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MICROMORPHOLOGICAL AND GENETIC MOLECULAR VARIATIONS IN SOME TAXA OF ASTERACEAE AND ITS IMPORTANCE AS GRAZING PLANTS

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Abstract

The present study used Randomly Amplified Polymorphic DNA (RAPD) marker and scanning electron microscope (SEM) for achene surface to characterize and detect morphological and genetical molecular markers in 15 taxa of family Asteraceae collected from Al-Jouf and Al-Ula regions in the northern of Saudi Arabia and give attention to the importance of this family as grazing plants. The results of achene surface showed great differences between studied taxa and gave important criteria to characterize them. RAPD analysis scored molecular genetic markers that helped to characterize and detect variation between studied taxa. Primers produced a total of 105 bands of which 59 were species specific markers. The dendrogram generated by combination between RAPD polymorphism and achene scanning electron microscope features produced a clear view about the genetic relationship between studied taxa of Asteraceae.

Key words: Asteraceae, Achene, RAPD marker, Genetic diversity, Grazing plants.

Introduction

Saudi Arabia has a great plant species diversity. Asteraceae is one of the largest families in the flora of Saudi Arabia, represented by 233 species which is about (10.7%) of the total plant species in the Kingdom (Al-Sodany *et al.* 2013).

The fruit in Asteraceae called achene, is a single seeded thin-walled fruit in which the seed coat is not part of the fruit coat. Achene morphology such as shape, size and color help in correct identification and classification of taxa at specific level (Kynclova, 1970; Haque & Godward, 1984; Kreitschitz & Vallés, 2007; Savadkoohi *et al*., 2012; Akcin & Akcin, 2014; Bona, 2014; Karanović *et al.*, 2016; Lazarević *et al*., 2016). Achenes of most species of Asteraceae have a ring of fine hairs or scales called pappus. Pappus is taxonomically helpful to distinguish certain taxa (Vít & Agáta, 2007). At the base of the achene there is a carpopodium. The carpopodium has different shapes like annular, cylindrical, multi-layered, cellular form, the cells rarely have hairs or glands (Michelli & Luiz, 2017). The fine structure of the seeds and achenes are useful in families in which delimitation of particular taxa on morphological basis is difficult (Johnson *et al*., 2004; Plaza *et al*., 2004; Zeng *et al*., 2004).

Molecular genetic markers have an important role in the identification and understanding the phylogenetic relationships between plant species. RAPD marker is one of the significant tools of genetic characterization of plants, revealing the genetic diversity and relationship among species (Henuka *et al*., 2015). These molecular parameters have an important role for the protection of genetic resources (Saiki *et al*., 1988; Welsh & McClelland, 1990; Williams *et al*., 1990; Echt *et al*., 1992; Nagaoka & Ogihara, 1997; Sivolap *et al*., 2000; Sreekumar, 2006; Abdel-Hamid, 2011). Moazzeni *et al*., 2010 opined that morphological characters of achenes and seeds should be correlated with the molecular data.

The floristic studies in Saudi Arabia are not conducted with reference to genetic and molecular bases that be useful for knowing the evolutionary relationships between different plant species (Rodr´ıguez-Garaya *et al*., 2009; Haq *et al*., 2013).

The aim of the present study is to investigate micromorphological features of achene surface and the molecular genetic markers of 15 taxa of Asteraceae from Al Jouf and Al-Ula regions in the northern part of Saudi Arabia and explain the genetic variations between them and shed light to its importance as grazing plants.

Materials and Methods

Fifteen taxa of family Asteraceae were collected from Al Jouf and Al-Ula regions in the northern of Saudi Arabia (Table 1). The taxa were identified following (Migahid, 1996; Collenette, 1999; Al-Hassan, 2006).

Scanning electron microscopic study of achene surfaces sculpturing (SEM): For SEM studies dry achenes were mounted directly onto clean stubs using double-sided adhesive tape, followed by coating with gold in a JEOL JFC 1100E ion-sputtering unit and examined using a JEOL JSM 5400 LV at a magnification of 35 X, 75 X, 100 X, 150 X, 200 X, 350, 500 and 1000 X scanning electron microscope. The terminology used for the description of the achene surface followed here is of (Stearn, 1966; Stant, 1973; Esau, 1977; Barthlott, 1981; Boesewinkel & Bouman, 1984; Koul *et al*., 2000; Abid & Qaiser, 2008; Zareh *et al*., 2016).

Following morphological parameter of the achenes were studied: size (length and width), color and surface pattern; pappus length, color and shape; Carpopodium shape was also noted (Fig. 1).

DNA isolation: DNeasy plant Mini Kit (Qiagen Inc. Dneasy plant mini handbook) protocol was used for isolation of plant DNA.

RAPD-PCR conditions: RAPD-PCR reactions were done by using seven arbitrary random 10-*mer* primers with sequences indicated in Table 2.

Reaction conditions and mixtures (50 µl total volume) consisting of dNTPs, $MgCl_2$, 10 X buffer, Primer and Template DNA were optimized. Amplification was

carried out in a Perkin Elmer 2400 thermocycler programmed for 44 cycles as follows: 94°C / 4 mins (1 cycle); 94°C / 1 min, 37°C / 1 min. 72°C / 2 mins. (40 cycles); 72°C / 10 mins. (1 cycle) and 4°C (infinitive).

Table 2. Names of random primers and their sequences for RAPD-PCR analysis.

Primer's name	Sequence
OP-O05	*5-CCCAGTCACT-3*
OP-O10	*5-TCAGAGCGCC-3*
OP-O20	*5-ACACACGCTG-3*
OP-Z12	*5-TCAACGGGAC-3*
OP-A10	*5-GTGATCGCAG-3*
OPA-02	*5-TGCCGAGCTG-3*
OPA-05	*5-AGGGGTCTTG-3*

Table 1. Serial numbers and names of studied Asteraceae taxa.

Serial No.	Species's name
1.	*Launaea capitate* (Spreng.) Dandy
2.	*Launaea mucronata* ssp. *Cassiniana* (Jaub. & Spach.) N. kilian
3.	*Launaea mucronata* ssp. *Mucronata* (Forssk.) Muschl.
4.	*Pulicaria undulata (*Forssk.) C.A. Mey.
5.	*Centaurea scoparia* Sieber. ex Spreng.
6.	*Centaurea pseudosinaica* Czerep.
7.	*Conyza bonariensis* (L.) Cronquist
8.	*Senecio flavus* (Decne.) Sch.-Bip. ex A. Rich.
9.	*Lactuca serriola* L.
10.	*Anthemis deserti* Boiss.
11.	*Phagnalon barbeyanum* Asch. & Schweinf.
12.	*Rhanterium epapposum* Oliv.
13.	*Artemisia sieberi* Besser
14.	*Artemisia judaica* L.
15.	*Artemisia monosperma* Delile

Gel electrophoresis: Agarose (12%) was used for resolving the PCR products a Kb DNA ladder (Stratagene) its molecular sizes in bp were: 3000, 2500, 2000, 1500, 1200, 1033, 900, 800, 700, 600, 500, 400, 300, 200 and 100 according to Maniatis *et al*., (1982).

The run was accomplished for one hour at 100 V in a Bio-RadTM submarine (12 cm x 8 cm). Bands were observed by UV-transilluminator and photographed by Gel Doc XR+ System Bio-Rad.

Data analysis: The analysis of the morphological features and RAPD data was performed using the band binary criterion by codifying the detected bands to 0 when absent and 1 when present, Band scoring and assortment was done using Peak scanner Applied Biosystems. Microsoft Excel 2013 FAMD software (Schluter & Harris, 2006) was used for phylogenetic analysis and Treegraph2 programs to visualize the produced tree (Stover & Muller, 2010).

Results

Achene morphology: Achene morphological characters includng (length and width), color, surface pattern, pappus length, pappus color, shape of bristles and shape of carpopodium of the 15 taxa of Asteraceae are listed in Table 3.

The results in Table 3 showed that the achene size of the studied taxa varied between 0.83–6.5 mm x 0.15–1.5 mm, and pappus length ranged from 2.8–5.50 mm. Out of 15 taxa following 8 taxa were epappose: *Launaea capitata*, *Pulicaria undulata*, *Anthemis deserti, Rhanterium epapposum*, *Artemisia sieberi, Artemisia judaica* and *Artemisia monosperma*.

***Launaea capitata* (Spreng) Dandy:** Achene size 3.8 mm x 1.5 mm, creamy to brown, surface pattern areolate with round borders, carpopodium was irregularly developed.

***Launaea mucronata* ssp. *cassiniana* (Jaub. & Spach.) N. Kilian:** Achenes size 5.50 mm x 0.50 mm, brown, irregular sulcate. Pappus bristles 5.00 mm long, white, filamentous, carpopodium rectangular.

***Launaea mucronata* ssp. *mucronata* (Forssk.) Muschl:** Achenes 4.75–5.25 mm x 0.40 mm, brown with creamy lines, irregularly sulcate with papillae. Pappus bristles length 5.25 mm long, white, capillary, carpopodium shape elliptic.

***Pulicaria undulata* (Forssk.) C.A. Mey.:** Achenes size 3.38 mm x 0.38 mm, biege, surface pattern reticulate. Cell borders clearly raised above the cell centers with a smooth structure, carpopodium elliptic.

***Centaurea scoparia* Sieber. ex. Spreng.:** Achenes size 3.75 mm x 1.50 mm, creamy to bronze, surface pattern smooth scalariform. The centers of the cells little higher than the borders. Pappus creamy to bronze, 3.75 mm long, scalely, homomorphic bristillate, carpopodium elliptic with scales.

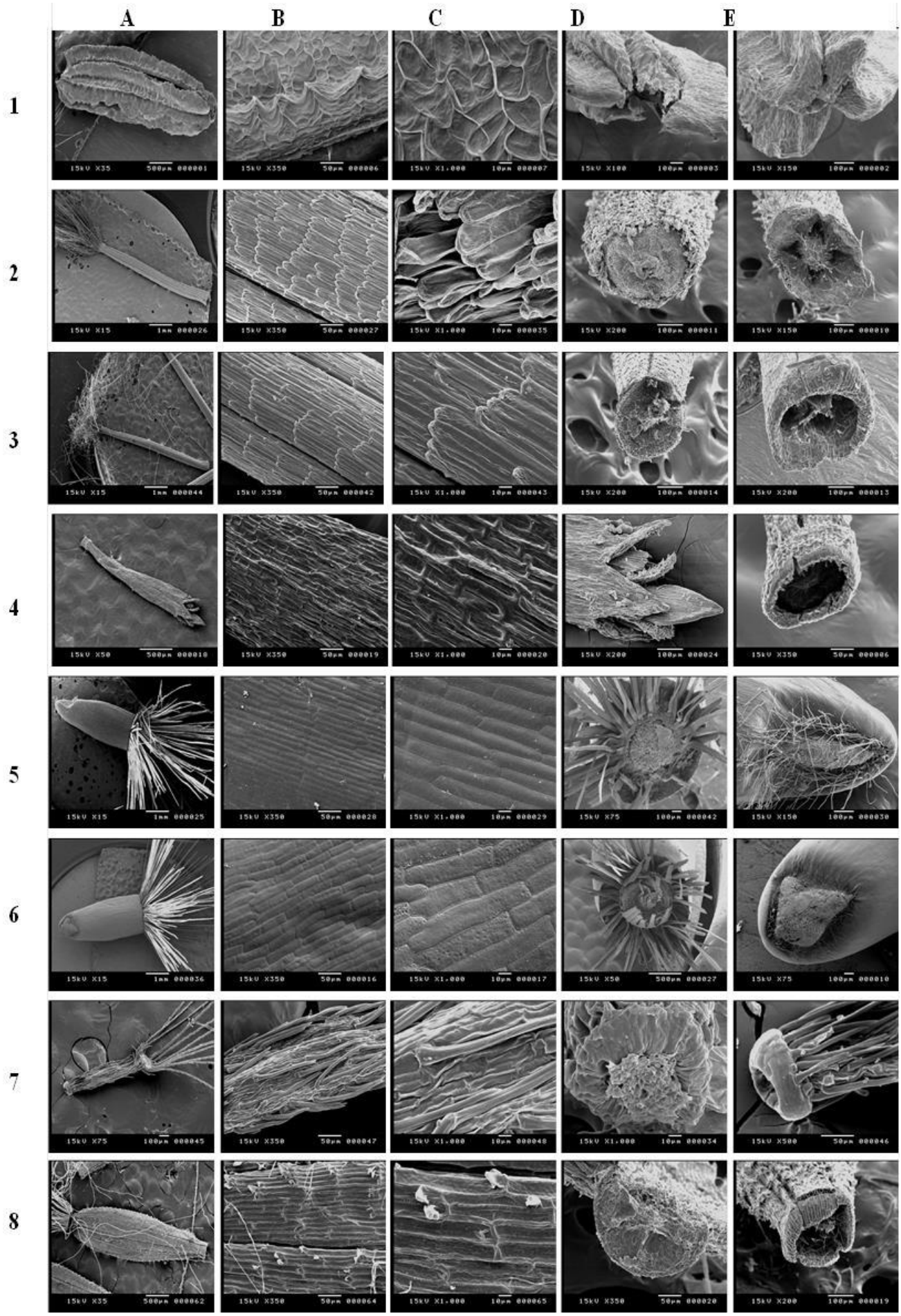
***Centaurea pseudosinaica* Czerep.:** Achene 4.00 mm x 1.50 mm, creamy, surface pattern falsafoveate and scalariform. The cell borders thin and the centers of the cells were at the same level with the borders, pappus white, ca 4.25 mm long, carpopodium triangular with scales.

***Conyza bonariensis* (L.) Cronquist.:** Achenes 0.83 mm x 0.15 mm. Surface cells pubescent, ribbed, vertically oriented with straight anticlinal walls and shallow periclinal walls and upwardly directed hairs. Pappus bristles ca 3.00 mm long, white, homomorphic, scabrous serrulate bristillate. Carpopodium circular disc-shaped.

***Senecio flavus* (Decne.) Sch.-Bip. ex A. Rich.:** Achenes 3.18 mm x 1.00 mm, wholly brownish, striate-scalariform, reticulate and warty. Pappus 5.50 mm long, white, scabrous-barbellate. Carpopodium circular with interruption.

***Lactuca serriola* L.:** Achenes 2.58 mm x 0.87 mm, obovate, brown, longitudinally ribbed on both sides, gradually narrowed towards the base. Pappus bristles 3.00 mm length, white, homomorphic capillary. Carpopodium round shaped with interruption.

***Anthemis deserti* Boiss.:** Achenes 2.00 mm x 0.67 mm, brown to black, surface pattern reticulate. Pappus absent. Carpopodium round shaped.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(Fig. 1. Cont'd.)

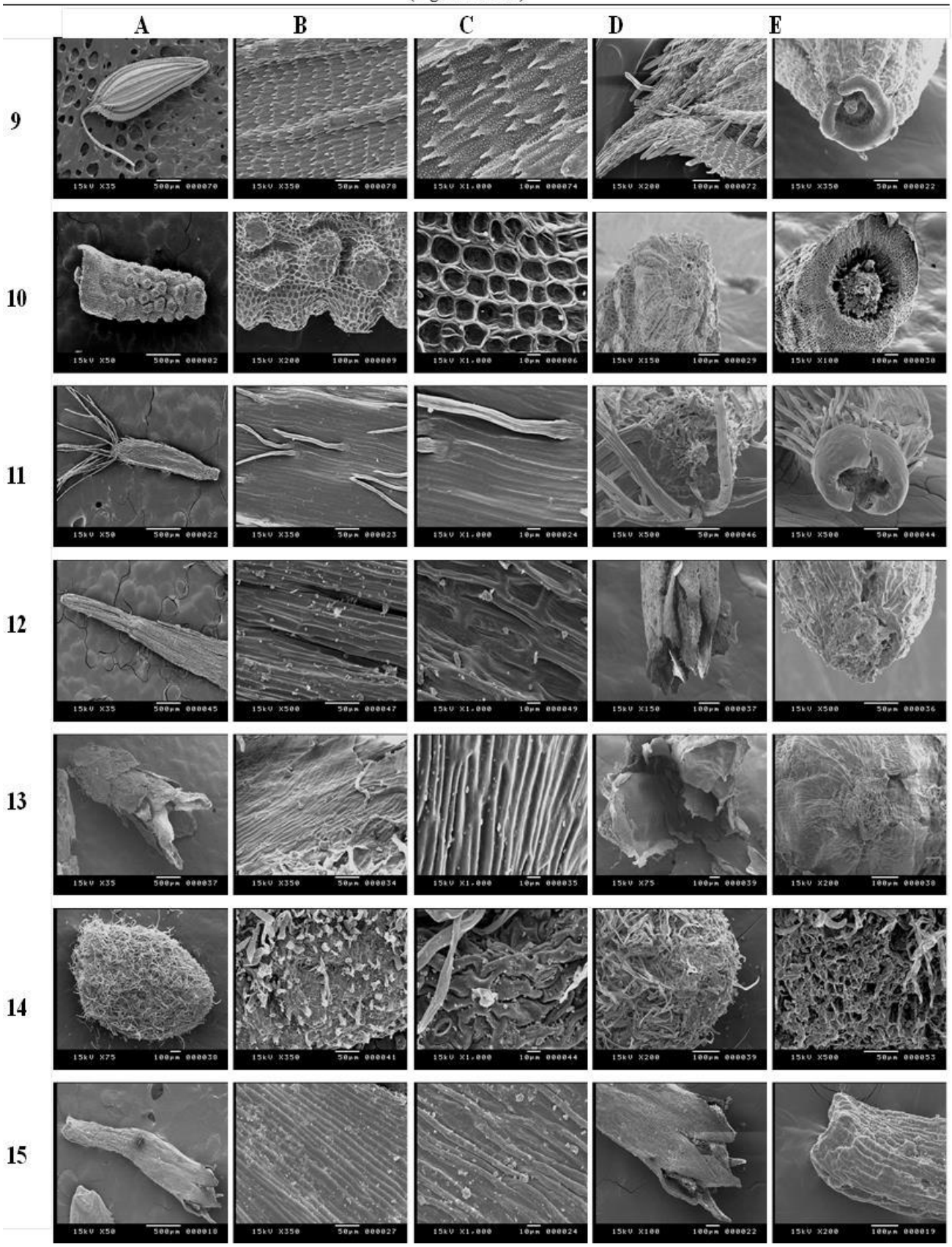

Fig. 1. Scanning electron micrographs (SEM) of achenes of studied Asteraceae taxa. A: Shapes of achenes. B: Achene surface. C: Achene surface. D: Achene tip. E: Shape of carpopodium. 1: *Launaea capitata*, 2: *Launaea mucronata cassiniana*, 3: *Launaea mucronata mucronata*, 4: *Pulicaria undulate*, 5: *Centaurea scoparia*, 6: *Centaurea pseudosinaica*, 7: *Conyza bonariensis*, 8: *Senecio flavus*, 9: *Lactuca serriola*, 10: *Anthemis deserti*, 11: *Phagnalon barbeyanum*, 12: *Rhanterium epapposum*, 13: *Artemisia sieberi*, 14: *Artemisia judaica*, 15: *Artemisia monosperma*.

Table 3. Description of achene characteristics for studied Asteraceae taxa.

Taxa	Achene				Pappus				Carpopodium
	Size		Color	Pattern	Length (m)	Color	Shape of bristles	Pappus Disc	Shape
	Length (m)	Width (m)							
1	3.80	1.50	Creamy to Brown	Areolate with round borders	-	-	-	Serrate	Irregularly developed
2	5.50	0.50	Brown	Irregular sulcate	5.00	White	Filamentous serrulate	Round	Rectangular
3	4.75–5.25	0.40	Brown	Irregular sulcate with papilla	5.25	White	Filamentous	Round	Elliptic
4	3.38	0.38	Beige	Reticulate	-	-	Capillary	Dentate crown	Elliptic
5	3.75	1.50	Creamy to Bronze	Scalariform	3.75	Creamy to Bronze	Homomorphic Bristilate	Round	Elliptic with scales
6	4.00	1.50	Creamy	False foveate and scalariform	4.25	White	Homomorphic Bristilate	Round	Triangular with scales
7	0.83	0.15	Beige to Brown	Pubescent Ribbed	3.00	White	Homomorphic Scabrous Serrulate Bristillate	Salient and round	Broad circular disc
8	3.18	1.00	Brown	Striate Scalariform and reticulate and warty	5.50	White	Scabrous barbellate	Round	Round with fissure
9	2.58	0.87	Brown	Longitudinal ribbed	3.00	White	Homomorphic Capillary ray	İnconspicuous	Round with interruption
10	2.00	0.67	Brown to Black	Ocellate and reticulate	-	-	-	-	Round
11	1.40	0.30	Brown	Lineate with hair like appendages	2.80	White	Homomorphic Capillary ray Scabrid barbellate	Entire	Broad circular disc subbasal
12	6.50	0.75	Beige	Striate Scalariform and reticulate	-	-	-	-	Irregularly developed
13	3.25	1.13	Beige	Glabrous Sulcate and Ribbed	-	-	-	-	Irregularly developed
14	1.30	0.82	Beige to Brown	Rugose with pits	-	-	-	-	-
15	2.50	0.53	Creamy to Brown	Glabrous Sulcate and Ribbed	-	-	-	Dentate crown	Round

***Phagnalon barbeyanum* Asch. & Schweinf.:** Achenes 1.40 mm x 0.30 mm, dark brown, long hairy, surface pattern lineate with hair like appendages. Pappus bristles 2.80 mm long, white, homomorphic scabrid barbellate, Carpopodium broad subbasal, circular disc-like.

***Rhanterium epapposum* Oliv.:** Achenes of disc-florets without pappus, 6.50 mm x 0.75 mm, beige, straight to slightly curved, striate scalariform and reticulate, glandular, with many narrow longitudinal sclerenchymatic ribs. Carpopodium irregularly developed.

***Artemisia sieberi* Besser:** Achenes 3.25 mm x 1.13 mm, pericarp wholly beige, glabrous sulcate and ribbed. Pappus absent, Carpopodium irregularly developed.

***Artemisia judaica* L.:** Achenes 1.30 mm x 0.82 mm small, beige to brown in colour, surface pattern rugose with pits, usually glabrous, pappus absent.

Artemisia monosperma Delile**:** Achenes 2.50 mm x 0.53 mm, creamy to brown in colour; glabrous sulcate and ribbed. The centers of the cells were higher than the borders. Pappus absent.

RAPD results: The RAPD analysis of 15 taxa of Asteraceae against seven different random 10-*mer* primers generated a total of 105 amplified fragments. (Fig. 2 and Table 4). The total number of bands produced by each primer ranged from 11 to 24 bands. The polymorphism percentage varied among the primers. The highest ratio of polymorphism generated by OP-A02 was 83.33%, while OP-O05 produced the lowest polymorphism (36.36%).

Species specific molecular markers for different Asteraceae taxa: The total number of species specific markers scored across Asteraceae taxa was 59. The highest number of species specific markers generated from RAPD analysis was twenty by OP–A02, while the lowest one was four generated by primer OP–O05 (Table 4).

Genetic relationships: The genetic similarities among the 15 studied taxa were based on Nei's method (Nei, 1978) and (Saitou & Nei, 1987). The highest pairwise similarity indices resulted from both RAPD analysis and achene features were between *Centaurea scoparia* and *Centaurea pseudosinaica*, while the lowest similarity indices were between *Launaea mucronata* ssp. *cassiniana* and *Phagnalon barbeyanum; Launaea mucronata* ssp. *mucronata* and *Artemisia monosperma* (Table 5).

In the UPGMA tree (Fig. 3), the studied taxa were divided into two main groups at distance 0.65, the first group divided into two clusters at distance 0.68, the cluster 1 had only one species *Launaeacapitata*, cluster 2 contained two taxa *Launaea mucronata* ssp. *cassiniana* and *Launaea mucronata* ssp. *mucronata*. The second group was also divided into two clusters at distance 0.71, the cluster 1 had 10 species, *Pulicaria undulata*, *Centaurea scoparia*, *Centaurea pseudosinaica*, *Lactuca serriola*, *Conyza bonariensis*, *Senecio flavus*, *Rhanterium epapposum*, *Artemisia sieberi*, *Artemisia judaica* and *Artemisia monosperma*. Cluster 2 contained two species *Anthemis deserti* and *Phagnalon barbeyanum.*

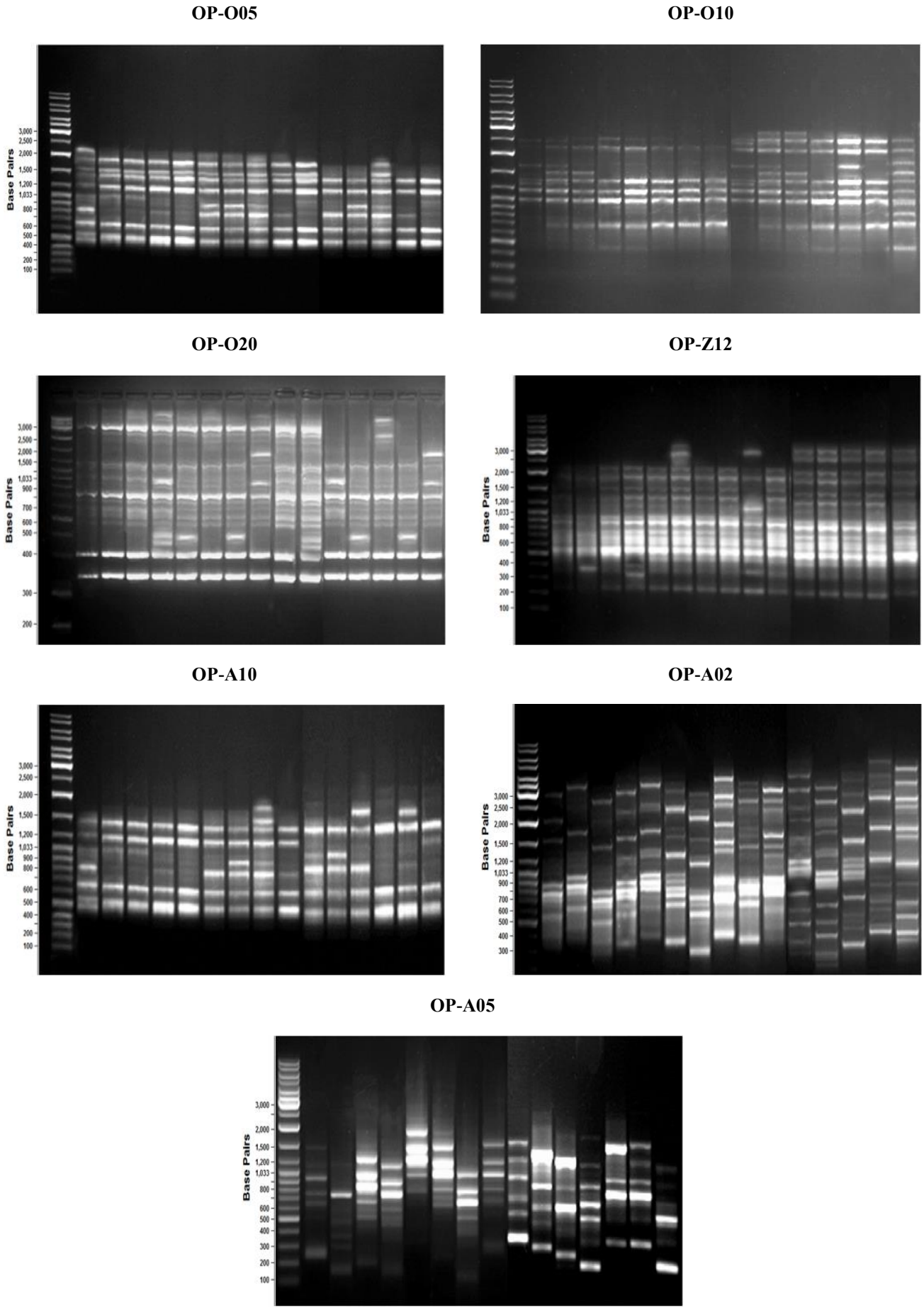

Fig. 2. DNA polymorphism of studied Asteraceae taxa generated by RAPD-PCR.

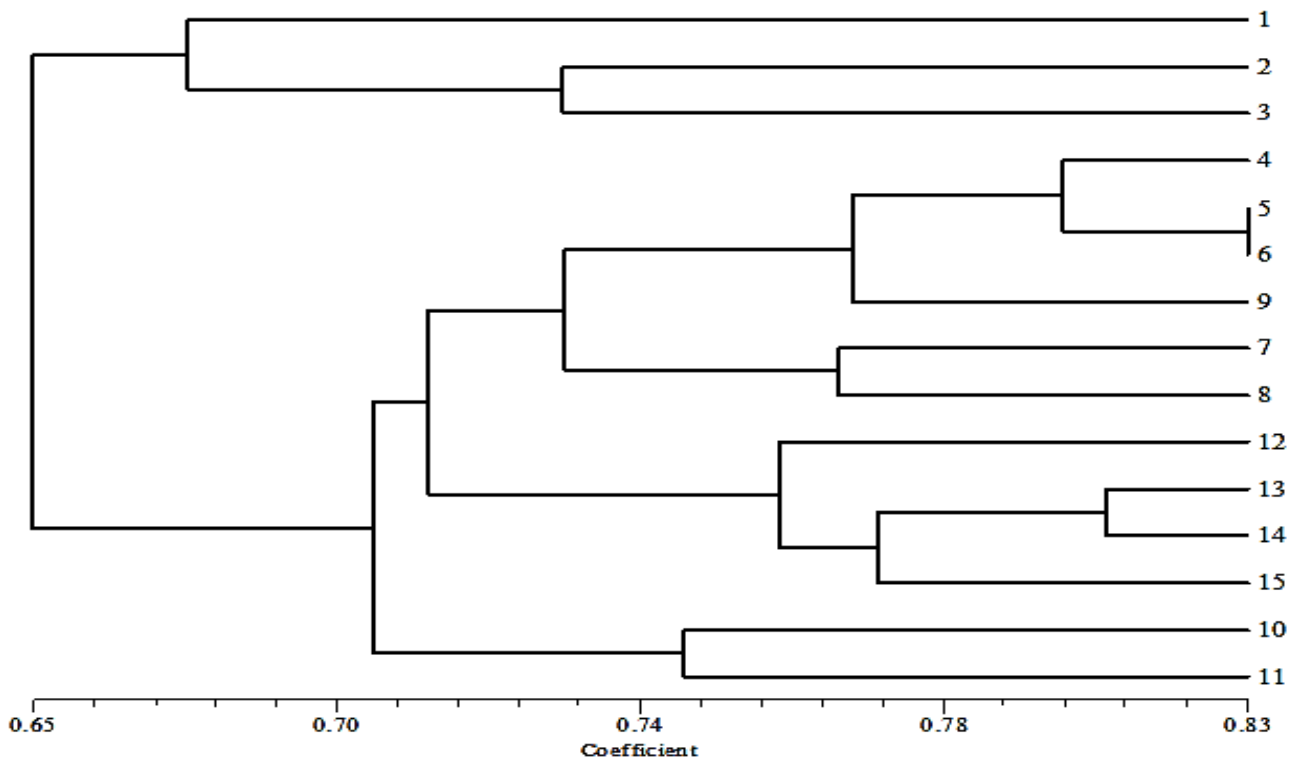

Fig. 3. UPGAMA dendogram based on achene features and RAPD-PCR analysis of studied Asteraceae taxa. 1: *Launaea capitata*, 2: *Launaea mucronata cassiniana*, 3: *Launaea mucronata mucronata*, 4: *Pulicaria undulate*, 5: *Centaurea scoparia*, 6: *Centaurea pseudosinaica*, 7: *Conyza bonariensis*, 8: *Senecio flavus*, 9: *Lactuca serriola*, 10: *Anthemis deserti*, 11: *Phagnalon barbeyanum*, 12: *Rhanterium epapposum*, 13: *Artemisia sieberi*, 14: *Artemisia judaica*, 15: *Artemisia monosperma*

Table 4. DNA monomorphic and polymorphic bands by using RAPD-DNA marker among studied Asteraceae taxa.

Primer's name	Monomorphic bands	Polymorphic bands		Total No. of bands	Polymorphism %
		Species specific bands	Non DNA markers		
OP-O05	4	4	3	11	36.36
OP-O10	4	6	1	11	54.55
OP-O20	8	6	2	16	37.50
OP-Z12	5	8	1	14	57.14
OP-A10	2	6	3	11	54.55
OP-A02	1	20	3	24	83.33
OP-A05	1	9	8	18	50.00
Total	25	59	21	105	56.19

Table 5. Similarity matrices for studied Asteraceae taxa generated by morphological features and RAPD -PCR.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.67	1.00													
3	0.68	0.73	1.00												
4	0.69	0.67	0.70	1.00											
5	0.67	0.66	0.72	0.80	1.00										
6	0.71	0.66	0.76	0.80	0.83	1.00									
7	0.67	0.61	0.69	0.73	0.69	0.75	1.00								
8	0.65	0.64	0.71	0.72	0.74	0.78	0.77	1.00							
9	0.68	0.70	0.63	0.79	0.75	0.77	0.71	0.71	1.00						
10	0.62	0.68	0.67	0.77	0.71	0.69	0.70	0.70	0.72	1.00					
11	0.62	0.58	0.62	0.73	0.66	0.69	0.68	0.67	0.66	0.75	1.00				
12	0.63	0.59	0.71	0.70	0.75	0.75	0.69	0.66	0.65	0.67	0.70	1.00			
13	0.73	0.60	0.63	0.76	0.74	0.78	0.72	0.71	0.72	0.72	0.78	0.75	1.00		
14	0.67	0.59	0.60	0.74	0.73	0.73	0.69	0.70	0.72	0.66	0.71	0.79	0.81	1.00	
15	0.62	0.59	0.58	0.69	0.66	0.67	0.69	0.67	0.70	0.68	0.73	0.74	0.76	0.78	1.00

Discussion

The aim of the present work was to characterize some wild taxa of Asteraceae growing in Al-Jouf and Al-Ula regions in the northern of Saudi Arabia by using micromorphological characters of achene surface and molecular genetic markers. These techniques succeeded in separating and distinguishing between studied taxa and explain the genetic variations between them and be useful to preserve the germplasm of these taxa to avoid disappearance by unrestricted grazing.

This study demonstrates that achene morphology is very useful for differentiating various Asteraceae taxa. The pattern of the achene surface gives important micromorphological characters that are useful to delimit the studied taxa. Pappus also showed considerable differences especially the shape of bristles and pappus rim (Table 3).

The results of RAPD marker revealed the utility of this technique for detecting molecular markers, elucidating relationships and variation between studied taxa. RAPD analysis (Tables 4 and 5) with seven different random 10-*mer* primers resulted a total of 105 fragments with 56.19% of polymorphism. The highest ratio of polymorphism generated by OP-A05 (83.33%), while OP-O05 primer produced the lowest polymorphism (36.36%).

The UPGMA tree resulted from both RAPD analysis and achene morphology of 15 taxa of Asteraceae revealed clear diversity in the studied taxa. The tree was divided into two main groups at distance 0.65, the first group was divided into two clusters at distance 0.68. The cluster 1 had only one species, cluster 2 contained two species. The second group was divided also into two clusters at distance 0.71, the cluster 1 had 10 species, Cluster 2 contained two species (Fig. 3).

Petropoulos *et al*., (2018) demonstrated that some members of the family Asteraceae had a nutrition value. It contains high levels of carbohydrates, antioxidant and phenolic compounds as well as some-minerals like K, Ca, Mg, and Fe.

Many shepherds depend on wild plants for animal grazing including the members of Asteraceae, especially it is one of the largest families in the flora of Saudi Arabia. Plant species diversity is great affected by herbivore grazing animals especially camels. The number of camels in Saudi Arabia is 0.12 camels/km^2 (Milchunas & Lauenroth, 1993; Proulex & Mazumder, 1998), over 90% of lands of the Arabian Peninsula is affected by camels because camels can graze freely throughout the desert (Gallacher & Hill, 2006; Liu *et al*., 2015).

Batanouny (1990) demonstrated that unrestricted grazing and increasing number of livestock exhausted over 30% of the Arab Gulf grazing lands. Disturbance in habitat area and habitat connectivity are the major factors influencing species diversity (Deng *et al.*, 2014). Many studies demonstrated there was an opposite correlation between the grazing pressures and the change of genetic diversity (Shan *et al.*, 2006; Komac *et al.*, 2014).

Heavy grazing had affected plant life forms, composition of plant community, vegetation structure that resulted in a significant reduction in species diversity plants and distribution of dominant species in the arid regions. (El-Keblawy, 2003; Zhao *et al.*,2007; El-Keblawy *et al.*, 2009; Al-Rowaily *et al.*, 2012; Louhaichi *et al.*, 2012). Species diversity is very important for plant as well as animal productivity (Feng *et al.*, 2016).

Conclusion

A combination of RAPD technique and scanning electron microscopic study of achene morphology generated a large number of polymorphisms for characterization and distinguishing the different taxa of Asteraceae and gave a clear view about the genetic relationship between them. It also explained the importance to preserve the germplasm of these species because its important value as grazing plants and avoid extinction due to overgrazing in Saudi Arabia and in other arid countries.

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