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Molecular and Phenotypic Evaluation of some Summer Squash Inbred Lines

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ABSTRACT

In order to evaluate molecular and phenotypic diversity and detecting molecular markers for six summer squash inbred lines belong to species [Cucurbita pepo L.], five RAPD and five ISSR primers were used as well as 12 economical traits were estimated. These primers succeeded in generating reproducible and reliable amplicons. RAPD revealed 88.1 % of polymorphism while ISSR techniques showed 80.5% polymorphism. The resolving power (Rp) value for RAPD technique was 5.00 which was higher than 3.40 of ISSR technique. Therefore, the RAPD technique was better than ISSR technique in evaluated molecular diversity and discrimination capacity among lines. But, the ISSR technique was better than RAPD technique in showing unique markers (21 for ISSRs and 9 for RAPDs). Also, the correlation between phenotypic distance (PD) and molecular distance (MD) based on ISSRs was 0.173 highest than with MD based on RAPDs (0.045). On the other hand, with the exception of P6 which gave significant desirable value in two traits (number of fruits and yield per plant), each of the other five strains gave a significant desirable value in one trait, thus the number of these traits which distinguished in the six inbred lines were 7 traits. These traits could be linked with all unique markers detected in this study. The inbred line P5 showed the highest number of unique markers (10, 9 of them were positive), one or some of which may be linked with NL trait that showed in this inbred line a significant desirable value. Followed by the inbred line P6 which showed seven unique markers (six of them were positive) one or some of which may be linked with NF and/or Y/P traits. This indicated that some of these markers may be used as markers assisting selection in the breeding and improvement of squash.

Keywords: RAPD, ISSR, Summer squash, Genetic diversity, Phenotypic distance, Molecular distance, correlation, Cluster analysis.

INTRODUCTION

Summer squash (*Curcurbita pepo* L.) is an important source of human food and the fruits are good sources of several nutrients and plants have medical uses (Burrows and Ronald, 2013). It has the constant and relatively high chromosome number (2x=40) (Al-Ballat, 2008). In Egypt, cultivated area was 71009.57 fed which harvested average yield (7.5156 *tons |fed)* with total production 543334 tones (FAO, 2013). Recently, plant breeders used modern methods such as molecular marker which one of the essential steps in every plant breeding program to asses genetic diversity, which achieve the greatest success in this field. Among the different types of molecular markers available, two of such useful markers are random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR), which are depended on polymerase chain reaction (PCR). RAPD is a type of PCR reaction using random segments of DNA which are amplified. The RAPD technique needs short primers (8-12 nucleotides), then performing with the PCR using a large template of genomic DNA to get amplified fragments. The RAPD marker is simple, fast, easy to perform, comparatively and cheaper than other molecular markers and require no prior knowledge of DNA sequences. Therefore, RAPD are useful technique to assess the genetic diversity. Using RAPD technique to study the genetic diversity within and between species of *C. pepo*, *C. moschata*, and *C. maxima*. All researchers showed that RAPD technique is the effective for determining the relatedness of different *Cucurbita* accessions (Brown, 2001). ISSR is technique based on PCR method. This technique uses microsatellites usually 16-25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the ISSR sequences of different sizes and involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction of chromosome. ISSR-PCR is a technique that has important role to overcome most of these limitation by the research community in various fields of plant improvement. Also, it is useful in areas of genetic diversity, phylogenetic studies gene tagging, genome mapping and evolutionary biology in a wide range of crops. (Reddy *et al*., 2002).

Aim of this study was to evaluate genetic diversity using two molecular marker techniques (RAPD and ISSR), phenotypic distances and the correlation relationships between molecular distances and phenotypic distances for six inbred lines of summer squash. Thus, it would be possible to determine the number of molecular markers that can be linked with distinguished traits in each studied inbred lines.

MATERIALS AND METHODS

Plant materials

Six summer squash inbred lines belong to species [*Cucurbita pepo* L.] were used in the present investigation and are shown in Table 1. The experiment was carried out in Sakha Horticultural Research Station, Kafr El-Sheikh Governorate, during summer season of 2013 to obtain 15 crosses from 6×6 half diallel mating system, these genotypes (parents and its hybrids) were evaluated in summer season of 2014.

Molecular evaluation

a. DNA isolation methods

Squash seeds were collected separately from lines under this study. The total DNA was isolated using DNeasy Mini Kit (QIAGEN). These DNA isolated were used form all studied inbred lines as a template for PCR amplification were performed in Techni TC-512 PCR System using 20 RAPD and 14 ISSR primers (Operon Technology, USA). These primers were used in detecting polymorphism among studied lines. Amplification reactions were performed in 30-µl volume tubes according Williams *et al*. (1990). The reaction in RAPD technique was programmed for one cycle at 94 ºC for 4min followed by 40 cycles of 1 min at 94 ºC, 1 min at 37 ºC, and 2 min at 72 ºC, followed by one cycle of 10 min at 72 oC. Also, the amplification reactions in ISSR technique were performed in 25 µl reaction volume according to Wolfe *et al*. (1998). The reaction in ISSR technique was programmed for one cycle at 94 ºC for 4 min followed by 40 cycles of 1 min at 94 ºC, 1 min at 57 ºC, and 2 min at 72 ºC, followed by one cycle of 10 min at 72 oC. 15

µl from each DNA amplified products, were loaded and separated on a 1.5 % agarose gel with 1.5 kb ladder markers (mix was used as standard DNA with molecular weights of 1.5, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 kb). The run was performed for about 30 min at 80 V in mini submarine gel BioRad.

Table 1. Inbred lines characteristics and source

Inbred line	Stem length	Fruit length	Fruit color	Source
Lungoditoscan (P_1)	Long	Long	Light green	I. E. Metwally[1]
S26 (P_2)	Long	Short	Light green	Manal A. Abd Alla[2]
S24 (P_3)	Short	Medium	Light green	Manal A. Abd Alla[2]
CGN11916 (P_4)	Medium	Medium	Light green	I. E. Metwally[1]
PI|512788 (P_5)	Long	Short	Dark green	I. E. Metwally[1]
Eskandrani (P_6)	Medium	Medium	Light green	Open market

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[2]: Sakha Hort. Station, Hort. Res. Ins., Agric. Res. Center, Giza, Egypt.

b. Molecular data analysis

Molecular data obtained from RAPD and ISSR PCR products banding patterns were analyzed by GelAnalyzer3 software. The efficiency of each primer to differentiate among inbred lines was evaluated by value known as resolving power (Rp) ,this value was calculated according to Saini *et al.* (2010). Based on binary data matrix, the molecular distances MD were performed using Nei and Li coefficients (Nei and Li, 1979) by computational package MVSP3.1. As well as, Cluster analysis was performed using the same program depended on this matrix.

Phenotypic evaluation

a. Phenotypic data recorded

The data were recorded on several randomly chosen plants with an each plot of the three replicates for the following traits. These traits were stem length (SL), number of branches per plant (NB), number of leaves per plant (NL), leaf area/leaf (LA),sex ratio (Sr), number of days to first female flower opening (DOF), fruit weight (FW),number of fruits (NF), yield per plant (Y/P),fruit length (FL), fruit diameter (FD) and shape index (SI).

b. Phenotypic distance

Based on data of mean performances of these traits between six inbred lines, phenotypic distances (PD) were carried out using computational software MVSP 3.1 by equation of normalized Euclidean morphological distance according to Roldan Ruiz *et al*., (2001). Cluster analysis by Phenotypic Distances PD were carried out based on traits data using computational software MVSP 3.1 according to Nei (1987).

Correlation relationships between MD and PD

The relationships between molecular distances (MD) and phenotypic distances (PD) were explained based on simple correlations using the computational software Minitab (El-Zanaty *et al*., 2013).

RESULTS AND DISCUSSION

Molecular evaluation

Five RAPD and five ISSR primers were succeeded for evaluating six inbred lines of *C. pepo*. Banding patterns and DNA Profiling of these primers were shown in Figure 1, 2 and 3.

Figure 3 showed that RAPD and ISSR primer generated 30 (11 negative and 19 positive) out of 83 amplicons (36.1 %) were found to be useful as unique markers. Moreover, all studied inbred lines were determined by unique markers based on RAPD and ISSR techniques. This indicates the possibility of using results for these techniques in signing genetic diversity and useful tool for molecular identification for studied inbred lines.

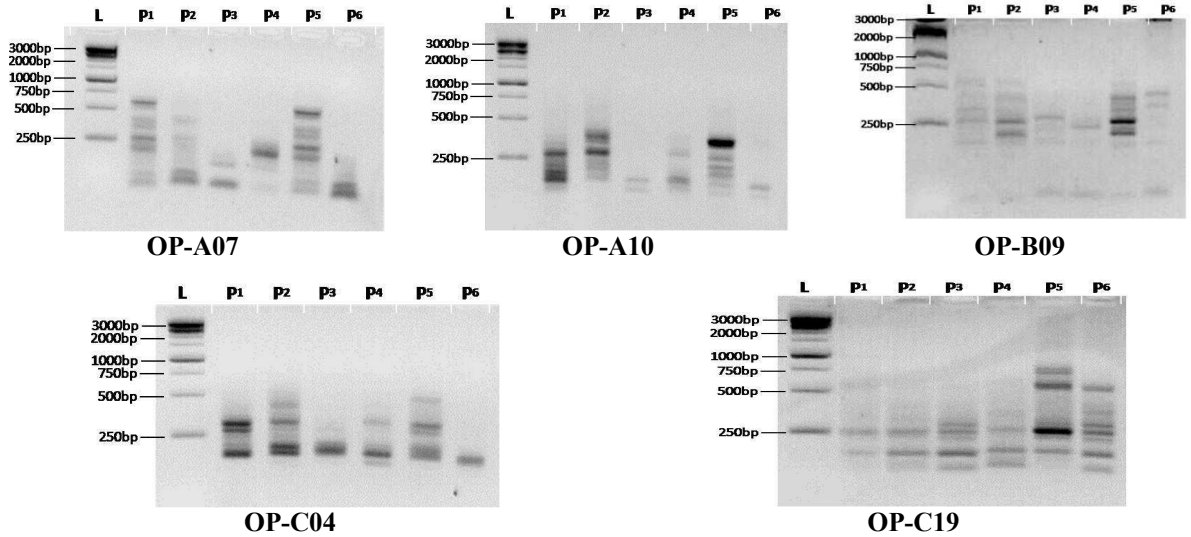

Figure 1. RAPD banding patterns obtained using five primers; L, 1.5 kb ladder and lanes 2 to 7 for six inbred lines of squash

These results were in agreement with Abd EL-Aziz and Habiba (2016 a) in canola and Abd EL-Aziz *et al.,* (2016 c) in tomato.

Molecular data from banding patterns of RAPD and ISSR techniques were recorded in Tables 2 and 3. These Tables revealed that in total of 83 amplicons, 70 of them were polymorphic. The ISSR primer HP-12 and RAPD

primer OP-B09 showed the highest number of amplicons (11). On the other hand, the ISSR primer 44B showed the lowest number of amplicons (2). Also, molecular size (bp) of these amplicons for RAPD and ISSR techniques were ranging from 42 to 848 bp and from 131 to 1447 bp, respectively. The percentage of polymorphism for RAPD and ISSRs techniques were ranging from 80.0 to 100.0 % and from 50.0 to 88.9 %, respectively.

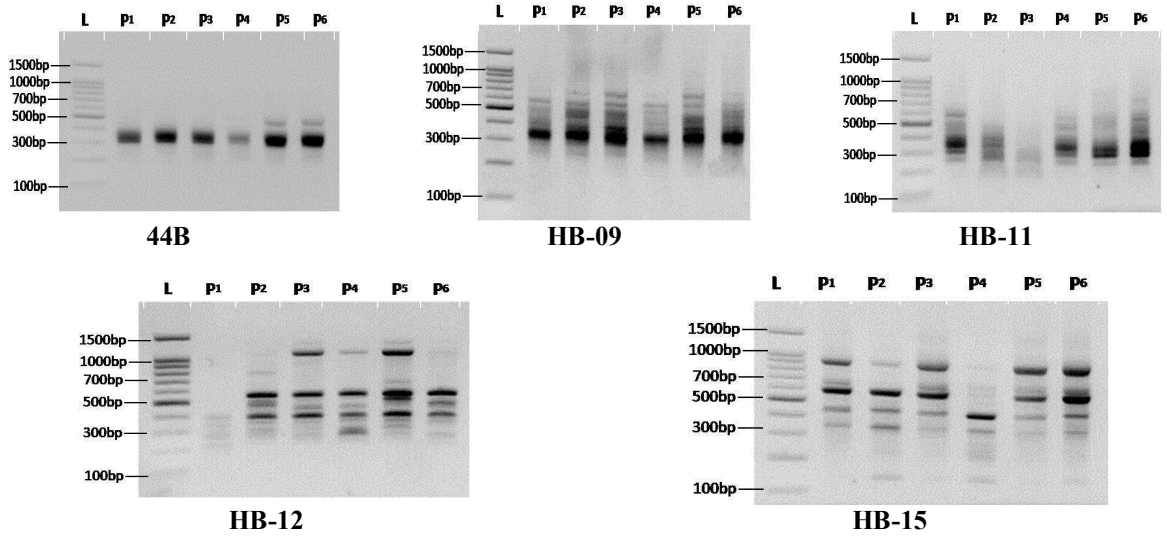

Figure 2. ISSR banding patterns obtained using five primers; L, 1.5 kb ladder and lanes 2 to 7 for six inbred lines of squash.

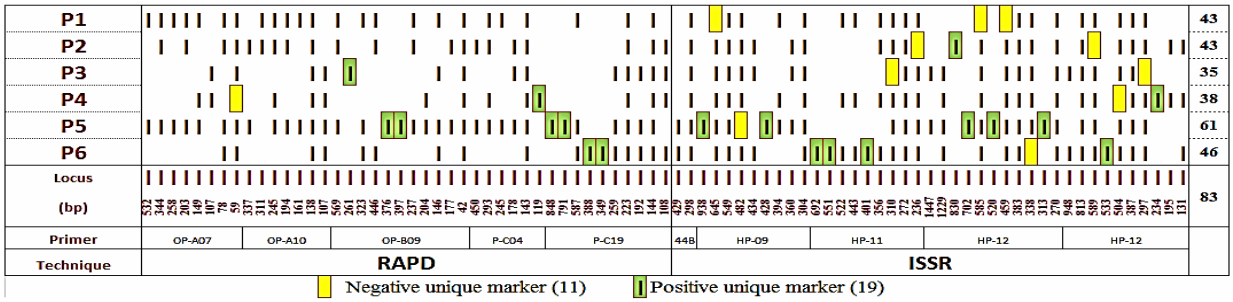

Figure 3. DNA profiling for the six parental lines of summer squash based on RAPD and ISSR according to Adhikari *et al.* (2015).

Table 2. Molecular data estimated from banding patterns of RAPD technique.

Primer			Amplicons						
Name	Sequence (5´→ 3`)	Molecular size range	Monomorphic	Polymorphic			Total	Polymorphism %	Resolving power Rp
				Polymorphic without unique	Unique +	Unique -			
OP-A07	GAAACGGGTG	59:532	-	7	-	1	8	100.0	6.00
OP-A10	GTGATCGCAG	107:337	1	6	-	-	7	85.7	4.33
OP-B09	TGGGGGACTC	942:569	1	7	3	-	11	90.9	7.00
OP-C04	CCGCATCTAC	119:450	1	4	1	-	6	83.3	3.33
OP-C19	GTTGCCAGCC	108:848	2	4	4	-	10	80.0	4.34
Total			5	28	8	1	42		
Mean								88.1	5.00

Table 3. Molecular data estimated from banding patterns of ISSR technique.

Primer			Amplicons						
Name	Sequence (5´→ 3`)	Molecular size range	Monomorphic	Polymorphic			Total	Polymorphism %	Resolving power Rp
				Polymorphic without unique	Unique +	Unique -			
44B	CT_8GC	298:429	1	1	-	-	2	50.0	0.67
HB-09	GT_6GC	304:938	2	3	2	2	9	77.8	4.34
HB-11	GT_6CC	236:692	1	3	3	2	9	88.9	4.34
HB-12	CAC_3GC	270:1447	2	2	4	3	11	81.8	3.67
HB-15	GTG_3GC	131:948	2	3	2	3	8010	80.0	4.00
Total			8	12	11	10	41		
Mean								80.5	3.40

Moreover, the resolving power values for RAPD and ISSRs techniques were ranging between 3.33 to 7.00 and 0.67 to 4.34, respectively. As well as, RAPD and ISSR primers generated unique markers except OP-A10 and 44B primers, respectively. The highest number of unique marker was generated by ISSR primer HB-12 (seven) .On the other

hand, the lowest number of unique marker was generated by RAPD primer OP-A07 and OP-C04 (one).

Comparison of RAPD and ISSR techniques

Data in Table 4 revealed comparison between RAPD and ISSR techniques used in this study which exhibited that the RAPD technique generated 42 of amplicons and ISSR technique generated (41). In RAPD analysis 37 out of 42 amplicons was polymorphic (88.1%) nine of them were unique (eight positive and one negative). While, in ISSR analysis 33 out of 41 amplicons were polymorphic (80.5%) 21 out of them were unique (11positive and 10 negative). The average numbers of polymorphic amplicons generated by these primers were 7.4 (88.1 % of polymorphism) and 6.6 (80.5 % polymorphism) for RAPDs and ISSRs, respectively.

Table 4. Comparison of genetic diversity assessment by RAPD and ISSR analysis.

Molecular marker technique	Unique amplicons			Total number of Polymorphic amplicon	Total number of amplicon	Average number of polymorphic amplicon	Average of Polymorphism (%)	Unique marker %	Average resolving power (Rp)
	Unique (+)	Unique (-)	Total						
RAPD	8	1	9	37	42	7.4	88.1	21.4	5.0
ISSR	11	10	21	33	41	6.6	70.5	51.2	3.4
Combined	19	11	30	70	83	7.0	84.3	36.1	4.2

These results were in agreement with Muthusamy *et al.* (2008) in rice bean, Gajera *et al.* (2011) in *Mangifera indica*, Giancarla *et al.* (2012) in barely, Guasmi *et al.* (2012) in South Tunisian Barley, Sadigova *et al.* (2014) in wheat and Bhagyawant *et al.* (2015) in Chickpea. On the other hand, these results disagreement with Fernández *et al.* (2002) in barely and Izzatullayeva *et al.* (2014) in sugar beet.

However, the average values of resolving power (Rp) for RAPD and ISSR were 5.00 and 3.40, respectively. So the RAPD technique was better than ISSR technique in discrimination capacity and efficiency for studied lines and assessment for genetic diversity among them. But The ISSR technique was better than RAPD technique in showing unique markers (51.2% for ISSRs and 21.4% for RAPDs)

These results were in accordance with Guasmi *et al.* (2012); Gajera (2014) in cowpea and Abd El-Aziz *et al.* (2016c) in tomato. In the contrary, these results disagree with Fernández *et al.* (2002) in barely; Tonk *et al.* (2014) in triticale and Abd El-Aziz and Habiba (2016 a) in canola.

Molecular distance among inbred lines

Data in Table 5 revealed that Molecular distance (MD) matrix for RAPD, ISSRs, and combined data. These results indicated that the highest MD for RAPD data was between lines P_1 and P_3 (0.632) but the lowest MD for the same data was between lines P_1 and P_5 (0.241). For ISSR data, the highest MD was between lines P_5 and P_6 (0.357) but the lowest MD for the same data was between lines P_1 and P_4 (0.163). Also, the highest MD for combined data was between lines P_1 and P_3 (0.487) while the lowest MD for the combined data was between lines P_3 and P_6 (0.284). These results showed that the P_1 and P_3 were the highest inbred lines in genetic diversity, indicating hybridization of obtaining the highest hybrid vigour from hybridization between them. These results matches with Giancarla *et al.* (2012) in barely.

Cluster analysis among inbred lines

Figure 4 showed UPGMA clustering dendrogram for six squash inbred lines based on MD values from combined data. The combined data are based on the fact that they improved the efficiency of RAPD and ISSR techniques because they help to provide more accurate information about genetic diversity (Abd El-Aziz *et al.*, 2016 c and Abd El-Hady *et al.*, 2010).

This dendrodram exhibited that these lines may be divided into two groups (A and B), each group consists of two subgroups. The group A involved three lines P_3, P_4 and P_6, respectively. While, the group B included P_1, P_2 and P_5. The MD between and B was 0.4, as well as the MD values between the two subgroups for the group A was 0.338, while the MD values between the two subgroups for group B was 0.324. This indicates that the cluster analysis based on combined data of MD for RAPD and ISSRs techniques succeeded in description of genetic diversity and heterogeneity within studied lines.

Table 5. Molecular distance (MD) matrix for six studied inbred lines of squash based on RAPDs, ISSRs, and combined data.

Inbred lines	P_1	P_2	P_3	P_4	P_5	Techniques
P_2	0.289					RAPD
	0.300					ISSR
	0.302					Combined
P_3	0.632	0.543				RAPD
	0.350	0.238				ISSR
	0.487	0.385				Combined
P_4	0.568	0.529	0.407			RAPD
	0.163	0.244	0.289			ISSR
	0.358	0.358	0.342			Combined
P_5	0.241	0.345	0.542	0.574		RAPD
	0.348	0.333	0.250	0.294		ISSR
	0.288	0.346	0.396	0.434		Combined
P_6	0.463	0.526	0.290	0.533	0.490	RAPD
	0.292	0.280	0.280	0.208	0.357	ISSR
	0.371	0.393	0.284	0.333	0.321	Combined

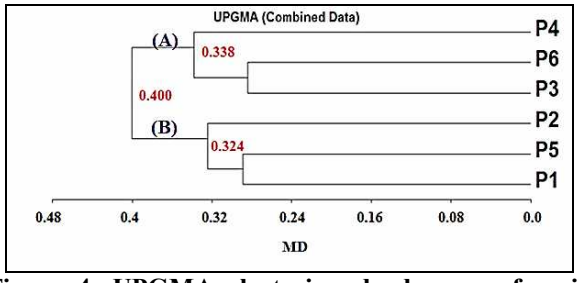

Figure 4. UPGMA clustering dendrogram for six squash inbred lines based on MD values from combined data of RAPD and ISSR.

The results also, indicates the presence of clear variance between all studied lines, this reflects the genetic diversity within these lines. This indicated that the possibility of obtaining hybrid vigours from hybridization between any inbred line from group A and any inbred line

from group B. These results were in agreement with, Giancarla *et al.* (2012) in barely.

Phenotypic evaluation

The results in Table 6 showed the lowest and highest mean performance values for studied phenotypic traits of six squash inbred lines. These phenotypic traits were very important in plant breeding programs and in estimation of phenotypic distance to assess the genetic diversity (Abd El-Aziz *et al.,* 2016c) in tomato. Also this Table shows the desirable values for all studied traits, which evaluated according to consumer needs in Egypt and in agreement with Abd El-Hadi *et al*., (2005); Al-Ballat (2008); AL-Araby (2010); Abd El-Raziq (2013) ; El-Khatib (2013); Abd El-Hadi *et al*., (2014 a) and Abd El-Hadi *et al*., (2014 b). From these data, it is indicated that each of the six inbred lines gave a significant desired value in one of the studied traits, except for P_6 which gave desirable values in two traits and they were NF and Y/P.

Table 6. Mean performance range and desirable values for all studied traits in six inbred lines.

Traits	Mean performance range of six inbred lines				Desirable value
	Low		High		
	Value	Inbred line	Value	Inbred line	
SL	18.3*	P_3	26.5*	P_1	Low
NB	1.10	P_6	1.80 **	P_1	High
NL	13.1**	P_1	21.7**	P_5	High
LA	175.5	P_1	244.2	P_6	High
Sr	0.42**	P_1	2.21*	P_2	High
DOF	35.5	P_3	41.0	P_1	Low
FW	70.9	P_3	86.6*	P_4	High
NF	4.75**	P_1	22.4**	P_6	High
Y/P	352.0**	P_1	1736.3**	P_6	High
FL	10.5	P_5	15.5**	P_1	Low
FD	2.47	P_5	2.95	P_3	Low
SI	3.99	P_2	6.08	P_1	High

*,**Significant difference at 0.05 and 0.01 with the closest value

Phenotypic distances (PD) among six Squash inbred lines

Phenotypic distance among six inbred lines based on mean performance for 12 traits were calculated. The results of phenotypic distances (PD) in Table 7 exhibited that the phenotypic distances ranged from 2.40 to 6.90 with the mean of 4.75. The highest PD values were between the lines P_1 and P_6, on the other hand the lowest PD values were between the lines P_3 and P_6. Also, Figure 5 showed cluster analysis based on PD this dendrogram revealed that the studied inbred lines could be divided into two main groups (A and B) with PD was 6.05.The second group (B) consists of P_1only, while the first group (A) included two subgroups (d and c) with PD was 4.58, the first subgroup (c) involved one line P_4. As well as, the other subgroup (d) included two sub-sub groups. The first sub-subgroup included P_3 and P_6 and the second one included the two lines P_2 and P_5. This indicated the possibility of obtaining hybrid vigours from hybridization between any inbred line from group A and any inbred line from group B.

Table 7. Phenotypic distance (PD) matrix for six studied inbred lines of squash based on mean performance data.

Inbred lines	P_1	P_2	P_3	P_4	P_5
P_2	5.75				
P_3	6.23	3.95			
P_4	4.83	4.52	5.00		
P_5	6.52	3.06	5.05	4.13	
P_6	6.90	4.04	2.40	4.67	4.23

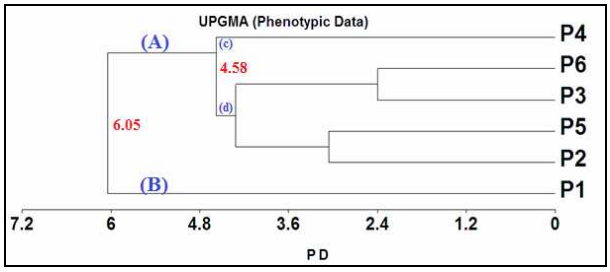

Figure 5. UPGMA clustering dendrogram for six squash inbred lines based on PD values from phenotypic data.

Correlation between MD and PD

Table 8 presented the correlation relationships among three types of MD and PD. These relationships indicated that insignificant positive correlations among all types of MD (based on RAPD, ISSRs and their combined data) and PD were detected with values 0.045, 0.173 and 0.121, respectively. These results were in agreement with Abd El-Aziz *et al*. (2016 b) in maize and Abd El-Aziz *et al.* (2016 c) in tomato. Whereas MD based on ISSRs was most positive in correlation value with PD. This result indicate that possibility of demonstrating the ISSR technique of unique molecular markers that can be linked to the distinguished traits in these studied inbred lines better than RAPD technique. So, plant breeders are recommended to study genetic diversity for lines which are used as parents in breeding improvement programs of squash requires to evaluate these lines at more than location and under different climatic conditions, used more than molecular markers techniques and used specific molecular markers (Abd El-Aziz *et al.*, 2016 c).

Table 8. Correlation relationship among the types of genetic distances (MD and PD)

Genetic distance	MDRAPD	MDISSR	MDcomb
MDISSR	-0.382		
MDcomb	0.824**	0.182	
PD	0.045	0.173	0.121

Association between unique molecular markers and distinguished traits in studied inbred lines.

Based on positive correlation values between MD and PD (Table 8), and also the inbred lines that gave desirable values in some of studied traits (Table 6), data presented in Table 9 clear that NB, SR, SL, FW, NL, Y/P and NF traits which showed significant desirable values could be linked with all unique markers detected in this study. These unique markers were 11 negative and 19 positive, 21 out of them were generated based on ISSR technique. It is evident that ISSR technique was better than RAPD technique in showing unique markers may be associated with desirable performance in these traits. The results showed that ISSR technique succeeded in showing unique markers for all inbred lines, the RAPD technique succeeded in showing unique markers for most inbred lines except P_1 and P_2. On the other hand, the inbred line P_5 showed the highest number of unique markers (10, 9 of them were positive), one or some of which may be linked with NL trait that showed in this inbred line a significant desirable value. Followed by the inbred line P_6 which showed seven unique markers (six of them were positive) one or some of which may be linked with NF and/or Y/P traits. These results indicated that some of these markers may be using as

markers assisting selection in the breeding and improvement of squash inbred lines . (Giancarla *et al*., 2012 in barely and Abd El-Aziz *et al*., 2017 in okra).

CONCLUSION

In this study, RAPD and ISSR primers were succeeded in generating reproducible and reliable amplicons. RAPD technique was better than ISSR technique in evaluating molecular diversity and discrimination capacity among lines. But, the ISSR technique was better than RAPD technique in showing unique markers may be associated with desirable performance in some of studied traits. Based on detecting positive correlation values between molecular and phenotypic distance as well as detecting of inbred lines which gave significant desirable values in some of studied traits, some of these markers may be used as selected markers in breeding programs for genetic improvement of these traits in squash.

Table 9. The relationship between molecular markers and desirable improvement of some economical studied traits.

Inbred lines	Unique markers				Distinguished traits	
	Primer	Molecular size	Type	Total	Trait	Mean performance
P_1	HP-09	645	-	3	NB	1.80**
	HP-12	585,459	-			
P_2	HP-12	830	+	3	SR	2.21*
	HP-11	236	-			
	HP-15	589	-			
P_3	OP-B09	261	+	3	SL	18.27*
	HP-11	310	-			
	HP-15	297	-			
P_4	OP-C04	119	+	4	FW	86.65*
	HP-15	234	+			
	OP-A07	59	-			
	HP-15	504	-			
P_5	OP-B09	370,397	+	10	NL	21.73**
	OP-C19	848,791	+			
	HP-09	938,428	+			
	HP-09	482	-			
	HP-12	702,520,314	+			
P_6	OP-C19	388,349	+	7	Y/P	1736.26**
	HP-11	692,551,401	+		NF	22.38**
	HP-12	338	-			
	HP-15	533	+			

*,**Significant difference at 0.05 and 0.01 with the closest value

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التقييم الجزيئي والمظهري لبعض السلالات المرباة داخليا في قرع الكوسه

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من أجل تقييم التنوع الجزيئي والمظهري والكشف عن العلامات الجزيئية لستة سلالات مرباة داخليا من قرع الكوسا الصيفى *Cucurbita pepo* [L.] ، تم استخدام خمسة بادئات عشوائية RAPD وخمسة بادئات للتتابعات البينية بين التتابعات المتكررة القصيرة ISSR فضلا عن تقدير 12 صفة إقتصادية. حيث نجحت هذه البادئات في استهداف تضاعف العديد من تتابعات DNA المتباينة بين السلالات الستة . حيث كشفت بادئات RAPD عن 88.1٪ تنوع جزيئى في حين أظهرت بادئات ISSR 80.5٪ تنوع جزيئى بين السلالات الستة. وكانت قيمة متوسط قوة التحليل Rp لتقنية RAPD 5.0 بينما كانت 3.4 لتقنية ISSR . ولذا فإن تقنية RAPD أظهرت تقييماً للتنوع الجزيئى والعلاقات الوراثية بين السلالات الستة أفضل من تقنية ISSR. إلا أن تقنية ISSR كانت أفضل من تقنية RAPD في إظهار واسمات جزيئية متنوعة ومتفردة (21 واسمة متفردة فى تقنية ISSR و 9 فى تقنية RAPD). أيضا كان الإرتباط بين المسافات المظهرية والمسافات الجزيئية على أساس ISSR بقيمة 0.173 أعلى منه مع المسافات الجزيئية على أساس RAPD بقيمة 0.045. ومن ناحية أخرى، فباستثناء السلالة P_6 التى أعطت قيما مرغوبة مختلفة معنوياً عن باقى السلالات في صفتين هما عدد الثمار لكل نبات ومتوسط محصول النبات، فقد أعطت كل سلالة من السلالات الخمس الأخرى قيمة مرغوبة مختلفة معنوياً عن باقى السلالات في صفة واحدة، وبذلك يصبح عدد الصفات التي تميزت فيها الستة سلالات 7 صفات. هذه الصفات ربما يمكن ربطها مع الواسمات الجزيئية المحددة في هذه الدراسة. أظهرت السلالة P_5 أعلى عدد من الواسمات (10: 9 منها كانت إيجابية) واحدة أو بعض من هذه الواسمات قد تكون مرتبطة مع الصفة التى أعطت فيها هذه السلالة قيمة مرغوبة مختلفة معنوياً عن باقى السلالات وهى صفة عدد الأوراق. تلى ذلك السلالة P_6 التى أظهرت سبعة واسمات متفردة، ستة منها كانت موجبة، واحدة أو بعض من هذه الواسمات قد تكون مرتبطة مع أى من الصفتين اللتين أعطت فيهما هذه السلالة قيماً مرغوبه مختلفة معنوياً عن باقى السلالات. وهذا يشير إلى أن بعض هذه الواسمات ربما يمكن إستخدامه كعلامات تساعد على الإنتخاب في تربية وتحسين قرع الكوسه.