38

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Genetic Diversity between Pumpkin Accessions Growing in the Northern Border Region in Saudi Arabia Based on Biochemical and Molecular Parameters

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THE GENETIC variation and relationships among 16 pumpkin accessions of *Cucurbita moschata* & *Cucurbita maxima* were assessed based on variation in fruit shape, skin color, flesh and size of fruits. Polyacrylamide gel electrophoresis of seed protein and molecular markers revealed by SCoT and ISSR techniques. The SDS-PAGE electropherogram showed 12 bands; two (75 and 145 kDa) were found in accessions (1-8) and one (85 kDa) was found in accessions 9-16. Other three bands were unique to accessions 1, 2 & 11. Five ISSR primers produced 79 markers ranging in molecular size from 130 to 2140 bp. Six SCoT primers produced 173 markers ranging from 135 to 2660 bp. The ISSR polymorphism among the examined accessions was 100% in the case of primers 49A, 44 B and HB-12, and was 92% for primer HB-15. Similarly, 100% polymorphism was scored for the primers SCoT8, SCoT11, SCoT12 and SCoT14. The lowest polymorphism was 93.94% in case of primer SCoT1. Our data based on protein, RAPD and ISSR data using the SYSTAT version 7.0 program clearly distinguished accessions from each other.

Keywords: Pumpkin, *Cucurbita* L., Isozymes, Protein, ISSR, SCoT.

Introduction

The genus *Cucurbita* L. family Cucurbitaceae comprises species that are considered as major vegetable crops that grown in all regions from the cool temperate to the tropical. *Cucurbita* is a new world genus of about 20 species of high importance in diets of world populations ranging from the tropics to warm, temperate regions. Cultivation of the domesticated *Cucurbita* species has spread beyond their new world origin. Three *Cucurbita* species, *C. pepo*, *C. moschata*, *C. maxima,* comprise the principal cultivated squash and pumpkin crops (Paris, 2005).

Cucurbita fruits are grown for human consumption of their entire young fruit or mature fruit flesh. *Cucurbita* species, have a mild flavor; eaten raw or cooked and have a short storage life compared to the strongly flavored winter squash. In contrast to summer squash, the postharvest life of winter squashes and pumpkins is much longer and the fruit are not eaten raw. Roasted seeds of some species are a favorite food (Abd El-Hamed, 2015). The three economically important species of *Cucurbita* L.; *C. pepo, C. maxima* and *C. moschata*, differ in their climatic adaptation, so they are distributed differently among the agricultural regions of the world (Andres & Robinson, 2002).

The use of both morphological and molecular markers is recommended for investigating the genetic diversity of plant populations because they provides complementary information with greater power of resolution for genetic diversity analyses (Gomez et al., 2004). Morphological and molecular characterization is essential for elucidating the genetic relationships among the different groups of the species of *Cucurbita* (Barzegar et al., 2013). Fruit shape morphology is one of the most diverse traits, and depends on geographic origin (i.e., adaptation to environmental factors), cultural traditions, culinary attributes, and market characteristics and requirements (Staub et al., 2000).

Seed storage proteins have been used as a genetic marker in comparison between accessions. The low level of protein polymorphism could be

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result from conservative nature of the seed protein (Soliman et al., 2014). However, its validity and simplicity made it useful for describing the genetic structure of crop germplasm (Akbar et al., 2012). Variation in SDS-PAGE of seed protein banding patterns has successfully been used to differentiate between *Cucurbita* cultivars (El-Adl et al., 2012).

Isozymes are useful biochemical markers for assessing genetic variability. They have been used in taxonomic, genetic, evolutionary and ecological studies. Isozymes have also been used for the identification of cultivars and lines (Hamrick et al., 1991). Allozyme polymorphisms revealed a primary division within *C. pepo* into three subspecies (Decker 1985 and 1988). Seed storage proteins offer a suitable tool for comparative studies at higher taxonomic levels in the family *Cucurbitaceae* (Castro et al., 2006). SDS polyacrylamide gel electrophoresis is useful to find out the interrelationships among four taxa of the genus *Cucurbita* (Singh & Matta 2008, 2010 and 2015).

The genetic variability within *C. pepo* has previously been assessed using allozymes and different DNA marker systems and inter simple sequence repeats (ISSRs) (Lebeda et al., 2007 and Esteras et al., 2011). It is well known that using of DNA based markers is very successful for assessment of genetic diversity and identification of plant accessions, at the level of plant genome. ISSR markers are PCR based technique that is easy and simple to use. It has been developed by Zietkiewicz et al. (1994). In addition, they do not require prior sequence information (Thimmappaiah et al., 2009). ISSR markers have been successfully used for assessment of genetic diversity and gene pool origin in common bean (Gelvan et al., 2003), wild populations of Chinese *Glycyrrhiza uralensis* (Yao et al., 2008), ISSR markers are also highly polymorphic, reproducible used for fingerprinting, diversity studies and saturating linkage maps (Reddy et al., 2015). Moreover, ISSRs are useful for distinguishing among individuals with limited genetic diversity such as watermelon (Levi et al., 2005). ISSR markers have been successfully used for assessment of genetic diversity of plants with reference to plant size and seed yield (Badr et al., 2017).

The start codon targeted polymorphism (SCoT) is novel and gene targeted molecular marker technique derived from flanking ATG translation codon in plant gene and is considered to be more authentic in assessing genetic homogeneity (Xiong et al., 2011). Like the ISSR method, SCoT is simple, resolvable, and cost-effective in assessing the genetic homogeneity (Rathore et al., 2014). SCoT primers are universal in plants, as validated in genetic diversity studies of *Cucurbita* (Bhawna et al., 2017).

In pumpkin, many landraces cannot be assigned to a given known morphotype; therefore, characterization based on the use of both molecular and morphological markers is essential for elucidating the genetic relationships of ecotypes within this species (Ferriol et al., 2003). The main objective of this work is to estimate diversity analysis of the landraces of 16 pumpkin accessions of *Cucurbita moschata* & *Cucurbita maxima* based on variation in fruit shape, skin color, flesh and size of fruits. Polyacrylamide gel electrophoresis of seed protein, and molecular markers revealed by SCoT and ISSR.

Materials and Methods

Samples collection

The sixteen accessions of pumpkin of combining the two species (*Cucurbita maxima* & *Cucurbita moschata*) were collected from different localities from Northern Borders Region, K.S.A; their locations are mapped on the map of the study area are shown in Table 1 and Fig. 1..

Isozymes electrophoresis

Extraction of isozymes was adopted as described by Jonathan & Weeden (1990). Native–polyacrylamide gel electrophoresis (Native-PAGE) was performed on 12% (W/V) slab gels (Davis, 1964). Then, gels were stained according to Tanksely & Rick (1980) for peroxidase (Px) isozyme and poly phenyl oxidase (PPO). The stained gels were incubated at 37˚C in dark conditions for complete staining after adding the appropriate substrates and staining solutions.

Protein analysis

The method for discontinuous SDS-PAGE technique, based on Laemmli (1970) percentage of acrylamide/bis acrylamide in the gel, was used to identify relationship between the selected studied accessions by their protein profile.

TABLE 1. The accession numbers, local name and collection site of the 16 studied accessions of pumpkin collected from Northern Border region of Saudi Arabia.

Accession Number	Accession	Local Name	Collection site	*Cucurbita species*
1	H_1	Alqara Hendiy	Rafha	*Cucurbita moschata*
2	B_1	Alqara Binghaly	Arar	*Cucurbita moschata*
3	A_1	Alqara Amirky	Turaif	*Cucurbita moschata*
4	Y_1	Alqara Yabany	Sakaka	*Cucurbita maxima*
5	P_1	Alqara Pakistani	Alowiqela	*Cucurbita moschata*
6	Su_1	Alqara Sudany	Turaif	*Cucurbita moschata*
7	Sh_1	Alqara Shamy	Arar	*Cucurbita moschata*
8	M_1	Alqara Masry	Arar	*Cucurbita moschata*
9	Y_2	Alqara Yabany	Rafha	*Cucurbita maxima*
10	P_2	Alqara Pakistani	Alowiqela	*Cucurbita moschata*
11	A_2	Alqara Amirky	Arar	*Cucurbita moschata*
12	Sh_2	Alqara Shamy	Rafha	*Cucurbita moschata*
13	M_2	Alqara Masry	Sakaka	*Cucurbita moschata*
14	Su_2	Alqara Sudany	Turaif	*Cucurbita moschata*
15	P_3	Alqara Pakistani	Rafha	*Cucurbita moschata*
16	Y_3	Alqara Yabany	Arar	*Cucurbita maxima*

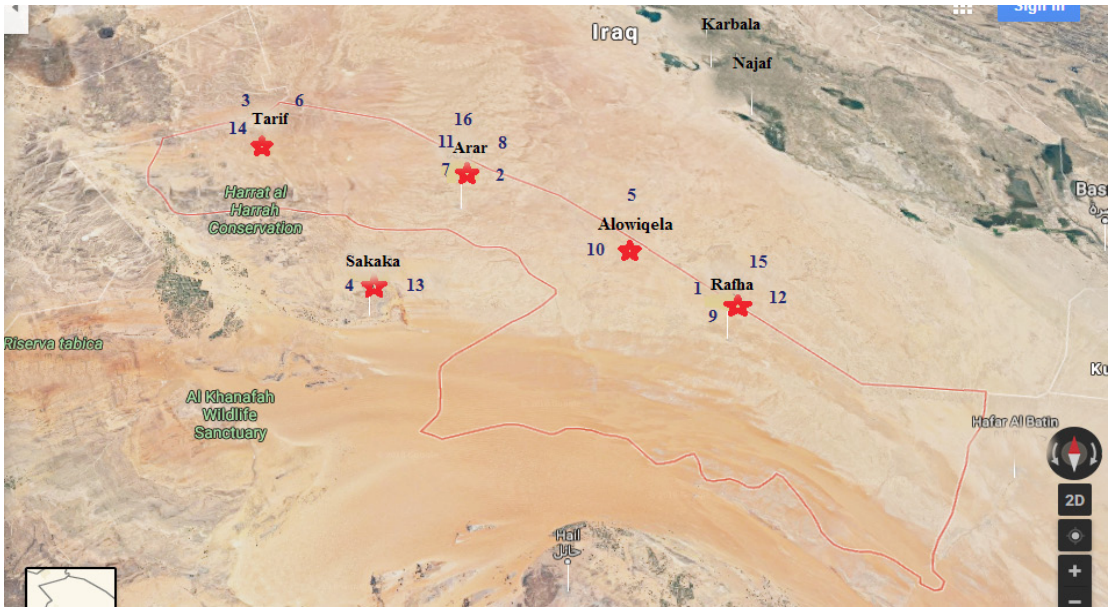

Fig. 1. Geographic map of sampling accessions (★) of two species of *Cucurbita* L. from Northern Border Region of Saudi Arabia.

DNA extraction and SCoT and ISSR markers

Genomic DNA was extracted from the fresh young leaves of the studied plant samples according to Dellaporta et al. (1983). Six primers sequences employed in the present study to generate the start codon targeted **(**SCoT) as designed by Collard & Mackill (2009) based on the consensus sequences of translation initiation codon region in higher plants. In addition, five ISSR primers were tested to amplify Inter-Simple Sequence Repeats DNA (ISSR) markers, as described by Zietkiewicz et al. (1994). The sequence of the five ISSR and six SCoT are listed in Table 2.

TABLE 2. Nucleotide sequences of primers used for SCoT and ISSR techniques.

SCoT		ISSR	
Code	**Sequence**	**Code**	**Sequence**
SCoT 1	ACG ACA TGG CGA CCA CGC	14A	5` CTC TCT CTC TCT CTC TTG 3`
SCoT 8	ACA ATG GCT ACC ACT GAG	49A	5` CTC TCT CTC TCT CTC TAG 3`
SCoT 11	ACA ATG GCT ACC ACT ACC	44B	5` CTC TCT CTC TCT CTC TAG 3`
SCoT 12	CAA CAA TGG CTA CCA CCG	HB-12	5´ CAC CAC CAC GC 3`
SCoT 14	ACA ATG GCT ACC ACT GCC	HB-15	5´ CAC CAC CAC GC 3`
SCoT 15	ACA ATG CTA CCA CCA AGC		

Data analysis

All gels were photographed and analyzed using Bio-Rad video documentation system, Model Gel Doc 2000. The presence coded "1" or absence coded "0" of each band was treated as a binary character in a data matrix. Cluster analysis was conducted to generate a dendogram demonstrating the relationships among the sixteen accessions of the studied *Cucurbita* accessions based on biochemical and molecular data. Data analyses were performed using the SYSTAT version 7.0 program (Wilkinson, 1997).

Results

Morphological features

Morphological characteristics of fruits of the 16 accessions of *Cucurbita* L. are recorded in Table 3. These illustrate fruit shape, shell, skin color, flesh color and fruit size.

Isozymes electrophoresis

The peroxidase and polyphenol oxidase isozymes analysis on the basis of the number, intensity and reproducibility of POD and PPO were used to distinguish among the accessions. Band intensity differences were found in the two isozymes. POD-isozymes analysis displayed a total 6 bands as shown in Table 4 with *Rf* of 0.3 to 0.9 with 6 polymorphic band. The total numbers of Polyphenol Oxidase isozymes were 4 bands as shown in Table 5. PPO displayed 6 bands with 6 polymorphic bands with *RF* of 0.3 to 0.9.

Seed protein electrophoresis

Table 6 illustrates the band pattern for the investigated accessions. Twelve bands were found in the investigated accessions ranging in size from 25kDa to 275kDa. Three positive unique bands were observed; a 65kDa band was characteristic for accession 1 (H_1), a 220kDa band was found in accession 2 (B_2), while a 275kDa was recorded in accession 11 (A_2). Bands with molecular weight 40kDa and 55kDa were present in all accessions except accession 12 (Sh_2); these are negative unique markers. Bands with molecular weight 75kDa and 145kDa were reported only in the accessions (H_1, B_1, A_1, Y_1, P_1, Su_1, M_1 and Y_2). On the other hand, an 85kDa band was illustrated only in the second eight accessions (Y_2, P_2, A_2, Sh_2, M_2, Su_2, P_3 and Y_3) as distinguished band. The highest percentage of polymorphism 58.33% was recorded in accessions 2, 4, 5, 6, 7, 8, 12 and 15, where the lowest percentage (8.33%) was found in accession Sh_2 (Table 6).

Molecular analysis

(SCoT and ISSR)

The ISSR fingerprinting generated by the five ISSR primers 14A, 49 A, 44 B, HB -12 and HB-15 is illustrated in Plate 1, the range of products, number of bands (common and polymorphic) and total number of bands produced by different primers were shown in Table 7. Out of 79 ISSR markers, four were common to all studied accessions and 75 bands were polymorphic. Primer 14 A markers with the molecular sizes 440, 700 and 1280bp were found in the nine accessions (9-16), while the 410bp was recorded only in the 1-9 accessions; markers with size of 290 and 1145bp were presented in accessions 7 and 8, respectively. The ISSR markers 280, 480, 670 and 766bp that have been recorded in accession 7 it could be used as positive unique markers to this accession. The primer 44 B marker with a molecular size of 230 bp was illustrated in accessions 8-16, whereas the 300bp was presented in the accessions 1-5. On the other hand, the markers with the sizes 290, 650, 860, 1370, 1730, 2140 bp generated by primer HB-15, were recorded as unique markers for accessions 9-16 and markers having 485bp, 595 bp and 930bp were found in accessions 1-8. Thus, these markers could be considered as positive markers for these accessions.

TABLE 3. The morphological description of fruit characteristics of the 16 pumpkin accessions collected from Northern Border region of Saudi Arabia.

Accession Number	Accession	Fruit shape	Shell	Skin color	Flesh color	Fruit size
1	H_1	Pyriform	Smooth	Dark Green	Yellow	Small (< 2kg)
2	B_1	Pyriform	Semi-grooved	Yellow	Yellow	Small (< 2kg)
3	A_1	Pyriform-globular	Semi-grooved	Light Copper	Light yellow	Small (< 2kg)
4	Y_1	Globular	Grooved	Light Green	Orange	Medium (2-4kg)
5	P_1	Globular	Semi-grooved	Green	Light yellow	Medium (2-4kg)
6	Su_1	Pyriform	Grooved	Light Copper	Light yellow	Large (> 4kg)
7	Sh_1	Pyriform	Grooved	Dark Copper	Deep Orange	Large (> 4kg)
8	M_1	Pyriform-globular	Semi-grooved	Light Orange	Deep Orange	Extra Large (> 6kg)
9	Y_2	Pyriform	Semi-grooved	Light Green	Orange	Medium (2-4kg)
10	P_2	Pyriform	Semi-grooved	Green	Light yellow	Medium (2-4kg)
11	A_2	Pyriform-globular	Semi-grooved	Light Copper	Light yellow	Small (< 2kg)
12	Sh_2	Globular	Semi-grooved	Light Green	Orange	Medium (2-4kg)
13	M_2	Pyriform	Smooth	Dark Copper	Deep Orange	Large (> 4kg)
14	Su_2	Pyriform-globular	Semi-grooved	Light Orange	Deep Orange	Extra Large (> 6kg)
15	P_3	Pyriform	Smooth	Light Copper	Light yellow	Large (> 4kg)
16	Y_3	Globular	Semi-grooved	Green	Light yellow	Medium (2-4kg)

TABLE 4. Peroxidase isozymes banding pattern and percentage of the developed bands in the studied pumpkin accessions.

Peroxidase groups	Relative Mobility	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PX 1	0.3	1^{+}	1^{+}	1^{-}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}	1^{++}	1^{+}	1^{-}	1^{-}	1^{++}	1^{+}	1^{++}	1^{++}
PX 2	0.6	1^{++}	1^{++}	1^{++}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{-}	1^{-}	1^{++}	1^{+}	1^{++}	1^{++}
PX 3	0.7	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{+}	1^{++}	1^{+}	1^{++}	1^{++}
PX 4	0.8	1^{+}	1^{+}	1^{-}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{-}	1^{-}	1^{+}	1^{++}	1^{+}	1^{++}	1^{++}
PX 5	0.85	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{++}	1^{-}	1^{+}	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
PX 6	0.9	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}

TABLE 5. Polyphenol Oxidase isozymes banding pattern and percentage of the developed bands in the studied pumpkin accessions.

Polyphenol Oxidase Groups	Relative Mobility	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PPO 1	0.3	1^{+}	1^{++}	1^{+}	1^{-}	1^{++}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
PPO 2	0.6	1^{+}	1^{++}	1^{+}	1^{-}	1^{++}	1^{+}	1^{++}	1^{+}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
PPO 3	0.7	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}	1^{-}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}
PPO 4	0.8	1^{+}	1^{+}	1^{-}	1^{+}	1^{++}	1^{++}	1^{+}	1^{-}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}
PPO 5	0.85	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}	1^{++}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
PPO 6	0.9	1^{-}	1^{-}	1^{+}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}

TABLE 6. Comparative analysis and molecular weight of the proteins revealed in the SDS-PAGE profile of the pumpkin accessions.

Protein Band No.	M.W k.Da.	Pumpkin accessions															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	275	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
2	220	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	215	0	0	0	1	1	1	1	1	0	0	0	0	1	0	1	1
4	155	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1
5	145	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
6	100	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
7	85	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
8	75	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
9	65	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	55	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
11	40	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
12	25	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0
Total	1455	6	7	6	7	7	7	7	7	6	6	6	1	7	4	7	6
Polymorphism%		50	58.33	50	58.33	58.33	58.33	58.33	58.33	50	50	50	8.33	58.33	33.33	58.33	50

The SCoT-PCR fingerprinting (Plate 2) illustrated 173 markers with 170 polymorphic and three common markers. Regarding primer SCoT 1, the 320bp marker was found in accessions 8-16. The SCoT 11 primer, produced 240, 260, 290 and 360bp in accessions 1-9, on the other hand 400 and 445bp markers were recorded in accessions 8-16. SCoT12 and SCoT 15 primers were distinguished the first from the last nine accessions by 8 unique bands. For SCoT 12, markers 975, 1500 and 1535bp were found, while bands with molecular weight 400, 430 and 1070bp were found in the accessions 9-16. SCoT 15, 400bp marker was unique for accessions 9-16, whereas the 680bp differentiated accessions 1-8, these bands could be considered as positive marker for these accessions (Table 8).

Genetic diversity

Cluster analysis was conducted to get a dendogram based on biochemical and molecular attributes that divided the studied accessions into two groups at 25 the first group contain the first nine accessions (1-8) and the second group includes accessions (9-16) (Fig. 2). Similarity indicates between the studied accessions of pumpkin in KSA according to biochemical and molecular profiles, the highest percentage of similarity was 1.00 recorded between accessions 2 (B_1) and 12 (Sh_2) and the lowest value 0.00 was reported between accessions 13 (M_2) and 15 (P_3) (Table 9).

The genetic diversity between the studied accessions of pumpkin based on morphological variation and polymorphism of seed protein, ISSR and SCoT markers is expressed by Biplot using the SYSTAT software (Fig. 3). PERMA-Biplot shows the importance of fruit characteristics to distinguish between all studied accessions of pumpkin into two groups; first group includes *C. maxima* (4, 9 and 16). The second group includes *C. moschata* (1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15). Scatter diagram also proved the result of Biplot the morphological characters, biochemical and molecular data can illustrate the distribution of the studied accessions all accessions of *C. moschata* was interposed, while the accessions of *C. maxima* in separate group (Fig. 4).

Discussion

Plant morphology is the study of the physical form and external structure of plants.

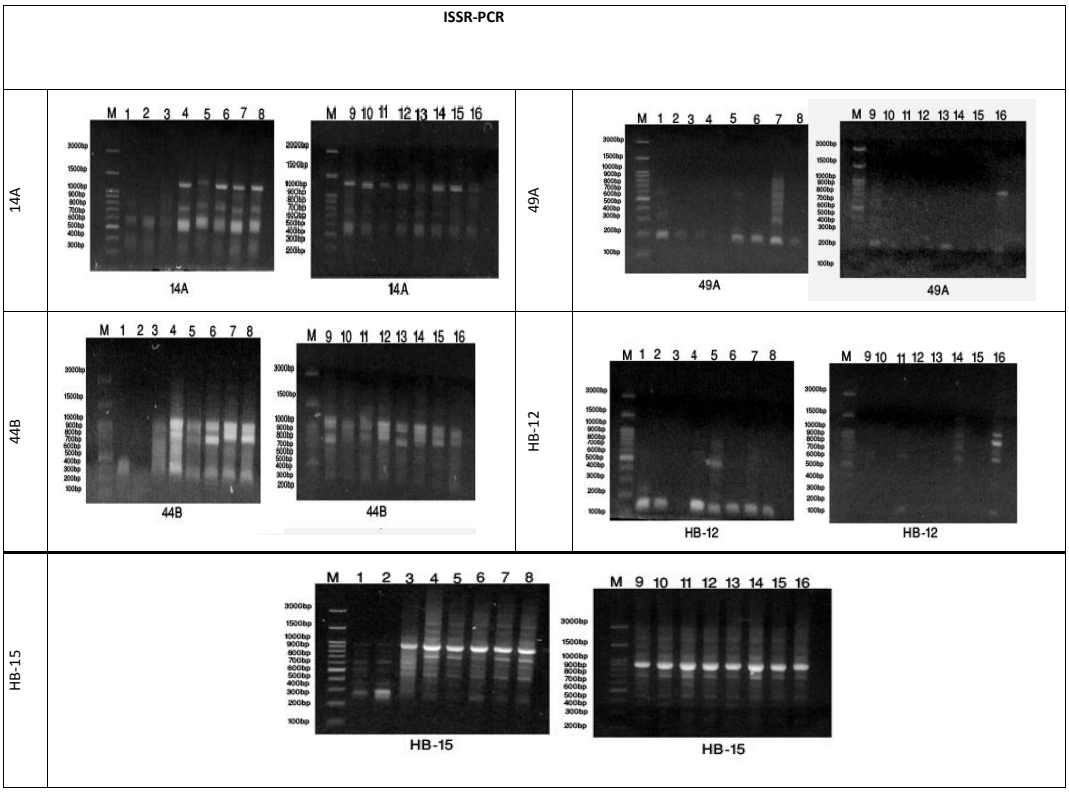

Plate 1. ISSR-PCR product profiles of 16 accessions of pumpkin.

TABLE 7. The range of band products, number of common and polymorphic bands produced by different ISSR primers for the studied accessions of pumpkin.

	Primers	14A	49A	44B	HB-12	HB-15
Bands	Range of products (bp)	20	20	13.39	26.67	26.67
	Total	27.27	0	0	0	27.27
	Monomorphic	9.52	4.76	14.28	28.37	19.05
	Polymorphic	14	14.29	14.29	28.57	28.57
	٪ Polymorphic	28	32	32	40	40
% Polymorphism for accessions	1	36	71.43	28.57	9.09	26.67
	2	32	0.0	23.81	0.0	20
	3	36	71.43	23.81	0.0	20
	4	32	0.0	23.81	0.0	20
	5	32	0.0	28.57	0.0	20
	6	36	28.57	23.81	0.0	20
	7	32	0.0	14.28	0.0	26.67
	8	36	14.29	28.37	36.36	26.67
	9	40	14.29	23.81	0.0	53.34
	10	40	14.29	19.05	72.72	40
	11	40	14.29	28.57	9.09	26.67
	12	40	28.57	19.05	27.27	26.67
	13	40	28.57	28.37	0.0	26.67
	14	32	14.29	14.28	0.0	13.39
	15	32	14.29	4.76	0.0	20
	16	28	14.29	9.52	27.27	20

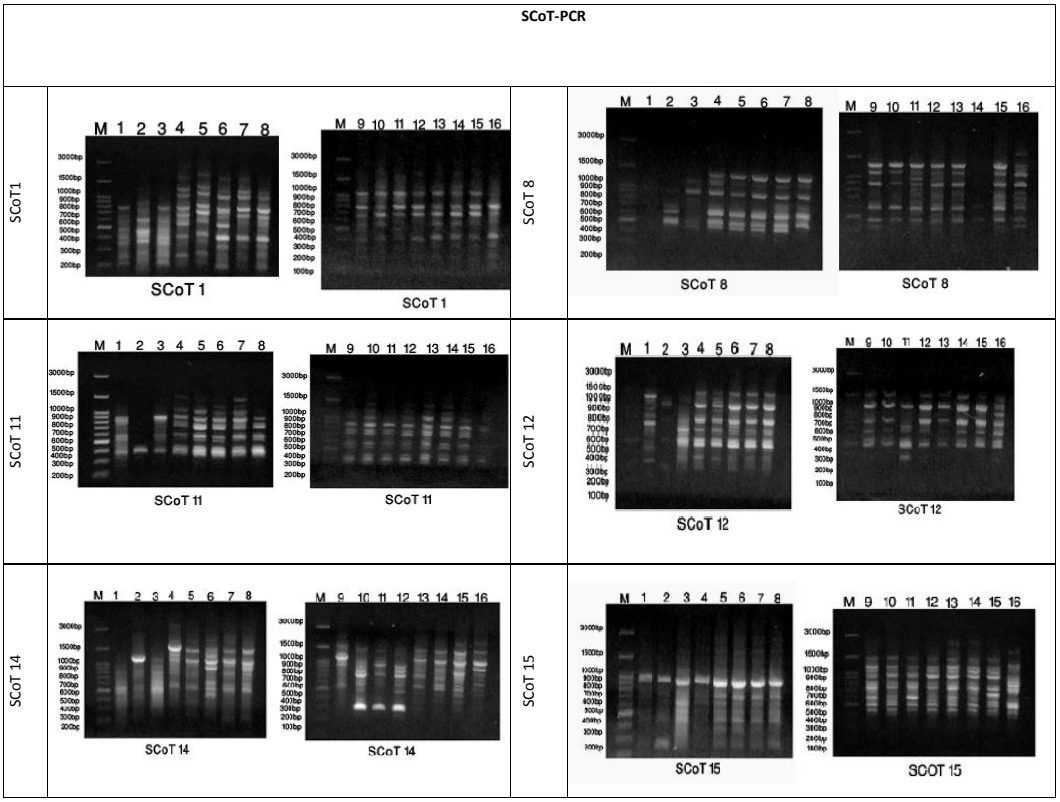

Plate 2. SCoT-PCR product profiles of 16 accessions of pumpkin.

TABLE 8. The range of band products, number of common and polymorphic bands produced by different Scot primers for the studied accessions of pumpkin.

	Primers	SCoT 1	SCoT 8	SCoT 11	SCoT 12	SCoT 14	SCoT 15
Bands	Range of products (bp)	2015-135	1595-335	1843-182	962-237	782-257	2660-205
	Total	33	22	27	28	37	26
	Monomorphic	2	0	0	0	0	1
	Polymorphic	31	22	27	28	37	25
	٪ Polymorphic	93.94	100	100	100	100	96.15
% Polymorphism for accessions	1	15.15	4.55	37.04	42.86	24.32	15.38
	2	33.33	27.27	18.52	17.86	24.32	23.08
	3	18.18	18.18	33.33	32.14	21.62	42.31
	4	24.24	27.27	48.15	35.71	24.32	11.54
	5	30.3	22.73	44.44	32.14	16.21	34.62
	6	27.27	22.73	51.85	35.71	35.14	38.46
	7	21.21	18.18	55.36	32.14	24.32	34.62
	8	27.27	22.73	48.15	35.71	35.14	34.62
	9	27.27	22.73	29.63	28.57	27.03	38.46
	10	27.27	31.82	29.63	21.43	27.03	34.62
	11	24.24	50	22.22	28.57	16.23	34.62
	12	18.18	50	29.63	28.57	18.92	34.62
	13	21.21	31.82	33.33	25	16.23	42.31
	14	15.15	9.09	33.33	25	27.03	42.31
	15	24.24	40.91	25.93	21.43	27.03	38.46
	16	24.24	50	14.81	39.29	21.62	34.61

TABLE 9. Similarity indices between the sixteen accessions of pumpkin according to biochemical and molecular profiles.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000															
2	.372	1.000														
3	.419	.413	1.000													
4	.488	.463	.353	1.000												
5	.508	.503	.359	.203	1.000											
6	.529	.542	.351	.236	.131	1.000										
7	.538	.586	.431	.279	.128	.086	1.000									
8	.547	.524	.386	.219	.163	.073	.056	1.000								
9	.934	.894	.812	.778	.824	.797	.849	.844	1.000							
10	.946	.923	.815	.815	.845	.781	.871	.815	.071	1.000						
11	.933	.891	.806	.752	.801	.773	.829	.807	.146	.103	1.000					
12	.982	1.000	.888	.901	.947	.895	.968	.930	.219	.102	.182	1.000				
13	.964	.941	.812	.830	.824	.829	.869	.829	.172	.071	.190	.113	1.000			
14	.966	.984	.815	.852	.880	.866	.888	.866	.233	.194	.234	.199	.052	1.000		
15	.933	.951	.786	.842	.836	.773	.829	.790	.181	.084	.144	.125	.000	.159	1.000	
16	.973	.915	.870	.781	.793	.815	.836	.847	.306	.310	.276	.352	.233	.257	.223	1.000

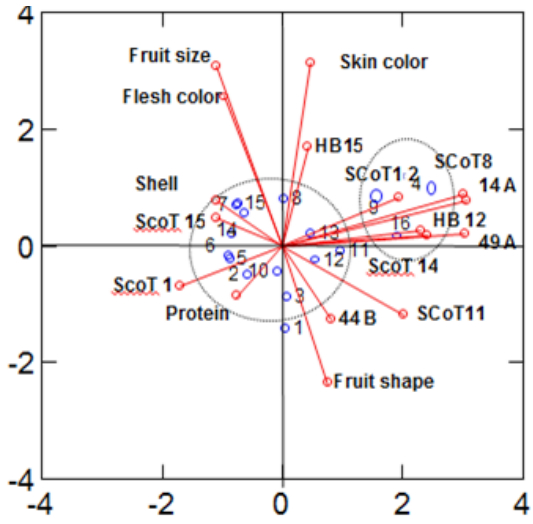

Fig. 3. (Biplot) Analysis Eigenvalues of the studied *Cucurbita* L. species based on morphological, biochemical and molecular parameters.

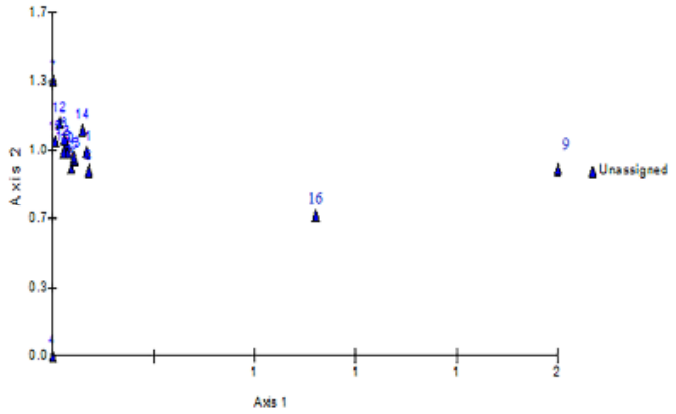

Fig. 4. Scatter diagram showing distribution of the sixteen accessions of *Cucurbita* L. based on morphological, biochemical and molecular data.

Understanding the characteristics and structures belong to each type is an important part of understanding plant evolution (Pitrat, 2013). On the other hand, genetic diversity, differentiation, characterization of genotypes and populations, provide valuable information for the detection of duplicates in collections and provide a reliable characterization of genetic resources for their utilization in plant breeding programs (Badr et al., 2014b). In the current study, the variability observed in size, shape, texture, and color of the skin in *C. maxima* and *C. moschata* is considerable. Such variation is also associated with great variability in the size and color of the seeds. These results are in agreement with Castro et al. (2006), Obiadalla et al. (2009) and Abdein et al. (2017).

The electrophoretic analysis of 16 accessions showed a total number of 12 bands. The lowest number of bands (1) was observed in accession 12 (Sh_2) with molecular weight 85kDa. There are three unique bands that distinguish eight accessions from the rest; these have molecular weights of 75, 85 and 145kDa. The SDS-PAGE banding patterns of the studied accessions indicated variability among landraces similar to the findings of Vladova et al. (2004) in the Cucurbitaceae. The application of isozyme polymorphism is important for population genetic studies and in addressing infra-specific relationships (Mustafa et al., 2005). The two isozymes peroxidase (POD) and polyphenol oxidase (PPO) were used to differentiate among the sixteen accessions of the two studied taxa; each of them has 6 polymorphic bands. The differences in protein and isozymes banding among the pumpkin indicated the landraces differ genetically, differences in the landraces due to differ in gene or gene expression. Proteins and isozymes markers used in many crops have been useful to some extent, but these do not - provide sufficient polymorphism among closely related cultivars unless large number of isozymes are used (Decker, 1985, 1988; Hamrick et al., 1991 and Beckmann & Soller, 1993).

ISSR involves amplification of DNA segments uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly inter simple sequence repeats of different sizes (Mishra et al., 2014). Five ISSR primers were used for the investigation species of 79 ranging in molecular size from 130 to 2140bp in addition out of 76 polymorphic bands.

The SCoT technique has been used successfully for the DNA fingerprinting, characterization of genetic variation and phylogenetic studies in some cultivated plants (Cabo et al., 2014). SCoT 12, markers having sizes of 975, 1500 and 1535bp were found, while bands with molecular weight 400, 430 and 1070bp were found in the accessions 9-16. SCoT 15, 400bp marker was unique for accessions 9-16, whereas the 680bp differentiated accessions 1-8, these bands could be considered as positive marker for these accessions. The markers with the sizes 290, 650, 860, 1370, 1730, 2140bp generated by primer HB-15, were recorded as unique markers for accessions 9-16 and markers having 485bp, 595bp and 930bp were found in accessions 1-8. Thus, these markers could be considered as positive markers for these accessions. The presence of unique ISSR markers may be regarded as markers for the authentication of genetic resources (Badr et al., 2014a).

The results obtained using 6 screened primers demonstrated that SCoT markers can be effectively used to estimate the genetic diversity of and to judge the genotype of pumpkin accessions with multiple morphological types were used to clarify the differentiation between the studied accessions, out of 173 total bands with 176 polymorphic.

The results clarified the ability of ISSR and SCoT analysis to differentiate among the investigated accessions of *Cucrbita*, in agreement with the study of Heikal et al. (2008). These results suggest that SCoT and ISSR molecular markers are an excellent choice for the evaluation of genetic diversity and the assessing differentiation among landraces belonging to different geographical regions in agreement with Xanthopoulou et al. (2015).

The result obtained from Biplot is similar to that outcome of scatter diagram that distinguish the studied accessions into two groups; group contains 3 accessions of *C. maxima* and the other group includes 13 accessions *C. moschata* based on morphological biochemical and molecular parameters.

Conclusions

The current study concluded that high variation for morphological, biochemical and molecular

profile in terms of different regions. The genetic resources characterization is a key for the management of gene banks and plant breeding. The three *Cucurbita* species evaluated in this study showed a high variability for some fruit parameters such as weight, fruit shape and predominant and secondary skin color at maturity. The high genetic diversity found could be used in breeding programs to obtain new cultivars and provide relevant information for the diversity conservation. Besides, Biplot was applied to classify the samples based on molecular composition and morphological properties and it was observed that 16 accessions was classified into two groups; first group with 3 species of *C. maxima* and the second group with the rest accessions revealed to *C. moschata*.

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التنوع الجيني بين طرز القرع العسلي في منطقة الحدود الشمالية بالمملكة العربية السعودية باستخدام القياسات البيوكيماوية والجزيئية

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استخدمت الدراسات البيوكيماوية والجزيئية لتقدير العلاقة والإختلافات الوراثية بين ستة عشر طرازاً جمعت من مناطق مختلفة في منطقة الحدود الشمالية بالمملكة العربية السعودية لجنسين من القرع العسلي وذلك من خلال دراسة الصفات الظاهرية للثمرة ودراسة نظائر الإنزيمات وأيضا دراسة البروتين لكل الأنواع محل الدراسة بطريقة التفريد الكهربي للبروتين حيث أنه أظهر 12 حزمة لكل الأنواع. وأوضحت النتائج حزمتين ذات الوزن الجزيئي 75 و145 كيلو دالتون مميزة للثمانية أنواع الأولى من القرع العسلي (8-1) بينما الحزمة ذات الوزن الجزيئي 85 كيلو دالتون وضحت في الثمانية أنواع الأخيرة (16-9) وتعتبر هذه الحزم كحزم مميزة. ولقد تم استخدام خمسة بوادىء للتفرقة بين الستة عشر أنواع من القرع حيث ظهر 79 حزمة مع طريقة تكرار التتابع الداخلي البسيط (ISSR) يتراوح الوزن الجزيئي لها من 130bp إلى 2140bp. وقد تم استخدام ست بادئات لطريقة (SCoT) وأظهرت 173 حزمة يتراوح الوزن الجزيئي لها من 135bp إلى 2660bp. وقد أظهرت النتائج أن أعلى نسبة للإختلافات 100% سجلت مع البادئات HB-12 , 44B ,49A بينما أقل نسبة للإختلافات 92% سجلت مع البادئ HB-15 بطريقة تكرار التتابع الداخلي البسيط (ISSR). على النقيض الأخر أظهرت أعلى نسبة للإختلافات 100% سجلت مع البادئات SCoT8, SCoT11, SCoT12,SCoT14 بينما أقل نسبة للإختلافات هي 93.94% سجلت مع البادئ SCoT 1 وذلك باستخدام طريقة SCoT .