Assessment of Genetic Diversity in Summer Squash Genotypes Using some Yield Traits and DNA Markers Analysis under Sinai Conditions

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Abstract: Fourteen summer squash genotypes were used to assess their genetic diversity and relationships using some yield traits (Fruit length, fruit diameter, fruit shape index, average fruit weight, No of fruits/plant, fruit yield, and total soluble solids %) and DNA markers (Random Amplified Polymorphic DNA, inter-simple sequence repeat, Start Codon Targeted polymorphism) analyses. Two open field trials were executed at two locations, *i.e.,* El-Arish (L_1) and Ras Sidr (L_2) in the North and South Sinai-Egypt, respectively. Significant differences were observed between both locations and indicated the superiority of (L_1) than (L_2) in all studied traits, while the interaction between genotype and location was significant for all studied traits. The location had a major effect on the relative genotypic potential of these traits, in which the genotype × location interaction was highly significant. SQ6 genotype ranked as the first in the fruit yield/plant and SQ2 for fruit diameter followed by SQ7 and SQ2 for fruit shape as well as SQ13 and SQ12 for the number of fruits/plant in L_1 and L_2 locations, respectively. Additionally, high polymorphism percentage (P %) was detected using RAPD (85%), ISSR (79.25%) and SCoT (69.44). Among the 15 studied primers, OP-B17 showed the maximum P% (100%) followed by primer OP-C17 (90.9 %) which also scored the highest values for polymorphism information content, effective multiplex ratio, and marker index. In addition, OP-K01 represented the maximum value of resolving power (12.56), indicated that these primers were highly informative. Intriguingly, 55 positive and two unique negative bands were amplified, out of which (33) bands were generated by RAPD followed by SCoT (15) and ISSR (9). In this regard, OP-C17 and SQ1 genotype produced the highest number of unique marker18 and 11amplicon, respectively. Moreover, the maximum similarity was found between SQ 11 and SQ 12 indicating that both genotypes were the most similar. Cluster and PCA analysis using RAPD, ISSR, and SCoT data grouped the 14 squash genotypes into four, three and two distinct clusters, respectively. Generally, the yield traits alongside molecular results showed significant information that may be utilized to sort and relationship between the studied summer squash genotypes under different conditions.

Keywords: Squash (*Cucurbita pepo*), Genetic diversity, Yield traits, RAPD, ISSR, SCoT, Sinai

INTRODUCTION

Summer squash (*Cucurbita pepo* L.) is one of the paramount vegetable crops of Cucurbitaceae family cultivated throughout the world. It is a cross pollinated plant, and its diploid chromosomal number is (2n=40). Its origin is Northern Mexico (Tropical America) and is amongst the most ancient cultivated crops in the America (Paris, 1996). Production of squash & pumpkins in Asia comprises half or more of the total area devoted to pumpkins and squash worldwide (Albrifcany, 2015). Squash is cultivated all over the year in Egypt, in the open field during spring and summer, where in tunnels or greenhouses in fall and winter (Abd-Alrahman *et al.,* 2020). According to Egyptian Ministry of Agriculture and Land Reclamation, the total production area of squash in 2016 was 73558 fed and produced 551023 tons of fruits (Nassar *et al.*, 2019). At the same time, it is considered an important source of human food, many nutrients, and medicinal uses (Matlob *et al*., 1989; Abd El-Hadi *et al*., 2017). The low production of summer squash in some production regions due to the deficiency in fruit setting resulted from unfavorable sex expression and bad cross-pollination flowers due to short blooming period (Albrifcany, 2015). Therefore, these restrictions need further studies including the environmental factors and cultural practices to increase crop production with desired quality (Mohammed, 1996). Genotype selection is also a key management component in plant breeding program in order to improve production quality characteristics. Several previous studies *i.e.,* Ferreira *et al*. (2003), Al-Araby (2004), Abdein (2005), Al-Araby (2010), Fayeun *et al*. (2012) Tamil *et al*. (2012), Abd El-Hadi *et al.* (2014) were interested in investigating the genetics performance under different conditions. When cultivars are grown in different locations, their performance was observed to vary according to environmental variations of these locations. This variation may refer to changes in environmental conditions such as temperature, soil type, moisture and so on (Robertson, 1959). Inconsistent genotypic responses to environmental factors from location to location, is a function of G × E interaction, hence resulting in alteration to the ordering of genotypes from one environment to another. Lately, plant breeders utilized modern methods such as molecular marker to evaluate genetic variability (Abd El-Hadi *et al.,* 2017). DNA fingerprinting is an indispensable tool towards tracing of lineages in plant lines. Unlike the morphological and biochemical markers that could be

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much influenced with environmental factor and growth practices, DNA markers could portray genomic sequences composition thus enabling to detect genetic differences carried by different individuals (Xiao *et al*., 1996; Ovesna *et al*., 2002). Three of such useful markers system have been employed: (i) Random Amplified Polymorphic DNA (RAPD) uses an arbitrary, short primer that anneals to the genome at complementary sequences, and sizes of amplified products reveal existing polymorphisms (Nadeem *et al.,* 2018) and have been applied successfully in diversity analyses of *C. pepo* (Athanasios *et al.,* 2009; Ntuli *et al.,* 2013; Mady *et al.,* 2013; Someh *et al.,* 2016) , (ii) ISSR markers (Zietkiewicz *et al.,* 1994) are based on the amplification of DNA regions located between two microsatellites loci and used successfully in diversity analyses of *C. pepo* (Katzir *et al.,* 2000; Heikal *et al.,* 2008; Inan *et al.,* 2012; Kiani and Siahchehreh, 2017) and (iii) Start Codon Targeted polymorphism (SCoT) is a 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; Abdein *et al*., 2018). SCoT analysis has been also used to study genetic relationships in *C. pepo* (Xanthopoulou *et al.,* 2015; Bhawna *et al.,* 2017). SCoT technique can be used as an effective complementary method to ISSR and RAPD (Abd El-Moneim, 2019). In the same context, different investigations studied the genetic diversity and relationships belonging to *Cucurbita* by comparing their molecular markers (SRAP and RAPD) (Ferriol *et al.,* 2003); ISSR, SRAP & RAPD (Yildiz *et al.,* 2011); ISSR & SRAP (Inan *et al.,* 2012); ISSR & SSR (Yildiz *et al.,* 2014) and SCoT & ISSR (Abdein, 2018). Phenotypic evaluation in Cucurbita has traditionally been based on seed and fruit characteristics. These have proved useful in distinguishing samples of related species but exhibit inadequate variation for intraspecific discrimination of cultivars and involves a limited number of phenotypic characters effected by environmental influences (Dijkhuizen *et al.,* 1996). In this regard, phenotypic markers in *Cucurbita* have been found to be unreliable and with no molecular basis (Wilson, 1989). Molecular information provides deeper insight into genetic structure, while detection of heterozygous loci utilizing marker techniques would give more realistic genetic relationships. Additionally, molecular evaluation was more favorable than phenotypic evaluation because it had more markers and represented neutral traits of simple inheritance (Sensoy *et al.,* 2007; Athanasios *et al.,* 2009). Some differences were observed between molecular and morphological studies, and it was concluded that only morphological or molecular analyses but a combination of both approaches are often more reliable in genetic variability studies in *Cucurbita* genus (Inan *et al.,* 2012). Therefore, the main objective of this work is to estimate diversity analysis of 14 genotypes of *Cucurbita pepo* based on variation in some yield traits attributes along with molecular markers revealed by RAPD, ISSR and SCoT markers. Finally, to provide recommendation for utilization of the important landraces in a breeding program to increase genetic diversity and to develop useful inbred lines of *C. pepo*.

MATERIALS AND METHODS

Field experiment:

Two open field trials were conducted in two different locations in Sinai Peninsula. Both locations have a desert climate (BWh) according to the Köppen-Geiger climate classification. 1st location (El-Arish) at the Research Farm of Fac. Environ. Agric. Sci., Arish Uni., Egypt that located at, 31°07'34.0"N /33°49'31.4"E. while, 2nd location (Ras Sidr) in South Sinai, Egypt and located at 29°37'32.5"N/32°42'48.6"E. The mechanical and chemical analyses of the experimental soil and irrigation water (average of two seasons) are presented in (Tables 1 and 2); their analysis was done according to the methods described by Piper (1947) and Jackson (1958).

Table (1): Initial physical and chemical properties of the experimental soil for two locations

Parameter		Soil depth 0-35 (cm)	
		El-Arish (L_1)	Ras Sidr (L_2)
Physical analysis			
Sand (%)		89.39	80.6
Silt (%)		4.51	8.61
Clay (%)		6.10	10.79
Soil texture		Loamy sand	Loamy sand
Chemical analysis			
Cations (meq.1^{-1})	Ca^{++}	6.72	12.3
	Mg^{++}	5.10	8.7
	Na^{+}	12.67	24.2
	K^{+}	0.40	0.8
Anions (meq.1^{-1})	CO_3^{--}	-	-
	HCO_3^{-}	2.45	3.00
	Cl^{-}	14.41	30.50
	SO_4^{--}	8.03	13.00
E.C (dS.m^{-1})		2.489	4.65
pH		7.9	7.7

According to (Jackson, 1958)

Table (2): Initial chemical analyses of irrigation water for two locations

Parameter	El-Arish (L_1)	Ras Sidr (L_2)
E.C ($dS.m^{-1}$)	3.22	7.85
Concentration (ppm)	2048	5024
pH	7.5	7.77
Soluble cations ($meq.l^{-1}$)		
Ca^{++}	8.64	20.50
Mg^{++}	6.03	8.60
Na^{+}	17.05	48.89
K^{+}	0.50	0.35
Soluble anions ($meq.l^{-1}$)		
CO_3^{--}	-	-
HCO_3^{-}	4.62	5.00
Cl^{-}	22.26	57.50
SO_4^{--}	5.34	16.20

According to (Jackson, 1958)

Plant materials and growth conditions:

Fourteen imported genotypes of summer squash (*Cucurbita pepo*) were evaluated for yield and some attributed traits during the 2017 and 2018 summer seasons under open field conditions in El-Arish, and Ras Sidr, Sinai, Egypt. The genotypes used in this study are presented in (Table 3). Average temperatures prevailing at (L_1) and (L_2) locations are given in (Fig. 1). Seeds were directly planted in the open field on the 3rd week of March in both locations. Two to four seeds were sown around emitters of dripper lines in a randomized complete block design (RCBD) with three replicates. The experimental unit was two ridges 5.0 m. long and 1.2 m. wide. Plants were set 50 cm apart in the bed and were subjected to common agricultural practices. After two weeks of sowing, plants were thinned to one plant. Thus, each plot contained 20 plants; fruits were harvested at a marketable stage at 50 days after planting and transferred to the laboratory. Eight fruits were randomly assigned from each plot to determine the following traits: 1) fruit length (FL, cm), 2) fruit diameter (FD, cm), 3) fruit shape index (FShI), 4) average fruit weight (WF, gm), 5) number of fruits per plant (NoFP), 6) fruit yield (FY, kg/plant) and 7) total soluble solids percentage (TSS%, measured by Brix refractometer).

Table (3): Genotypes code, origin and names of 14 squash genotypes

Genotypes		Origin	Name
Number	**Code**		
1	**SQ1**	EGYPT	ESKANDARANI
2	**SQ2**	FRANCE	WHITE BUSH SCALLOP
3	**SQ3**	USA	ZUCCHINE KRITI
4	**SQ4**	TURKI	SIYAH KABUK
5	**SQ5**	IRAQ	FIVE STAR
6	**SQ6**	TURKI	KABAK DOLMALIK KOLSUZ
7	**SQ7**	IRAQ	ERBIL GARDEN
8	**SQ8**	GERMANY	ZUCCHINI DIAMANT
٩	**SQ9**	SYRIA	HALAB
10	**SQ 10**	TURKI	BEYAZ SAKIZ KABAK
11	**SQ11**	ITALY	ZUCCHINO ALBERALLO DI SARZANA
12	**SQ12**	TURKI	SAKIZ KABGI
13	**SQ13**	USA	ALEXANDIA
14	**SQ14**	ITALY	ZUCCHINO BIANCO DI TRIESTE

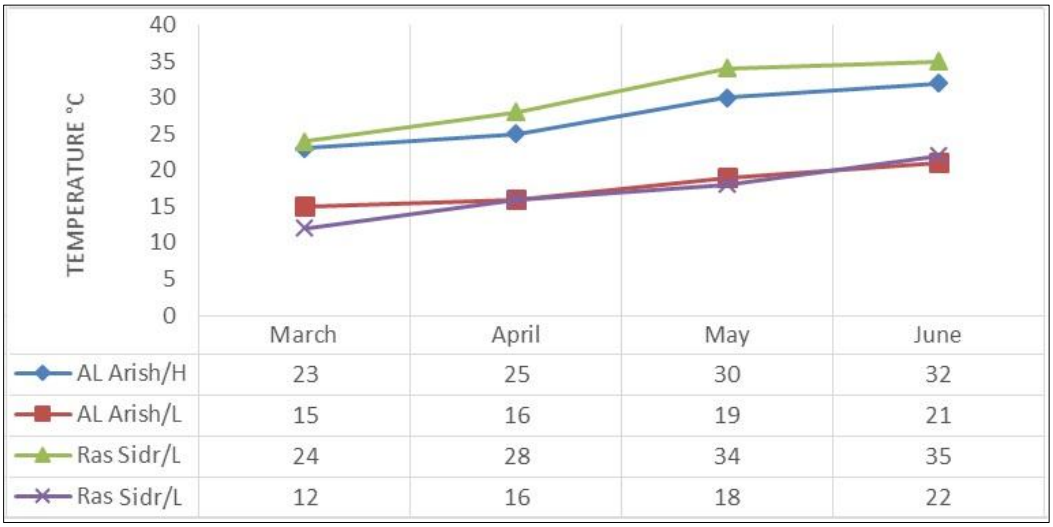

Figure (1): Average of high and low temperatures prevailing at (L_1) and (L_2) locations

Statistical analysis:

Data collected were analyzed using ASSISTAT Version 7.7 en, UFCG-Brazil (Silva and Azevedo, 2016a,b) computer program package. The data were first subjected to analysis of variance for each location using the procedure illustrated by Gomez and Gomez (1984) for a randomized complete block design over both years. A combined analysis of variance was computed using the same software (ASSISTAT) to study the genotype × location interaction. Phenotypic (PCV%) and genotypic (GCV%). Coefficient of variability was calculated according to Singh and Chaudhury (1985). Heritability based on (Stansfield, 1983).

Molecular Marker analysis:

Genomic DNA extraction and PCR procedures:

The total genomic DNA was extracted from young leaf pieces using a DNA Plant Kit (Qiagen) USA. Five primers/markers (Table 4) were screened for the studied genotypes. RAPD, ISSR, and SCoT markers amplification were performed as described by (Hussein *et al*., 2006) and (Collard and Mackill, 2009), respectively. PCR products were visualized by conventional agarose gel electrophoresis.

Table (4): List of the primers names and their nucleotide sequences used in the study for RAPD, ISSR, and SCoT procedure

Name	RAPD Sequence (5→3)	Name	ISSR Sequence (5→3)
OP-A9	GGGTAACGCC	**44B**	CTC TCT CTC TCT CTC TAG
OP-B17	CTCACCGTCC	**49A**	CTCTCTCTCTCTCTCTTG
OP-C13	GGACCCAACC	**HB-8**	GAGAGAGAGAGAGG
OP-C17	AAG CCT CGT G	**HB-11**	GTGTGTGTGTGTTGTCC
OP-K1	TGC CGA GCT G	**HB-13**	GAGGAGGAGC
	SCoT Sequence (5→3)		
SCoT 1	ACGACATGGCGACCACGC		
SCoT 2	ACCATGGCTACCACCGGC		
SCoT 3	ACGACATGGCGACCCACA		
SCoT 4	ACCATGGCTACCACCGCA		
SCoT 6	CAA TGG CTA CCA CTA CAG		

DNA banding pattern analysis:

The DNA banding patterns generated from ISSR and SCoT markers were analysed by Gel works ID advanced software. The presence or absence of each recorded band for each genotype is indicated by (1) or (0). Genetic similarity was calculated by Jaccard's coefficient. A dendrogram was generated with the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the computational package MVSP V. 3.1. To assess the informativeness of the markers, the following parameters were calculated for each primer according to (Anderson *et al*., 1993; Powell *et al*., 1996; Prevost and Wilkinson, 1999): polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (Rp). The principal coordinate analysis PCA (Davis, 1986) was used to find the eigen values and eigenvectors of a matrix containing the similarities between all genotypes.

RESULTS

Yield traits

Highly significant differences were observed between the two locations for fruit length, average fruit weight, fruit yield/plant, and TSS %, while fruit diameter, fruit shape index, and number of fruits/plant exhibited only significant differences between the two locations (Table 5). Data in (Table 6) showed a wide range of variation among the tested genotypes regarding these traits in both locations. The performance values of traits ranged from 4.08 cm (SQ2) to 14.2 cm (SQ10) for fruit length, 2.05 cm (SQ3) to 7.18 (SQ2) for fruit diameter, 0.64 (SQ2) to 4.20 (SQ7) for fruit shape index, 92.750 g (SQ3) to 221.083 g (SQ9) for average fruit weight, 15.08 (SQ2) to 30.42 (SQ12) for the number of fruits/plant, 1.655 kg (SQ4) to 2.769 kg (SQ6) for fruit yield/plant and 1.85 (SQ4) to 7.20% (SQ11) for total soluble solids percentage.

Table (5): Mean squares of combined analysis of variance over two locations for yield traits

VS	df	FLcm	FDcm	FShI	WFg	NoFP	FYPkg	TSS%
Location (L)	1	29.39**	19.98*	12.97*	25.47**	24.00*	29.32**	28.30**
Error-a	2	0.2953	0.8041	0.3042	0.1267	1.2232	0.2205	0.2647
Genotype (G)	13	50.25**	14.72**	10.70**	11897.19**	104.62**	0.73**	18.46**
G×L	13	0.174**	0.532*	0.325*	3.655**	2.968*	0.370**	0.600**
Error-b	52	0.018	0.2777	0.1787	1.477	1.4828	0.1476	0.2442

*,** Significant and highly significant at 0.05 and 0.01% probability levels, respectively

Table (6): Combined means of yield traits as affected by locations (L) and genotypes (G) of squash

Treatment	FLcm	FDcm	FShI	WFg	NoFP	FYPkg	TSS%
Location							
L_1	11.02	4.76	2.75	167.952	24.05	2.830	5.04
L_2	9.83	3.79	1.97	166.851	23.05	1.649	3.88
LSD	0.51	0.84	0.52	0.335	0.81	0.441	0.92
Genotypes							
SQ1	12.35	2.55	3.60	116.75	21.92	2.145	2.75
SQ2	4.08	7.18	0.64	129.083	15.08	1.694	5.05
SQ3	10.35	2.05	4.07	92.75	23.58	1.748	2.95
SQ4	8.05	5.95	1.06	140.75	15.58	1.655	1.85
SQ5	8.71	5.21	1.25	197.458	24.25	2.380	5.56
SQ6	13.24	3.67	3.67	118.375	25.42	2.769	3.63
SQ7	12.90	2.38	4.20	205.458	25.58	2.173	3.10
SQ8	9.11	4.96	1.37	212.875	26.58	2.434	6.97
SQ9	8.45	5.76	1.16	221.083	20.58	2.110	5.87
SQ 10	14.20	3.80	2.79	194.625	23.58	2.373	3.23
SQ11	8.20	4.98	1.17	192.167	25.42	2.560	7.20
SQ12	13.13	2.43	4.00	121.542	30.42	2.474	3.95
SQ13	9.65	5.17	1.38	208.292	26.75	2.196	6.80
SQ14	13.52	3.75	2.67	192.417	24.92	2.644	3.58
LSD	1.06	1.07	0.86	5.615	2.47	0.683	1.00
Mean	10.4	4.3	2.4	167.4	23.5	2.2	4.5
Minimum	4.08	2.05	0.64	92.750	15.08	1.655	1.85
Maximum	14.2	7.18	4.20	221.08	30.4	2.769	7.20

Results obtained from each location (over seasons) as shown in (Tables 7 and 8), indicated It is interested to note that, some genotypes constantly behaved at both locations: *i.e.,* SQ7 and both SQ6 and SQ9 in fruit length and fruit diameter, respectively and most genotypes in both fruit shape and number of fruits/plant as well as all genotypes in average fruit weight (Table 7). Some genotypes fluctuated from one location to another with certain traits, i.e., fruit shape index (Table 7), where SQ1 was 4.24 in 1st location while it was 2.97 in the 2nd one. Fruit diameter in the same genotype was 3.13 cm in the 1st region and 1.97 cm in the second. Fruit yield (Table 8) for SQ2 was 2.446 kg in 1st location, while it was 0.943 kg in the 2nd one. TSS

percentage in the same genotype was 5.63% in the 1st region and 4.47% in the second. The interaction between genotype and location was highly significant for fruit length, average fruit weight, fruit yield/plant and TSS%, and significant all other studied traits.

The best genotype which ranked the first in the studied traits was genotype SQ2, SQ6, SQ9, SQ10, and SQ11 in both locations for fruit diameter, fruit yield/plant, average fruit weight, fruit length and TSS%, respectively; SQ7 and SQ2 for fruit shape as well as SQ13 and SQ12 for the number of fruits/plant in 1st and 2nd localities, respectively. The pertinent of variance components in addition to genotypic (GCV) and phenotypic (PCV) coefficients of variability for yield traits are presented in Table (9). Genetic variation (σ^2g) was large in magnitude compared to error one (σ^2e) and $\sigma^2g\times l$ in all studied traits. Estimates of the genotypic and phenotypic coefficients of variation (Table 8) were determined with slight differences between them for all studied traits except WFg in the 1st region and NoFP in both regions. However, heritability in broad sense was high for all traits.

Table (7): Combined means of fruit length, fruit diameter, fruit shape index, and the average fruit weight as affected by the interaction of locations and genotypes of squash

Item	FLcm		FDcm		FShI		WFg	
	L_1	L_2	L_1	L_2	L_1	L_2	L_1	L_2
SQ1	12.93	11.77	3.13	1.97	4.24	2.97	117.333	116.167
SQ2	4.67	3.50	7.77	6.60	0.60	0.69	129.667	128.500
SQ3	10.93	9.77	2.63	1.47	4.15	3.99	93.333	92.167
SQ4	8.63	7.47	6.53	5.37	1.32	0.81	141.333	140.167
SQ5	9.29	8.13	5.79	4.63	1.60	0.89	198.222	196.694
SQ6	13.83	12.65	3.26	4.09	4.25	3.08	118.778	117.972
SQ7	13.48	13.32	2.96	1.81	4.56	3.83	206.222	204.694
SQ8	9.69	8.53	5.54	4.38	1.75	0.98	213.444	212.306
SQ9	9.02	7.87	5.89	5.64	1.53	0.78	221.556	220.611
SQ10	14.78	13.62	4.38	3.22	3.38	2.20	195.444	193.806
SQ11	8.83	7.58	5.63	4.33	1.57	0.76	192.667	191.667
SQ12	13.73	12.53	3.07	1.79	4.52	3.47	122.667	120.417
SQ13	10.27	9.04	5.73	4.60	1.79	0.96	208.333	208.250
SQ14	14.13	12.90	4.37	3.14	3.24	2.10	192.333	192.500
LSD	0.86		0.93		0.7124		4.522	

Table (8): Combined means of fruits number/plant, fruit yield/plant, and TSS% as affected by the interaction of locations and genotypes of squash

Item	NoFP		FYPkg		TSS%	
	L_1	L_2	L_1	L_2	L_1	L_2
SQ1	22.67	21.17	2.729	1.562	3.33	2.17
SQ2	15.33	14.83	2.446	0.943	5.63	4.47
SQ3	24.33	22.83	2.331	1.164	3.53	2.37
SQ4	16.00	15.17	2.236	1.074	2.43	1.27
SQ5	24.67	23.83	2.962	1.798	6.14	4.98
SQ6	24.67	26.17	3.347	2.191	4.21	3.04
SQ7	26.33	24.83	2.746	1.600	3.68	2.52
SQ8	26.33	26.83	2.971	1.898	7.56	6.39
SQ9	20.67	20.50	2.689	1.531	6.46	5.29
SQ10	24.00	23.17	3.131	1.614	3.81	2.64
SQ11	26.67	24.17	3.005	2.115	7.77	6.64
SQ12	30.67	30.17	3.045	1.903	4.53	3.38
SQ13	27.67	25.83	2.772	1.620	7.37	6.24
SQ14	26.67	23.17	3.216	2.071	4.17	2.99
LSD	**2.12**		**0.6422**		**1.42**	

Table (9): Pertinent of variance components from combined analysis over two locations, genotypic (GCV) and phenotypic (PCV) coefficients of variation and heritabilities (h^2) in both locations for yield traits

Parameter		FLcm	FDcm	FShI	WFg	NoFP	FYPkg	TSS%
$\delta^2 g$		8.346	2.364	1.745	1982.3	16.92	0.061	2.977
$\delta^2 e$		0.082	0.278	0.179	1.877	1.483	0.1476	0.244
$\delta^2 l$		0.696	0.463	0.303	0.519	0.501	0.689	0.660
$\delta^2 g \times l$		0.031	0.085	0.016	0.593	0.495	0.074	0.119
L_1	PCV%	25.49	17.96	25.03	393.91	26.75	1.80	20.28
	GCV%	25.29	17.42	24.14	391.32	24.36	1.01	20.20
	h^2 %	0.99	0.98	0.98	0.99	0.96	0.79	0.99
L_2	PCV%	25.37	17.80	24.54	394.96	24.91	1.79	20.41
	GCV%	25.35	17.73	24.43	394.66	23.63	1.73	20.41
	h^2 %	0.99	0.99	0.99	0.99	0.98	0.98	0.99

Polymorphisms detected using RAPD, ISSR, and SCoT Markers

In the present study, five primers of RAPD, ISSR, and SCoT were used to examine the genetic diversity among 14 summer squash genotypes. All primers - except (ISSR-HB-11) - demonstrated polymorphism in the tested genomic DNA as indicated by the banding patterns (Fig. 2 and Tables 10, 11, 12). Out of 80 amplified RAPD bands, 68 and 12 bands were polymorphic and monomorphic, respectively (Fig. 2A and Table 10). The total bands per primer varied from 9 to 33 for OP-B17 and OP-C17, respectively. The size of amplified products varied from 122 to 1623 bp. In addition, OP-B17 and OP-K01 primers had the highest (30) and lowest (9) number of polymorphic bands. The average of (P%) was 85% across all accessions. The highest P% was 100% for primer OP-B17, and the lowest was (61.54%) for primer OP-C13. The average of PIC, EMR, and MI values were 0.72, 11.80, and 9.31 respectively. Meanwhile, primer OP-K01 recorded lowest values for PIC, EMR, and MI (0.48, 6.75, and 3.22) while, primer OP-B17 recorded the highest values (0.89, 27.27, and 24.19). The Rp values ranged between 10.17 (OP-C13) to 12.56 (OP-K01) while, the average was 7.77.

Table (10): Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the five RAPD primers

RAPD	MB	UB	PB	TAB	FS (larger)	FS (smaller)	PIC	EMR	MI	P (%)	Rp
OP-B09	1.00	3.00	12.00	13.00	1312	122	0.80	11.08	8.88	92.31%	5.16
OP-B17	0.00	4.00	9.00	9.00	1495	261	0.81	9.00	7.25	100.00%	3.50
OP-C13	5.00	5.00	8.00	13.00	1456	356	0.61	4.92	3.00	61.54%	10.17
OP-C17	3.00	18.00	30.00	33.00	1623	167	0.89	27.27	24.19	90.91%	7.47
OP-K01	3.00	2.00	9.00	12.00	983	207	0.48	6.75	3.22	75.00%	12.56
Total	12.00	32.00	68.00	80.00			3.58	59.02	46.53	85.00%	38.87
Average	2.40	6.40	13.60	16.00			0.72	11.80	9.31	0.84	7.77

MB monomorphic band, UB unique band, PB polymorphic band, TAB total amplified bands, FS fragment size, PIC polymorphic information content, EMR effective multiplex ratio, P%, percent of polymorphism, Rp resolving power

In the same context, the size of amplified ISSR bands ranged from 114 to 1815 bp. A total of 36 bands were produced (Fig. 2B and Tables 11). Primers (49A and HB-11) amplified the lowest number of bands (3), and primer (HB-13) amplified the highest number of bands (15). From the detected bands, 25 and 11were polymorphic and monomorphic bands, respectively. The number of polymorphic bands ranged from 0 (HB-11) to 13 (HB-13), with a mean of 5 bands per locus. The average of (P%) was 69.4% across all the studied genotypes. The highest (P%) was (86.66%) for primer (HB-13), and the lowest was (0%) for primer (HB-11). Besides, the average of PIC, EMR and MI parameters were (0.43, 3.86, and 2.29) respectively. Moreover, the lowest values were 0 for (HB-11), while the highest values were (0.62, 11.27, and 6.99) for (HB-13) respectively. Finally, the Rp value varied from 3.72 (49A) to 11.29 (HB-13), whereas the mean value was 6.65 distinguishing the studied genotypes.

Table (11): Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the five ISSR primers

ISSR	MB	UB	PB	TAB	FS (larger)	FS (smaller)	PIC	EMR	MI	P (%)	Rp
44B	2.00	2.00	5.00	7.00	1259	305	0.63	3.57	2.25	71.42%	5.12
49A	1.00	0.00	2.00	3.00	406	248	0.37	1.33	0.49	66.66%	3.72
HB-08	3.00	0.00	5.00	8.00	682	114	0.55	3.13	1.72	62.50%	7.13
HB-11	3.00	0.00	0.00	3.00	564	303	0.00	0.00	0.00	0.00%	6.00
HB-13	2.00	6.00	13.00	15.00	1815	189	0.62	11.27	6.99	86.66%	11.29
Total	11.00	8.00	25.00	36.00			2.17	19.30	11.45	69.44%	33.26
Average	2.20	1.60	5.00	7.20			0.43	3.86	2.29	0.57	6.65

MB monomorphic band, UB unique band, PB polymorphic band, TAB total amplified bands, FS fragment size, PIC polymorphic information content, EMR effective multiplex ratio, P%, percent of polymorphism, Rp resolving power

On the other hand, SCoT primers generated 53 markers; among these, 42 were polymorphic, and 11 were monomorphic (Fig. 2C and Table 12). However, primer SCoT 3 scored the lowest number of bands (5) and primer SCoT 4 scored the highest number of bands (18). Amplified band product size varies from 107 bp to 1482 bp. Overall, the percent band polymorphism (PB) identified by these five SCoT primers have a range from 60 (SCoT 3) to 88.8 (SCoT4) with an average of 79.2%. The bands identified by the 42 polymorphic marker pairs varied from3 (SCoT 3) to 16 (SCoT 4) with an average of 8.40 bands per locus. Within the examined germplasm, Rp varied from 3.12 (SCoT 6) to 10.20 (SCoT1) with a mean of 6.76 for the 5 SCoT markers, indicating a wide variation in resolving power of the markers, indicating that the primers with high Rp can be further used for DNA fingerprinting and varietal discrimination studies. On the other hand, in the studied germplasm, SCoT 4 showed the maximum PIC, EMR and MI values of 0.79, 14.22, and 11.30, respectively, and SCoT 3 shows the least PIC, EMR and MI values of 0.45, 1.80, and 0.81, respectively.

Table (12): Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the five SCoT primers

SCoT	MB	UB	PB	TAB	FS (larger)	FS (smaller)	PIC	EMR	MI	P (%)	Rp
SCoT 1	3.00	5.00	12.00	15.00	883	107	0.66	9.60	6.33	80.00%	10.20
SCoT 2	3.00	1.00	6.00	9.00	1482	263	0.58	4.00	2.31	66.67%	7.61
SCoT 3	2.00	2.00	3.00	5.00	351	158	0.45	1.80	0.81	60.00%	5.49
SCoT 4	2.00	6.00	16.00	18.00	1049	205	0.79	14.22	11.30	88.89%	7.40
SCoT 6	1.00	1.00	5.00	6.00	830	279	0.73	4.17	3.08	83.33%	3.12
Total	11.00	15.00	42.00	53.00			3.22	33.79	23.84	79.25%	33.82
Average	2.20	3.00	8.40	10.60			0.64	6.76	4.77	0.76	6.76

MB monomorphic band, UB unique band, PB polymorphic band, TAB total amplified bands, FS fragment size, PIC polymorphic information content, EMR effective multiplex ratio, P%, percent of polymorphism, Rp resolving power

In this study, it was intriguing to observe the positive and negative markers. Tables (13 and 14) showed that 55 positive and 2 unique negative bands were amplified using RAPD, ISSR and SCoT markers in the studied genotypes. RAPD markers recorded the highest unique bands (33) followed by SCoT (15) and ISSR (9). It was clear that primer OP-C17 amplified the highest number of unique markers (18 amplicons); meanwhile, the lowest number of unique markers was detected by primers SCoT 2 and SCoT6 (1 amplicon) while, primers 49A, HB-08, and HB-11 do not generate any unique bands. Notably, across the studied genotypes it was obvious that genotype number 1 produced highest number of unique marker (11) out of which six, one and ten markers was amplified by RAPD, ISSR and SCoT markers respectively. Followed by genotypes 10 and 14 generated seven unique bands for each of them. Furthermore, genotypes number 3 and 11 were produced the lowest number of unique bands (1) for each of them. Generally, the obtained results clearly show a significant amount of polymorphism among the tested genotypes.

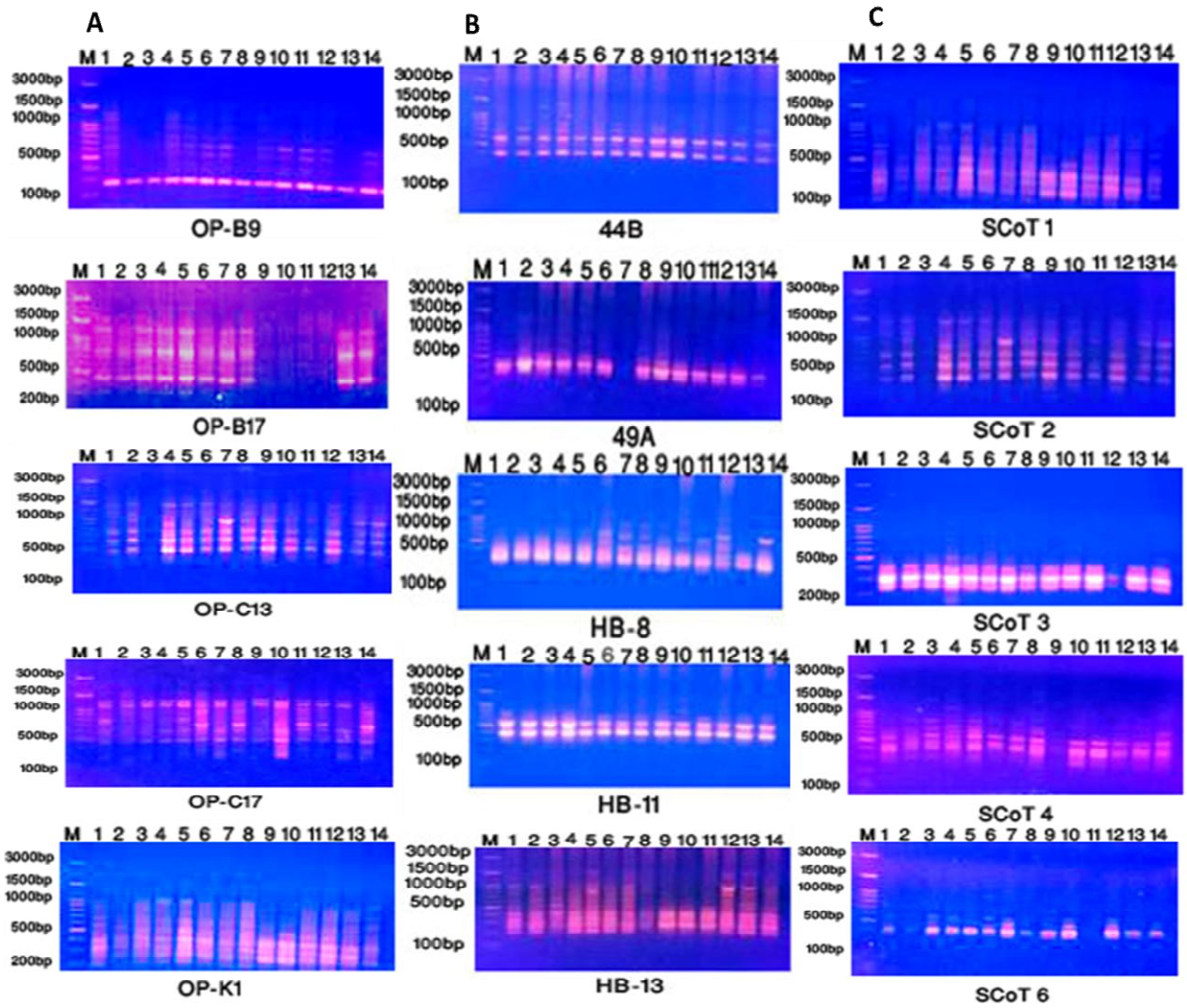

Figure (2): (A) RAPD (B) ISSR (C) SCoT patterns of the fourteen squash genotypes revealed by five primers per each

Dendrogram construction and principal component analysis

A similarity matrix based on the combined RAPD, ISSR, SCoT data was used to estimate the level of relatedness among the studied squash landraces (Table 15). The obtained matrix showed that the Jaccard's coefficient of similarity values (GS), ranging from 0.45 to 0.80, figured a moderate genetic diversity level within the studied germplasm. The smallest similarity value (0.45) suggested the high variance between SQ1 and SQ10. The maximum similarity value (0.80) was found between SQ11 and SQ12, indicating that both cultivars were the most similar. On the other hand, genetic relationships as determined by cluster analysis and principal component analysis (PCA) for RAPD, ISSR, SCoT, and pooled allelic data (RAPD+ISSR + SCoT), are shown in (Fig 3A, 3B, 3C, 3D) respectively. Cluster analysis using RAPD, ISSR, and SCoT data grouped the 14 squash genotypes into four, three, and two distinct clusters, respectively (Fig. 3A, 3B, 3C). UPGMA dendrograms obtained by the three markers were relatively similar, and most of the landraces were placed in their respective groups.

Table (13): Number of positive and negative unique RAPD, ISSR and SCoT markers recorded in squash genotypes

	Marker type	Primer	SQ1	SQ2	SQ3	SQ4	SQ5	SQ6	SQ7	SQ8	SQ9	SQ10	SQ11	SQ12	SQ13	SQ14
Positive marker	RAPD	OP-B09	122	-	-	-	-	-	-	-	321,250	-	-	-	-	-
		OP-B17	1495	-	-	-	-	-	-	-	-	558	405	-	1018	-
		OP-C13	-	-		1087	-	-	-	1117	965	1024	-	1053	-	-
		OP-C17	679,34, 167	-	-	-	849	1056	219	893	545	610,323,2 59,202	-	-	239	871,718 ,484,33
		OP-K01	976	382	-	-	-	-	-	-	-	-	-	-	-	-
	ISSR	44B	-	-	1221	1259	-	-	-	-	-	-	-	-	-	-
		49A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		HB-08	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		HB-11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		HB-13	1027	963	-	-	1815, 746	-	873	-	-	-	-	-	-	822
	SCoT	SCoT 1	107	-	-	-	122	-	703	802,660	-	-	-	-	-	-
		SCoT 2	-	-	-	-	-	-	-	-	-	-	-	650	-	-
		SCoT 3	266	-	-	351	-	-	-	-	-	-	-	-	-	-
		SCoT 4	262,205	-	-	1049	-	-	702	659	-	-	-	-	-	470
		SCoT 6	-	-	-	-	-	830	-	-	-	-	-	-	-	-
Negative marker	RAPD	OP-K01	-	-	-	-	-	-	-	-	-	501	-	-	-	-
	ISSR	HB-13	-	-	-	-	-	-	-	267	-	-	-	-	-	-
	Total		11	2	1	4	4	2	4	6	4	7	1	2	2	7

Table (14): Comparison among studied RAPD, ISSR and SCoT primers

Primer Name	Total Bands	Monomorphic Bands	Polymorphic Bands	Unique Bands	Polymorphism %
RAPD	80	12	68	33	85.00 %
ISSR	36	11	25	9	69.44 %
SCoT	53	11	42	15	79.25 %
Total	169	34	135	57	79.88 %

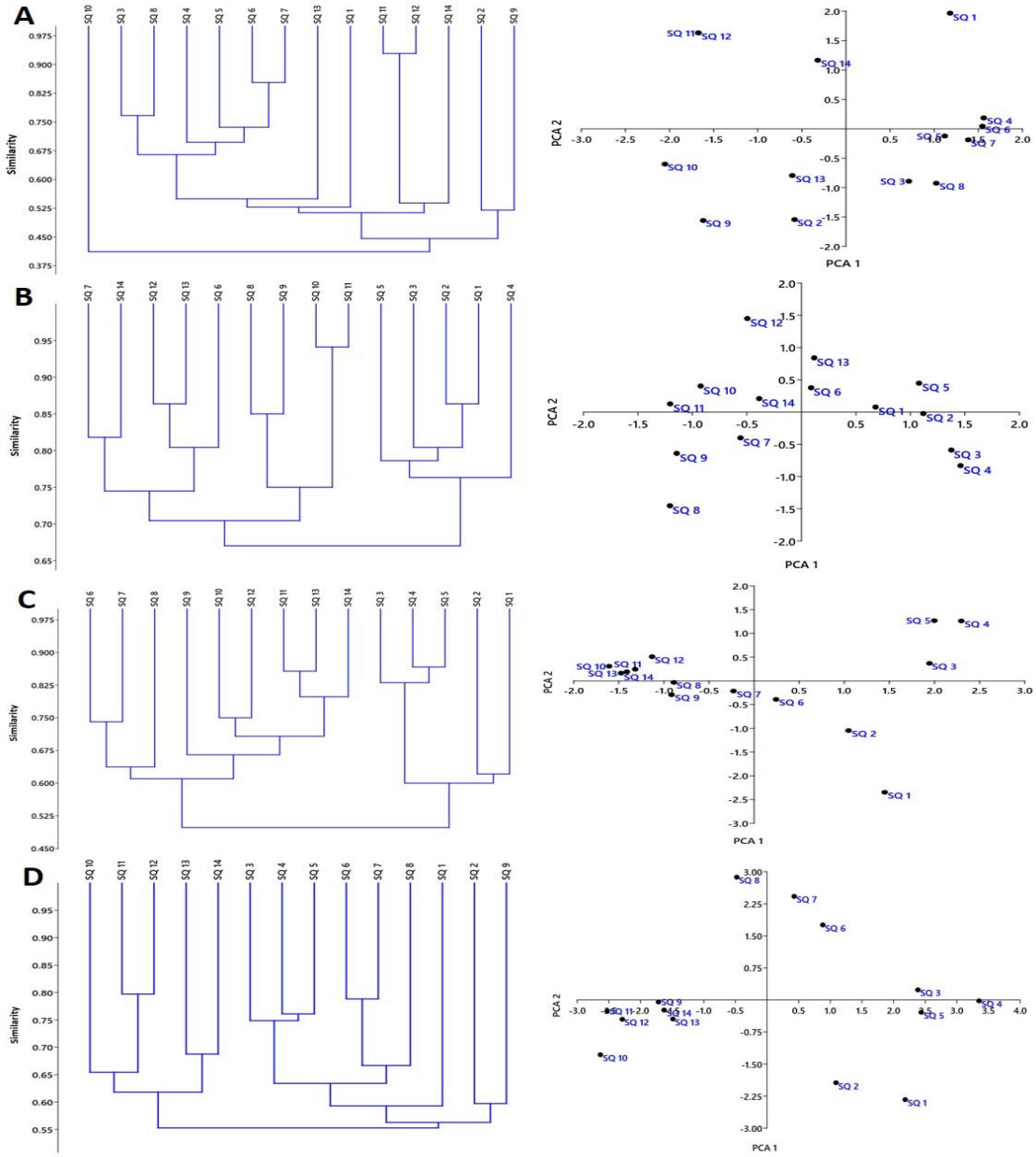

Figure (3): Dendrogram and PCA of (A) RAPD (B) ISSR (C) SCoT (D) combined data analysis of 14 squash genotypes

Cluster analysis on a combined set of RAPD, ISSR, and SCoT genotyping data classified summer squash landraces in two main distinct groups, which was further divided into four sub-clusters as depicted in (Fig. 3D). Subcluster I comprised two germplasm, subcluster II comprised seven germplasm, subcluster III includes two genotypes, and subcluster IIII involves 3 different genotypes. Notably, the PCA results further confirmed the grouping identified from the constructed UPGMA dendrogram for all the studied markers as well as the combined one. Additionally, (Fig. 4) explained cluster analysis based on the combination of yield and molecular data; the results revealed that all studied genotypes clustered into two main groups and five subgroups. In this regard, some of the clustered genotypes as revealed by pooled allelic data were similar to the clustered genotypes in (Fig. 3D) such as genotypes (SQ2 & SQ9) that represents highest values of fruit diameter and average fruit weight. Also, genotypes (SQ6 & SQ7) represent the highest fruit yield/plant and fruit shape values.

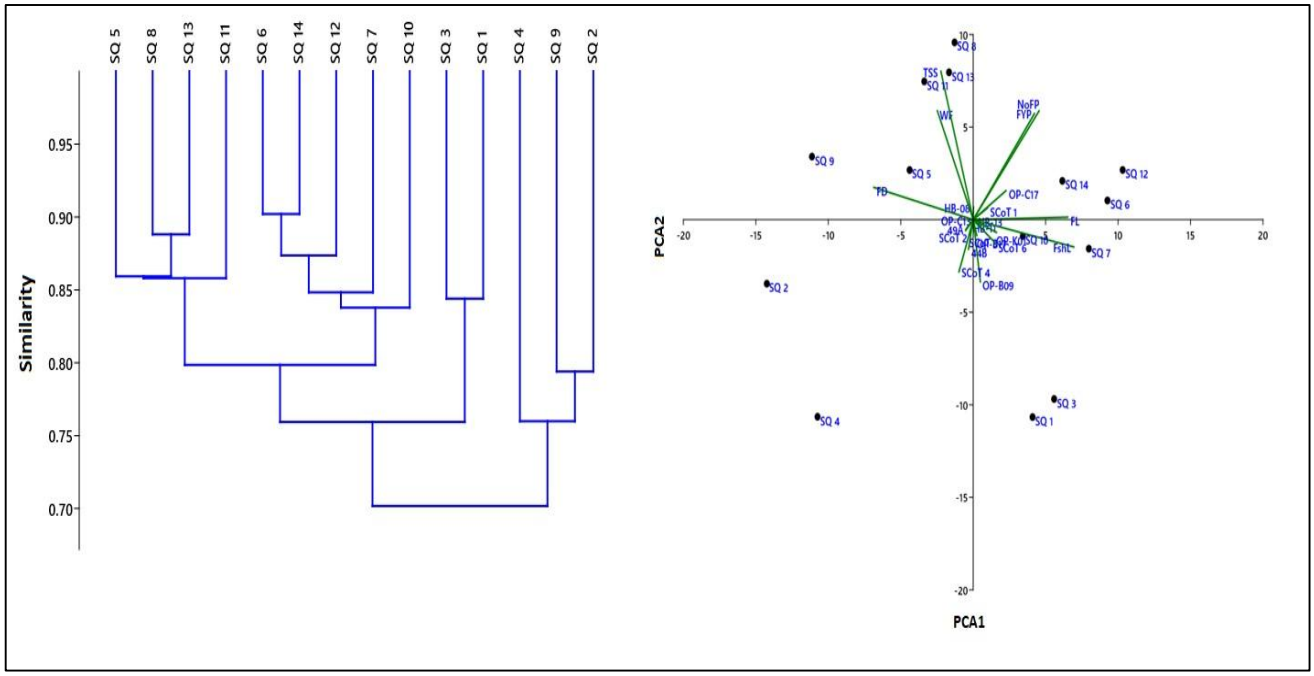

Figure (4): Cluster and PCA analysis based on combined molecular and yield data for 14 squash genotypes

Table (15): Similarity index of RAPD, ISSR, and SCoT combination analysis of 14 squash genotypes

	SQ 1	SQ 2	SQ 3	SQ 4	SQ 5	SQ 6	SQ 7	SQ 8	SQ 9	SQ 10	SQ 11	SQ 12	SQ 13	SQ 14
SQ 1	1													
SQ 2	0.59	1												
SQ 3	0.62	0.67	1											
SQ 4	0.63	0.59	0.74	1										
SQ 5	0.61	0.64	0.76	0.76	1									
SQ 6	0.62	0.59	0.69	0.66	0.69	1								
SQ 7	0.59	0.54	0.66	0.65	0.64	0.79	1							
SQ 8	0.50	0.52	0.62	0.54	0.56	0.66	0.68	1						
SQ 9	0.46	0.60	0.53	0.48	0.51	0.59	0.59	0.59	1					
SQ 10	0.45	0.50	0.47	0.46	0.51	0.53	0.56	0.48	0.62	1				
SQ 11	0.52	0.49	0.55	0.50	0.55	0.62	0.60	0.60	0.63	0.68	1			
SQ 12	0.52	0.48	0.55	0.50	0.56	0.62	0.60	0.55	0.55	0.63	0.80	1		
SQ 13	0.53	0.60	0.61	0.52	0.58	0.60	0.61	0.61	0.62	0.60	0.65	0.65	1	
SQ 14	0.54	0.53	0.53	0.50	0.56	0.62	0.62	0.57	0.55	0.55	0.64	0.62	0.69	1

DISCUSSION

Knowing the genetic divergence of a crop is vital for parental selection to maximize genetic improvement. More precise and integral characterization of the genotypes and genetic variability patterns could help determine future breeding strategies. In this study, 14 summer squash genotypes were evaluated for the genetic diversity using some yield traits along with 15 RAPD, ISSR and SCoT primers. Morphological and molecular characterization is essential for elucidating the genetic relationships among the different groups of the species of *Cucurbita* and provides complementary information with greater power of resolution for genetic diversity analyses (Barzergar *et al*., 2013).

Some genotypes constantly behaved at both locations and others fluctuated from location to another, meaning that the detected differences due to genotypes were so pronounced compared with locations, reflecting the relatively high stability of the various genotypes over locations and suggesting that the superior genotypes can be selected and recommended for growers (Valdés-Restrepo *et al.,* 2013; Rani, 2014; Abdein *et al.,* 2017). Genotype × location interactions were significant for all traits reflecting the drastic effect of varying environments among locations besides the differential response of genotypes in these environments. The location had a significant impact on the relative genotypic potential of these traits. These results are in general agreement with those reported by Abd El-Salam *et al*. (2010), Kumar and Wehner (2011), Blessing *et al*. (2012) and El-Khatib (2013) were found similar results, and reported significant differences for all studied characters among the different genotypes, These results suggest that it is necessity for evaluation squash genotypes in large diverse environments in breeding program to select the best genotype suitable for particular environment (Rani, 2014). Salama *et al.* (2019) found that the varietals differences among the studied cultivars may be due to the heredity differences. They may also be due to the differences among them in their yield attributes.

Genetic variation (σ^2g) was large in magnitude compared to error one (σ^2e) in all studied traits, reflecting the genetic differences among genotypes. On the other hand, small differences were observed between PCV% and GCV% for most traits, indicating the importance of the genetic effects in controlling the inheritance of traits under these environments and referring to highly genotypic variances resulted in high or moderate estimates of broad-sense heritabilities which, in turn, suggesting that phenotypic selection could be efficient. The high heritability for studied characters indicates there is less influence of environment. It reveals that these characters were governed by additive genes and selection for improvement under these environments would be beneficial. Results are in general agreement with those reported by Shamloul (2002); Abd El-Hadi and El-Gendy (2004); Abd El-Hadi *et al*. (2005) and El-Khatib (2013). In cucumber, Mishra *et al*. (2007) reported maximum heritability for yield per plant followed by number of fruits per plant. These finding led to the conclusion that the selection for such traits must be done under both regions in different seasons and sowing dates.

On the other hand, the three types of DNA markers employed in this study, RAPD, ISSR, and SCoT, differ in the nature of the evolutionary mechanisms underlying their variation and their distribution in the genome (Osman and Ali 2020). According to our results, the detected average of polymorphism percent was 85%, 79.25%, and 69.44 for RAPD, ISSR, and SCoT markers, respectively, indicating the very high discriminating ability of the studied techniques and genotypes. The recorded polymorphism percentage in this research was higher than Al-Tamimi (2014) (63.6), Kiani and Siahchehreh (2017) (61.6%), and Xanthopoulou *et al*. (2015) (62.82), while was lower than the polymorphism percentage reported by Panyanitikoon *et al*. (2018) (93%), Abd El-Hadi *et al.* (2017) (80.5%) and Bhawna *et al*. (2017) (87.63%) in Cucurbitaceae. Apparently, from (Table 14) RAPD marker revealed the highest polymorphism percentage and the total amplified bands as well as the unique bands, followed by SCoT and ISSR markers. The latter point indicates RAPD marker's potential for identifying and assessing genetic variations among squash genotypes and provide several choices for developing a successful breeding program to improve summer squash. Yildiz *et al*. (2011) found that polymorphism percentage of RAPD primers was higher than ISSR and SRAP marker when studied the genetic diversity among 63 melon (*Cucumis melo* L.) genotypes. In the same trend, Xanthopoulou *et al*. (2015) found that SCoT and ISSR markers were able to discriminate between 36 summer squash landraces. The level of polymorphism generated by SCoT markers was higher ISSR markers. Four parameters (PIC, EMR, MI and Rp) we recomputed to assess the efficiency of studied markers. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands and therefore the higher polymorphism provides higher EMR. Across all the studied primers, OP-B17 showed the maximum P% (100%) followed by primer OP-C17 (90.9%) which also recorded the maximum values for (PIC, EMR, MI), while primer HB-11 showed the least values for (P%, PIC, EMR, MI) parameters. The Marker index results are higher as compared with other investigations (Barzergar *et al*., 2013) SSR; 8.69, (Xanthopoulou *et al*., 2015) SCoT; 3.094 and ISSR; 1.33. Likewise, our estimated PIC values is more than those reported by Panyanitikoon *et al*. (2018) RAPD; 0.45; Kiani and Siahchehreh (2017) ISSR; 0.32 and Xanthopoulou *et al*. (2015) SCoT; 0.309. The resolving power of each primer/marker was estimated in order to determine the most informative ones for the discrimination between studied summer squash landraces.OP-K01 primer showed the maximum Rp value 12.56 while, SCoT 6 showed the least Rp value 3.12, indicating that OP-K01 primer can be further used for DNA fingerprinting and varietal discrimination studies. Our estimates are higher than Rp values presented in similar *Cucurbita* studies. For

example, (Inan *et al.,* 2012) ISSR; 6.6, (Xanthopoulou *et al*., 2015) SCoT; 10.0 and ISSR; 5.66.Very interestingly, in the present research, the higher unique bands detected in the studied squash genotypes that reflect higher genetic distinctness and could be used in the genotype discrimination. The presence of unique ISSR markers may be regarded as markers for the authentication of genetic resources (Badr *et al*., 2014). UPGMA dendrogram is accessible in (Fig 3), which can be used to recognize the genetic relationship between the germplasm. Kiani and Siahchehreh (2017) reported that the similarity values ranging from 0.14 to 0.70 using ISSR markers in *C. pepo*. landraces which indicates less diversity comparing with our results, while similarity index that represented by Yildiz *et al.* (2011) was ranging between 0.46 and 0.96 using ISSR, SRAP, and RAPD which was higher than our recorded similarity index. Furthermore, in this study the dendrograms and genetic distance matrices produced from the RAPD, ISSR and SCoT data were almost similar, this result is in line with that of (Xanthopoulou *et al.* 2015) and (Panyanitikoon *et al.* 2018). It is worthy to mention that the dendrogram showed clustering of germplasm was not according to geographical concordance, this is probably due to cross pollinated nature of summer squash. This result was congruence in line with Bhawna *et al*. (2017) in bottle gourd. Contrary, Panyanitikoon *et al.* (2018) showed that the 38 cucumber accessions were divided into two main clusters and this grouping was in good agreement with country of origin. The results obtained by principal coordinate analysis were consistent with those of cluster analysis. However, cluster analysis could supply more abundant information's than principal coordinate analysis on revealing the relationship among the closely related genotypes. It was concluded from the findings of (Fig. 4) that genotypes that had highest values of some yield traits located together in the same clusters, these result showed that molecular analysis were correlated with the yield characteristics. In addition, the obtained PCA further supported the dendrogram results in a robust way, similar results were found by Kiani and Siahchehreh (2017). Overall, marker analysis showed a number of differences in the size and number of bands among the species, which means that there are genetical differences among the studied cucurbit lines. Besides, the data deduced that the RAPD primers (OP-B17 and OP-C17) were the most efficient for discriminating the studied genotypes genetically.

CONCLUSION

The current study presented high variations for yield and molecular profile in terms of different genotypes. The 14 *Cucurbita pepo* genotypes evaluated in this study showed highly significant variability in both locations for all studied yield traits. In the same context, the studied markers generated 57 unique bands, out of which (33) bands were developed by RAPD followed by SCoT (15) and ISSR (9). Furthermore, OP-C17 and ESKANDARANI genotype produced the highest number of unique markers. The high genetic diversity found could be used in breeding programs to obtain new cultivars and provide relevant information for diversity conservation. Moreover, this research revealed that characterization based on both yield and molecular markers is essential for elucidating the genetic relationships of ecotypes within this *Cucurbita* species.

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تقييم التنوع الوراثى لبعض التراكيب الوراثية للكوسة باستخدام تحليل بعض صفات المحصول والواسمات الجزيئية تحت ظروف سيناء

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لدراسة تقييم التنوع الوراثي لبعض التراكيب الوراثية في الكوسة تحت ظروف سيناء - مصر، تم إجراء تجربتين حقليتين لدراسة بعض صفات المحصول في منطقتين هما: العريش (L_1) رأس سدر (L_2). الموقع الأول (العريش) في المزرعة البحثية لكلية العلوم الزراعية البيئية، جامعة العريش، شمال سيناء، مصر. والموقع الثاني (رأس سدر) في منطقة رأس سدر، جنوب سيناء، مصر. وذلك لدراسة أثر تفاعل (التركيب الوراثي × الموقع) على أداء ١٤ تركيباً وراثيًا مختلفًا من الكوسة. لوحظت فروق إحصائية بين الموقعين لجميع صفات المحصول المدروسة، كما بينت مقارنة النتائج التي تم الحصول عليها من كل موقع إلى تفوق موقع العريش علي موقع رأس سدر في جميع الصفات المدروسة في معظم التراكيب الوراثية. من الجدير بالملاحظة أن بعض التراكيب الوراثية كانت ذات سلوك ثابت باستمرار في كلا الموقعين، حيث أن معظم التراكيب الوراثية في كل من شكل الثمرة وعدد الثمار/النبات وكذلك جميع التراكيب الوراثية في متوسط وزن الثمرة كان التفاعل بين التركيب الوراثي والموقع كبيرًا بالنسبة لجميع الصفات مما كشف عن أن بعض التراكيب الوراثية كانت متفوقة في كلا الموقعين. علاوة على ذلك، كان للموقع تأثيراً كبيراً على السلوكيات الوراثية النسبية لهذه الصفات، حيث كان تفاعل التركيب الوراثي × الموقع مهمًا للغاية. كان أفضل التراكيب الوراثية الذي احتل المرتبة الأولى في الصفات المدروسة هو التركيب الوراثي SQ6 (KABAK DOLMALIK KOLSUZ) لمحصول الثمار/نبات، SQ2 (WHITE BUSH SCALLOP) لقطر الثمرة في كلا الموقعين، كذلك SQ7 (ERBIL GARDEN) و SQ2 (WHITE BUSH SCALLOP) لشكل الثمرة وكذلك SQ13 (ALEXANDIA) و SQ12 (SAKIZ KABGI) لعدد الثمار/النبات في المنطقة الأولى والثانية، على التوالي.على الجانب الآخر تم استخدام خمسة عشرة بادئا من الواسمات الجزيئية (RAPD) و (ISSR) و (SCoT) بغرض تقييم الاختلافات الوراثية الجزيئية بين تراكيب الكوسة الوراثية المدروسة. أظهرت النتائج أن عدد الحزم الكلية الناتجة (١٦٩) وكان منها (٨٠) حزمة ناتجة من تقنية (RAPD)، منها (٥٨٪) أليلات متباينة، وأظهرت تقنية (ISSR) (٣٦) حزمة منها (٦٩.٤٤٪) آليلات متباينة بينما أظهرت تقنية (SCoT) (٥٣) حزمة، منها (٧٩.٢٥٪) آليلات متباينة. وقد أظهرت تلك النتائج القدرة العالية لتقنية (RAPD) في الكشف عن التباين الوراثي بين التراكيب الوراثية المدروسة، وعلى جانب آخر أظهرت النتائج (٥٧) حزمة مميزة للتراكيب الوراثية المدروسة منها (٣٣) حزمة ناتجة من تقنية (RAPD)، و(١٥) حزمة ناتجة من تقنية (SCoT)، و (٩) حزمة ناتجة من تقنية (ISSR). وقد أظهر البادئ OP-C17 والصنف ESKANDARANI أعلى عدد من الحزم المميزة. وبشكل عام أظهرت نتائج دراسة صفات المحصول جنبًا إلى جنب مع النتائج الجزيئية معلومات مهمة يمكن استخدامها لفهم العلاقة بين تفاعل التراكيب الوراثية المختلفة مع اختلاف الموقع المدروس مع التوصية بزراعة أفضل التراكيب الوراثية في الموقع المناسب.