.1397	34° 10′ 35′′	49° 41′ 21′′	1200
48	CAN_{53}	*Lepidium persicum* Boiss var. *persicum*	Arak-Bagh-e-Fadak	28.01.1397	34° 07′ 18′′	49° 57′ 37′′	1764
49	CAN_{54}	*Lepidium persicum* Boiss var. *persicum*	Pang Ali	21.03.1397	34° 02′ 31′′	49° 18′ 08′′	2400
50	CAN_{55}	*Lepidium sativum* L.	Karahrod	12.02.1397	34° 03′ 24′′	49° 37′ 55′′	1834
51	CAN_{56}	*Lepidium vesicarium* L	Farmahin	02.03.1397	34° 31′ 47′′	49° 44′ 34′′	1852
52	CAN_{57}	*Lepidium vesicarium* L.	Saveh	17.03.1397	34° 53′ 50′′	50° 09′ 16′′	1155
53	CAN_{58}	*Litwinowia tenuissima* (Pall.) Woron. ex Pavlov.	Hezaveh	13.01.1397	34° 11′ 21′′	49° 33′ 11′′	1846
54	CAN_{59}	*Litwinowia tenuissima* (Pall.) Woron. ex Pavlov.	Hosseinabad	12.02.1397	34° 03′ 17′′	49° 47′ 10′′	1757
55	CAN_{60}	*Malcolmia africana* var. *africana* (L.) R. Br.	Hak-e-Bolagh	20.03.1397	33° 58′ 21′′	49° 22′ 41′′	1861
56	CAN_{61}	*Malcolmia africana* var. *africana* (L.) R. Br.	Tafresh	29.02.1397	34° 41′ 38′′	50° 00′ 55′′	1909
57	CAN_{62}	*Mathiola longipetala* (Vent.) DC.	Hosseinabad	31.02.1397	34° 03′ 16′′	49° 47′ 11′′	1760
58	CAN_{63}	*Mathiola longipetala* (Vent.) DC.	Arak-Khomein Road	02.03.1397	34° 02′ 35′′	49° 48′ 29′′	1713
59	CAN_{64}	*Nasturtium officinale* R. Brown	Sefidkhany	21.01.1397	33° 58′ 52′′	49° 35′ 10′′	2243
60	CAN_{65}	*Nasturtium officinale* R. Brown	Toreh	10.03.1397	34° 02′ 35′′	49° 17′ 28′′	1868
61	CAN_{66}	*Neslia apiculata* Fisch	Farmahin	12.02.1397	34° 32′ 55′′	49° 40′ 23′′	1812
62	CAN_{67}	*Neslia apiculata* Fisch	Tafresh-Moeenabad	28.02.1397	34° 41′ 13′′	49° 58′ 35′′	2010
63	CAN_{68}	*Rapistrum rugosum* (L.) All.	Astaneh	10.01.1397	33° 55′ 40′′	49° 24′ 12′′	1910
64	CAN_{69}	*Rapistrum rugosum* (L.) All.	Saveh	18.03.1397	34° 58′ 29′′	50° 23′ 05′′	985
65	CAN_{70}	*Raphanus sativus* L.	Karahrod	12.02.1397	34° 03′ 24′′	49° 37′ 55′′	1834
66	CAN_{71}	*Sisymbrium irio* L.	Arak-Park-e-Shahr	16.01.1397	34° 05′ 20′′	49° 41′ 57′′	1741
67	CAN_{72}	*Sisymbrium brassiciforme* C. A. Mey	Tafresh-Moeenabad	28.02.1397	34° 41′ 13′′	49° 58′ 35′′	2010
68	CAN_{73}	*Sisymbrium brassiciforme* C. A. Mey	Hak-e-Bolagh	11.03.1397	33° 59′ 16′′	49° 21′ 39′′	1846
69	CAN_{74}	*Thlaspi perfoliatum* L.	Sefidkhany	07.02.1397	33° 58′ 52′′	49° 35′ 10′′	2243
70	CAN_{75}	*Thlaspi perfoliatum* L.	Hezaveh	13.01.1397	34° 11′ 21′′	49° 33′ 12′′	1846
71	CAN_{76}	*Torularia torulosa* (Desf) O. E. Schultz	Arak-Bagh-e-Fadak	24.02.1397	34° 07′ 25′′	49° 57′ 30′′	1729
72	CAN_{77}	*Torularia torulosa* (Desf) O. E. Schultz	Arak-Zamenjan	03.03.1397	34° 01′ 55′′	49° 36′ 48′′	1873

* CAN: Collection Number Amirreza Nezamabadi

Table 3. Leaf epidermis anatomical data of 72 collected Brassicaceae populations from central Iran.

No.	Code	Taxon	Leaf ad trichomes	Leaf ab trichomes	Leaf trichomes type	Leaf stomata type ad	Leaf stomata type ab
1	*CAN_1	*Aethionema carnemum*	-	-	none	angular anisotricytic	circular anisotricytic
2	CAN_3	*Alyssum alysoides*	+	+	Stellate->3-armed	circular anisotricytic	anisotricytic
3	CAN_4	*A. desertorum*	+	+	Stellate->3-armed	angular anisotricytic	anisotricytic
4	CAN_5	*A. dasycarpum*	+	+	Stellate->3-armed	angular anisotricytic	angular anisotricytic
5	CAN_6	*A. longystylum*	+	+	Stellate->3-armed	angular anisotricytic	angular anisotricytic
6	CAN_7	*A. longystylum*	+	+	Stellate->3-armed	angular anisotricytic	angular anisotricytic
7	CAN_8	*A. linifolium*	+	+	Stellate->3-armed	angular anisotricytic	folded anisocytic
8	CAN_9	*A. linifolium*	+	+	Stellate->3-armed	angular anisotricytic	folded anisocytic
9	CAN_{10}	*Brassica deflexa* Boiss	-	-	none	anisotricytic	anisotricytic
10	CAN_{11}	*B. elongata*	-	-	none	angular anisotricytic	angular anisotricytic
11	CAN_{12}	*B. napus*	-	-	none	angular anisotricytic	angular anisotricytic
12	CAN_{13}	*Camelina rumelica*	+	+	1-armed long/Bifid multicellular	anisotricytic	angular anisotricytic
13	CAN_{14}	*C. rumelica*	+	+	1-armed long/bifid multicellular	anisotricytic	angular anisotricytic
14	CAN_{15}	*Capsella bursa pastoris*	+	+	dendroid (3-armed)	anisotricytic	folded anisocytic
15	CAN_{16}	*C. bursa pastoris*	+	+	dendroid (3-armed)	anisotricytic	folded anisocytic
16	CAN_{17}	*Cardamin hirsute*	-	-	none	circular anisotricytic	anisotricytic
17	CAN_{18}	*C. hirsute*	-	-	none	circular anisotricytic	anisotricytic
18	CAN_{19}	*C. impatience*	-	-	none	circular anisotricytic	folded anisocytic
19	CAN_{20}	*C. impatience*	-	-	none	circular anisotricytic	folded anisocytic
20	CAN_{21}	*Choriospora iberica*	+	+	glandular	folded anisocytic	folded anisocytic
21	CAN_{22}	*C. tenella*	-	-	none	folded anisocytic	folded anisocytic
22	CAN_{23}	*Clypeola jonthlaspi*	+	+	Stellate->3-armed	angular anisotricytic	angular anisotricytic
23	CAN_{24}	*Conringia perfoliata*	-	-	none	folded anisocytic	anisotricytic
24	CAN_{25}	*C. perfoliata*	-	-	none	folded anisocytic	anisotricytic
25	CAN_{26}	*C. persica*	-	-	none	folded anisocytic	anisotricytic
26	CAN_{27}	*Crambe orientalis*	-	-	none	anisotricytic	anisotricytic
27	CAN_{28}	*C. orientalis*	-	-	none	anisotricytic	anisotricytic
28	CAN_{29}	*Cymatocarpus pilosissimus*	+	+	2 armed basifixed	angular anisotricytic	angular anisotricytic
29	CAN_{30}	*Descurainia sophia*	+	+	dendroid (3-armed)	circular anisotricytic	circular anisotricytic
30	CAN_{31}	*D. sophia*	+	+	dendroid (3-armed)	circular anisotricytic	circular anisotricytic
31	CAN_{32}	*Diplotaxis harra*	-	-	none	angular anisotricytic	anisotricytic
32	CAN_{33}	*Draba minima*	+	+	bifid multicellular	folded anisocytic	folded anisocytic
33	CAN_{34}	*Eruca sativa*	-	-	none	circular anisotricytic	anisotricytic
34	CAN_{35}	*E. sativa*	-	-	none	circular anisotricytic	anisotricytic
35	CAN_{39}	*Euclidium syriacum*	+	+	2-armed basifixed	anisotricytic	folded anisocytic
36	CAN_{40}	*E. syriacum*	+	+	2-armed basifixed	anisotricytic	folded anisocytic
37	CAN_{41}	*Fibigia macrocarpa*	+	+	Stellate->3-armed	anisotricytic	anisotricytic
38	CAN_{42}	*Goldbachia laevigata*	-	-	none	folded anisocytic	folded anisocytic
39	CAN_{43}	*G. laevigata*	-	-	none	folded anisocytic	folded anisocytic
40	CAN_{44}	*Isatis armena*	-	-	none	folded anisocytic	folded anisocytic
41	CAN_{45}	*I. zarrei*	-	-	none	folded anisocytic	folded anisocytic
42	CAN_{46}	*I. kotschyana*	-	-	none	anisotricytic	anisotricytic

43	CAN_{47}	*I. kotschyana*	-	-	none	anisotricytic	anisotricytic
44	CAN_{48}	*I. minima*	-	-	none	folded anisocytic	folded anisocytic
45	CAN_{50}	*Lepidium draba* ssp.*draba*	+	+	1-armed-long	anisotricytic	anisotricytic
46	CAN_{51}	*L. draba* ssp.*draba*	+	+	1-armed-long	anisotricytic	anisotricytic
47	CAN_{52}	*L. perfoliatum*	-	-	none	anisotricytic	anisotricytic
48	CAN_{53}	*L. persicum* var. *persicum*	+	+	1-armed-short	anisotricytic	anisotricytic
49	CAN_{54}	*L. persicum* var. *persicum*	+	+	1-armed-short	anisotricytic	anisotricytic
50	CAN_{55}	*L. sativum*	-	+	1-armed-long	folded anisocytic	folded anisocytic
51	CAN_{56}	*L. vesicarium*	-	-	none	anisotricytic	anisotricytic
52	CAN_{57}	*L. vesicarium*	-	-	none	anisotricytic	anisotricytic
53	CAN_{58}	*Litwinowia tenuissima*	+	+	1-armed-long/grandular	anisotricytic	folded anisocytic
54	CAN_{59}	*L. tenuissima*	+	+	1-armed-long/grandular	anisotricytic	folded anisocytic
55	CAN_{60}	*Malcolmia africana* var. *africana*	+	+	Dendroid (3-armed)	anisotricytic	folded anisocytic
56	CAN_{61}	*M. africana* var. *africana*	+	+	Dendroid (3-armed)	anisotricytic	folded anisocytic
57	CAN_{62}	*Mathiola longipetala*	+	+	Dendroid (3-armed)	angular anisotricytic	folded anisocytic
58	CAN_{63}	*M. longipetala*	+	+	Dendroid (3-armed)	angular anisotricytic	folded anisocytic
59	CAN_{64}	*Nasturtium officinale*	-	-	none	folded anisocytic	folded anisocytic
60	CAN_{65}	*N. officinale*	-	-	none	folded anisocytic	folded anisocytic
61	CAN_{66}	*Neslia apiculata*	+	+	Dendroid(3-armed)	folded anisocytic	folded anisocytic
62	CAN_{67}	*N. apiculata*	+	+	DEndroid (3-armed)	folded anisocytic	folded anisocytic
63	CAN_{68}	*Rapistrum rugosum*	+	+	1-armed-long	folded anisocytic	folded anisocytic
64	CAN_{69}	*R. rugosum*	+	+	1-armed-long	folded anisocytic	folded anisocytic
65	CAN_{70}	*Raphanus sativus*	-	-	none	angular anisotricytic	angular anisotricytic
66	CAN_{71}	*Sisymbrium irio*	-	+	1-armed-long	angular anisotricytic	angular anisotricytic
67	CAN_{72}	*S. brassiciforme*	-	-	none	anisotricytic	folded anisocytic
68	CAN_{73}	*S. brassiciforme*	-	-	none	anisotricytic	folded anisocytic
69	CAN_{74}	*Thlaspi perfoliatum*	-	-	none	folded anisocytic	folded anisocytic
70	CAN_{75}	*T. perfoliatum*	-	-	none	folded anisocytic	folded anisocytic
71	CAN_{76}	*Torularia torulosa*	-	-	none	folded anisocytic	folded anisocytic
72	CAN_{77}	*T. torulosa*	-	-	none	folded anisocytic	folded anisocytic

*For synonyms and authors' names refer to table 1.

Table 4. Total variance explained for principal component analysis leaf epidermis anatomical characters of the studied Brassicaceae populations in central of Iran.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.222	46.912	46.912	4.222	**46.912**	46.912	4.049	44.984	44.984
2	2.421	26.903	73.815	2.421	**26.903**	73.815	2.579	28.658	73.642
3	1.137	12.635	86.450	1.137	**12.635**	86.450	1.153	12.808	**86.450**
4	.541	6.016	92.465						
5	.355	3.947	96.412						
6	.183	2.034	98.446						
7	.068	.753	99.199						
8	.055	.611	99.810						
9	.017	.190	100.000						

Extraction Method: Principal Component Analysis.

Table 5. Two components of PCA test and correlating leaf epidermis anatomical characters of the studied Brassicaceae populations in central Iran.

Rotated Component Matrix

	Component	
	1	**2**
Leaf trichomes adaxial	***.966**	
Leaf trichomes abaxial	**.940**	
Leaf trichomes type	**.875**	
Leaf trichomes 2 sides	**.691**	
Leaf stomata type abaxial		**.881**
Leaf stomata type adaxial		**.874**

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 2 iterations

***** Bold values are positive significant $P<0.05$.

Discussion

Studies on epidermal leaf anatomy characters in Brassicaceae showed that trichomes and stomata represent valuable characters for separating the taxa and populations. Doaigey et al. (2013) in their studies on 34 species of Brassicaceae growing in the Saudi Arabia desert divided these species into four groups on the basis of the presence or absence of trichomes and trichomes and stomata types (Doaigey et al., 2013). Also, Khalik (2005) found trichomes as being valuable characteristics for the identification of tribes, genera, species, subspecies, and varieties in Brassicaceae (Khalik, 2005). Our studied taxa can be divided into 3 groups based on the presence or absence of trichomes on 1 or 2 leaf surfaces.

Factor analysis of epidermal leaf anatomy characters showed that the first three factors describe 86% of the total variance. First components with 47% total variation were found positively correlated with the presence of leaf trichomes in adaxial and abaxial and also trichomes types. Component 2 with 27% total variation was positive and significantly correlated with leaf stomata types ad/ab. Component three with 12% total variation was correlated positively and significantly with all of the studied characters.

Cluster analysis (Ward method) of epidermal leaf anatomy characters data using cophenetic correlation showed two main clades: first clade (A) consists of all populations without trichomes containing 37 populations. The second main clade (B) consists of all samples with trichomes in their both adaxial and abaxial epidermal leaf surfaces with exception of *Lepidium sativum* and *Sisymbrium irio* species having trichomes just in their abaxial leaf surface (Table 3). Our results showed that two *Choriospora* species can be separated by their trichomes characters. *C. iberica* has glandular trichomes, while *C. tenella* has not any trichomes. Both species have similar stomata types (folded anisocytic) in their ad and ab leaf surfaces. These results confirmed that trichomes as being valuable characteristics for the identification of species, subspecies, and varieties in Brassicaceae (Khalik, 2005). Leaf stomata types in leaf adaxial and abaxial surfaces

showed that all studied samples have anisocytic stomata. But based on the subsidiary cell wall, four types, anisotricytic, angular, circular, and folded anisotricytic stomata were observed.

Conclusions

Based on the Brassicaceae leaf epidermis anatomical characteristics, the studied populations were divided into three parts: 1. without trichomes in both adaxial and abaxial leaf surfaces, 2. having trichomes just on the abaxial side (*Lepidium sativum* and *Sisymbrium irio*), and 3. trichomes in both adaxial and abaxial surfaces. It is believed that just epidermal leaf anatomy characters are not sufficient to identify members of the Brassicaceae, but can be helpful along with other features.

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