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The Determination of the Genetic Distance of Various Snake Melon *Cucumis melo var. flexuosus* Cultivars Using Inter Simple Sequence Repea Technique (ISSR)

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Abstract: The experiment have been done on the winter farming season 2020 in one of the farms that belongs to Faris company in Basrah governorate, the technique ISSR have been used to study the genetic distance for twenty one isolates of snake melon *Cucumis melo* var. *flexuosus* species. The variations between amplified samples have been revealed after running them on a gel of agarose which have been previously stained by ethidium bromide. Five primers which gave varied product on the agarose have been selected. Those five primers produced 713 bands, both primers UBC 813 and UBC 815 showed the higher numbers of bands reached to 177 while the primer UBC862 showed the least numbers of bands (100) and the bands which showed multi-variations showed (46) bands, and the results primers amplification unique bands their number reached to (14) and five of those bands belong to the primer UBC842 while the primer UBC862 produced three bands while the primer UBC807 did not produced any bands. While it has shown 100% polymorphisms with the primers 813, 815, 842 and 862, and the least polymorphism percentage have shown with the primer UBC807 reached 75%. According to the efficiency primers, the highest efficiency percentage shown with the primer UBC813 and 815 reached to 24.82% and the least percentage shown 14.02% by the primer UBC862. Cluster analysis showed the effect on the variance of the studied cultivars.

Keywords; *Cucumis melo* Var. *flexuosus*, ISSR, Genetic Distance.

Introduction

The snake melon *Cucumis melo var. flexuosus* crops belong to the family of Cucurbitaaceae which produced in summer and this family can be used as fresh, cooked or pickled (Besirli & Yanmaz, 1997). It is noticeable that the production of *C. melo* are low in Iraq and the increment in the production of *C. melo* can be achieved by the study of the environmental factors that effect on its growth such as the salinity which effect numerous of the main metabolic pathways, the salinity can affect the on the photosynthesis, fats and proteins metabolism (Munns, 2002; Paridaa & Dass, 2005).

The study of genetic variations between the different sup-species of *C. melo* to estimate the genetic distance or similarity which useful in growth and production by determination of

the breeding program according to their goals. The studying of apparent characteristics is not enough to study the genetic variations, especially when there is a strong close similarity between the studied groups. But it can be possible by using the molecular techniques and one of the most important technique is the Inter Simple Sequence Repeats (ISSR) which is based on the PCR (Semagn *et al*., 2006).

The ISSR technique amplify the simple inner repetitions region and it showing more repeats than RAPD because of the length of the primers which is imply the high temperature for the denaturation step of the double strand DNA to single strand (Chowdhury *et al*., 2002).

A previous study was done by Bashandy & El-Shaieny (2016) they studied five subspecies of peas (which are Cream7, Chinese reds, IT82C-12, Sudany and black Crowder) and their response to the salinity stress and analyse their ISSR and they concluded that both Sudany and Chinese Red are the most resisted to salinity and also the least crops production rate while the Creamy7 showed the higher rate of production and also sensitive to the salinity. The rate of polymorphisms was %82.08 and the genetic similarity rate was 0.48-0.67. The tree diagram shows that the genotypes were divided into two main groups. Group one: consists of the genotypes less tolerant of salinity.

While the second group included: the genotypes that are more tolerant of Salinity, which can be used in the breeding and improvement process to produce new varieties that tolerate salinity and have high production.

Another study was done by Majeed *et al*. (2018) in which they revealed three genotypes of wheat that improves salt tolerance by breeding the chosen plant breeding, in that study they used the ISSR technique to revealed the variation of cultivars that grown under salinity conditions. To render ISSR labels, they picked three primers (UBC 809, UBC 810 and UBC 811). According to the effects of the markers for amplification and ISSR. Their findings revealed that genetic variations occur between the selected genotypes and local cultivars in certain particular segments of various sizes (bp) for all the primers included in this analysis, with the exception of the primers (OPE-16), which revealed that no bands existed in all of the selected genotypes and local cultivars. In conclusion, the genotypes selected (salt tolerant) varied from those of the local (salt sensitive) cultivars. Five varieties of quadruple wheat were evaluated under salt stress and 14 varieties of barley were evaluated under drought stress to assess the genetic pathways correlated with molecular markers responsible for salinity tolerance in wheat and water deficiency in barley, Heiba *et al.* (2019) obtained new findings. The approaches employed were RAPD, ISSR and SSR-PCR, and molecular methods were used to analyse the data collected from the objects tested. Effects: The SSR effects indicated the presence of five water stress resistance molecular markers in barley, three of which were positive for toughness and resilience relative to regulation.

This study aims to determine the genetic relation and similarity between the studied species and also to determine gene expression, in order to guide the planter to choose the correct parents.

Materials & Methods

This study held during the winter season of 2020 in one of the farm that belong to Faris Company, Basrah, Iraq. 21 plants species have been included in this study (16 of them are local from Basrah, Missan, Thi Qar, Anbar,

Muthana, Babylon, Baghdad, Kirkuk. and the other five plants species are foreign (Iranian, Tarouzi from the French company Flmourine, Taarzi produced by the Dutch company Seimens, Egyptian by the company Alandalus and Italian produced by the company PAGANO COSTANTINO). At the end of the season 42 samples have been taken, 21 samples of them irrigated by water it's salinity 1 dSm^{-1}, and 21 samples irrigated with water its salinity equal to 5 dSm^{-1} then DNA isolation were done for the 42 samples according to manufacturer protocol of Zymo-USA then five primers were ordered from Integrated DNA technologies /USA, as listed in the table (1).

Table (1): The neocleotides sequences of primers that used in ISSR technique.

Primer	Sequence	Tm	GC%
UBC813	CTCTCTCTCTCTCTCTT	53	47
UBC815	CTCTCTCTCTCTCTCTG	54	53
UBC842	GAGAGAGAGAGAGAGAYG	53	52
UBC807	AGAGAGAGAGAGAGAGT	54	47
UBC862	AGCAGCAGCAGCAGCAGC	69	66

Table (2): Components of ISSR PCR reaction.

Components	Concentration
Taq PCR PreMix	5µl
Primer	10 picomols.μl^{-1} (2 µl)
DNA	1.5 µl
Distilled water	16.5 µl
Final volume	25µl

Table (3): The ISSR-PCR program steps.

Steps	Temperature (^oC)	Time (minute)	Cycles
Initial Denaturation	94	3	1
Denaturation -2	94	1	40
Annealing	35	1	
Extension-1	72	1	
Extension -2	72	1	1

Gel electrophoresis were done the gel electrophoresis according to protocol mentioned by Sambrook, & Russell (2001). The agarose was 2% concentration and the samples ran with presence of the DNA ladder to estimate the DNA bands sizes (Cerasela, *et al*., 2011) and then the results were

analysed by converting the electrophoresis equal 1 and absence equal 0 and then the characterization data converted to similarity according to Nei & Lei (1979).

$$similarity = \frac{2nxy}{nx + ny} * 100$$

The genetic distance calculated by using the Nie's 72 equation (Nei & Lei. 1979). As follow;

$$\text{Genetic distance (G.D)} = 1-\left(\frac{2*Nxy}{Ny+Nx}\right)$$

In which G.D. represent the genetic distance, r Nxy represent the number of bands that interfere between the two groups X and Y the samples, Nx represents the number of total bands in x sample while Ny represents the number of total bands in Y results into numbers the presence of band sample. After finding the G.D. between the samples the cluster tree were drawn according to UPGMA (Sneath & Sokal., 1973). The software NTSYS0-pc were used to draw the phylogenetic tree by using the following equation; the percentage of polymorphisms for each primer

$$\text{Primer} = \frac{\text{umber of varied bands}}{\text{total bands numbers}} * 100$$

$$\text{The ability of primers to distinguish} = \frac{\text{the number of varied bands for each primer}}{\text{the number of varied bands for all primers}} * 100$$

$$\text{Efficiency of primer} = \frac{\text{the total number of bands resulted by the primer}}{\text{the total numbers of bands resulted by all primers}} * 100$$

(Grundmann *et al*., 1995)

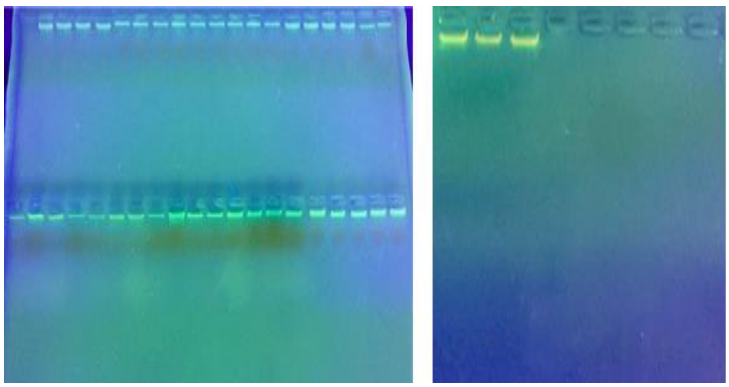

Fig. (1): Gel electrophoresis of genomic DNA extraction from Plant, 1% agarose gel at 5 vol .cm^{-1} for 1:15 hours.

Results & Discussion

As shown in table (1), five primers of the ISSR technique were used to assess the tolerance of the varieties in our study of *Cucumis sativus* (21 types irrigated with freshwater and themselves irrigated with saltwater). The PCR reaction products showed different bands, some of which could differentiate between fresh-water irrigated varieties from salt-water irrigated ones. The primer that records a single band in most fresh water irrigated varieties and not present in saltwater irrigated varieties or vice versa is a candidate of characteristic primer for varieties tolerant or sensitive to salinity.

Table (4): The products of ISSR primers their efficiency ratios and their discriminatory ability in the studied varieties.

Primer	813	807	815	842	862	Total	Mean
Number of total bands	177	146	177	118	100	713	142.6
Number of bands	10	4	11	-	9	34	6.8
Mono morphism bands	-	1	-	-	1	2	0.4
Poly morphism bands	10	3	11	14	8	46	9.2
Unique bands	4	-	2	5	3	14	2.8
Poly morphism	100%	75	100	100	100	-	94
Primer Efficiency	24.82	19.77	24.82	16.54	14.02	-	19.99
Primer Discrimination	21.73	6.52	23.91	30.45	17.39	-	19.99

The results showed that the primer UBC813 (Fig. 2) showed the existence of bands typical of salt-tolerant cultivars in two groups 34 and 42 of salt water of saltwater irrigated cultivars and 450bp of molecular weight. Although these bands did not appear in other cultivars which could be used as an indicator for distinguishing between these cultivars for salt tolerance from the rest of the cultivars in our study, as the bands appeared at 725bp molecular weight in varieties,

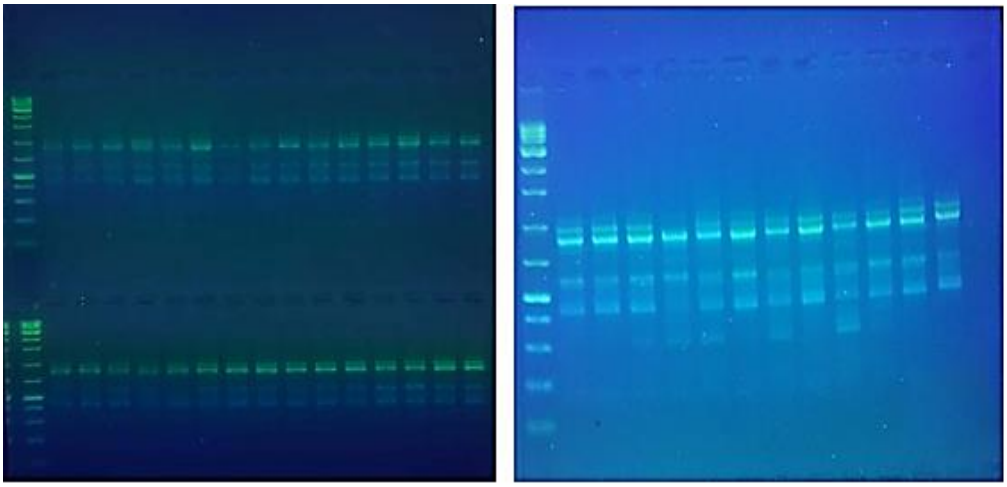

Fig. (2): Agarose gel electrophoresis primer UBC813. Bands were fractionated by electrophoresis on a 2% agarose gel, and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

Which may reflect some of these salinity-bearing varieties as well as the presence of irrigated with saltwater, which are 32, 34, 35, 36 and 41 and also appeared in class 13 irrigated with freshwater molecular weight bands 300 bp in cultivars in study 35, 37 and 39 appeared in cultivar No. 1, which is also indicative of cultivars with high salt tolerance and high gene expression compared to other studied cultivars. Through this analysis and amplification of

the primer UBC813, the existence of specific bands appeared at a molecular weight of 600 bp for freshwater irrigated varieties 3, 11, 12, 13, 14 and 15 that did not appear in saltwater irrigated cultivars that could be characteristic of salinity-

sensitive cultivars, as well as the appearance of bands at molecular weight 475bp 1, 3, 4, 6, to Class 15.

As for the primer UBC815 (Fig . 3), the pcr products display distinct bands , and for the cultivars tested, some may be special and characteristic bands for the cultivars irrigated with salt water, the molecular weight 650bp appeared in 23, 24, 25 and 26. In addition to 900 bp molecular weight in Varieties 23, 24, 26, 27, 30, 31, 32, 33, 34, 45, 36, 27, 38, 39, 40, 41, 42,which may be characteristic in the salt tolerance by 85 %.

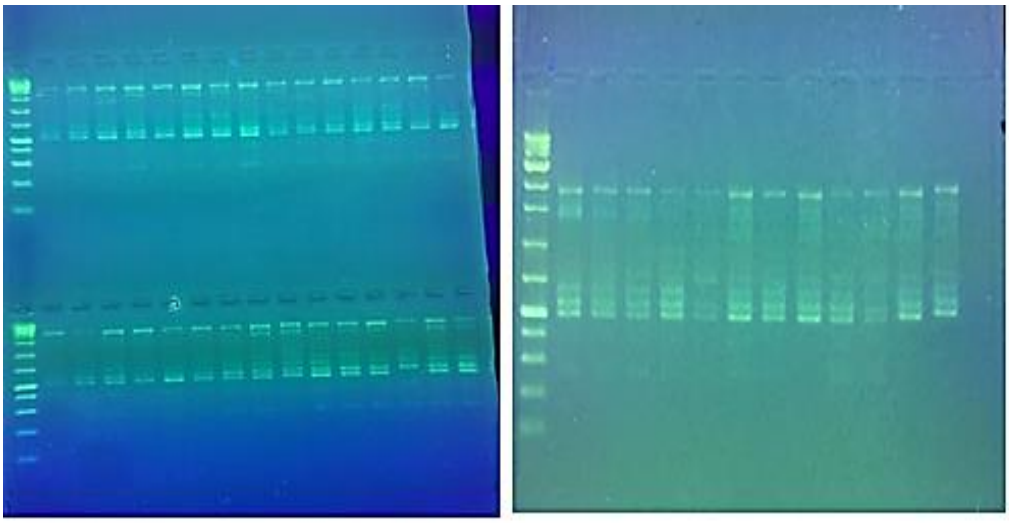

Fig. (3): Agarose gel electrophoresis primer UBC815. Bands were fractionated by electrophoresis on a 2% agarose gel, and visualized under U. V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

As for the primer UBC842 (Fig. 4), it is noted that single bands appeared at 800 bp molecular weight for the cultivars irrigated with salt water 26, 28 and did not appear in the other study cultivars as well as at 400 bp molecular weight for Varieties 25, 27 and 29. Even the molecular weight was shown 350 and 650bp for Varieties 1, 2, 3, 4, 5, 6, 7 and 8. In addition to their appearance in Varieties 10,11, 12, 13, 14 , 15, 16, 17 and 18 for 350 bp molecular weight and their appearance in classes 11, 13 and 14 about 650 bp molecular weight. As for the amplification products of the UBC807 primer (Fig. 5), no unique bands appeared in the studied varieties. While the primer of UBC862 (Fig. 6) is the presence of single bands at a molecular weight of 700 bp for classes 24, 25, 26, 27, 28 and 29 and not present for other cultivars that may reflect a state of salt tolerance or an increase in gene expression, as specific

bands appeared for irrigated taxa with fresh water at molecular weight 525 and 450 bp for classes 4 and 13 respectively in the study and not appearing in the other cultivars.

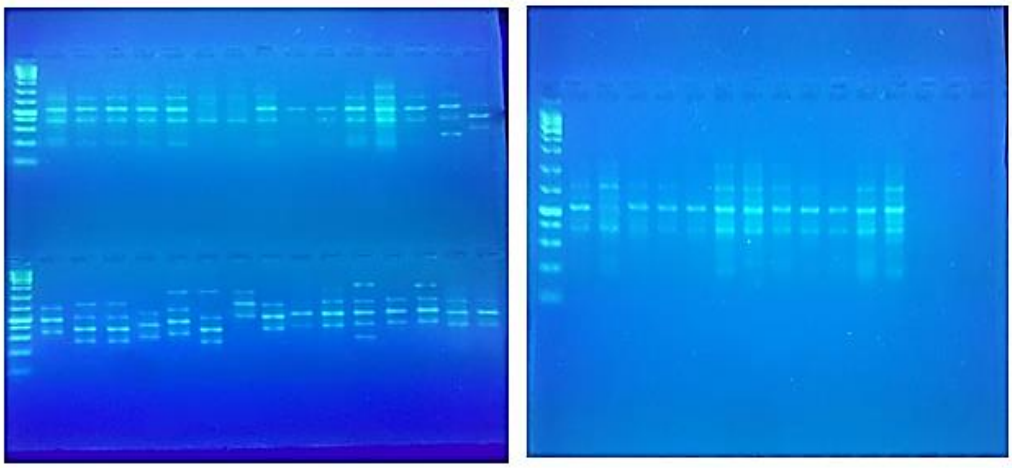

Fig. (4): Agarose gel electrophoresis primer UBC842. Bands were fractionated by electrophoresis on a 2% agarose gel, and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

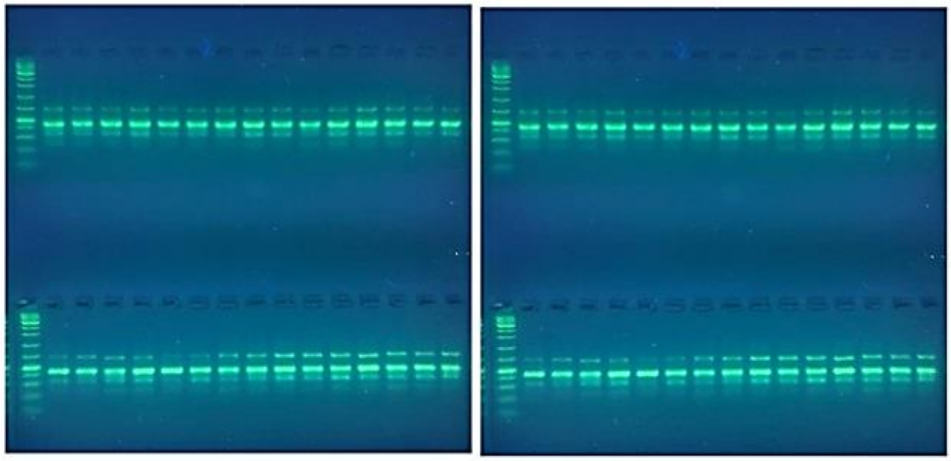

Fig. (5): Agarose gel electrophoresis primer UBC807. Bands were fractionated by electrophoresis on a 2% agarose gel, and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

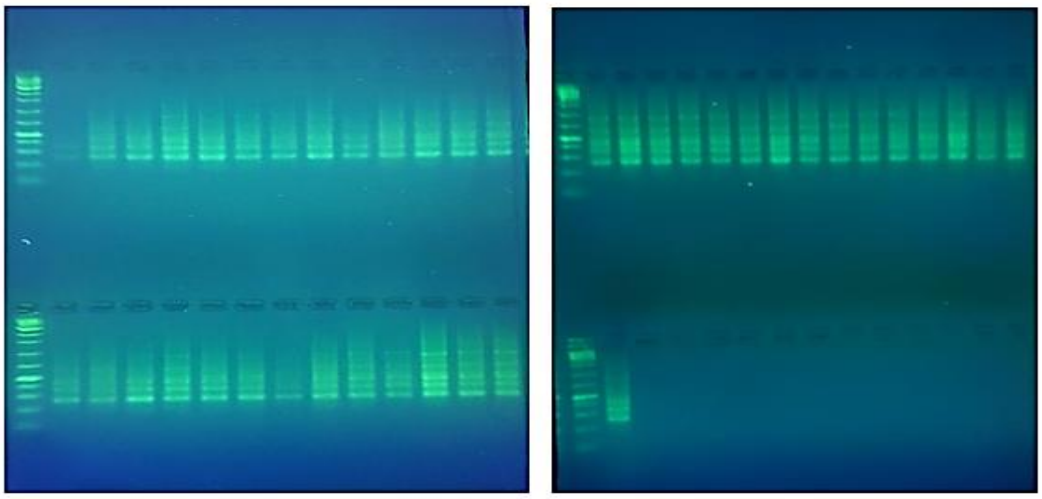

Fig. (6): Agarose gel electrophoresis primer UBC862. Bands were fractionated by electrophoresis on a 2% agarose gel, and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

This study showed the efficacy of all primers used in the polymerase chain reaction (PCR) amplification products, since the five primers provided a total of 713 amplified bands in all samples, with a mean of 142.6. The highest number of bands were provided by the primers 813 and 815, was 177 bands, while 862 provided the lowest number of bands 100. The number of bands with polymorphism bands resulting from primers reached 46 bands with a mean of 9.2, while the primer 842 was 14 bands, the highest number in the 807, the lowest number of bands was 3. As noted in table (4), the number of monomorphic bands generated by the primers amounted to 2 that appeared in the primers results 807 and 862, while the rest of the primers provided no Mono morphic bands. Similarly, the results of the amplification showed that unique bands with a mean of 2.8 as it showed 5 bands for primer 842 while the primer 862 generated three unique bands and the primer 807 generated no unique primer. As for the Polymorphism bands, the primers 813, 815, 842 and 862 reported the highest percentage of the variance by 100% because the number of disparate bands is equal to the number of their total bands, the lowest percentage was 75% for the primers 807 because the number of disparate bands is less than one band from the total bands and the percentage is 94%. The primer efficiency and its discriminatory ability, as the highest efficiency was reported in the primers 813 and 81 by 24.82 %. The primer 842 can therefore be selected to differentiate between the salt-tolerant and the salt-sensitive varieties.

Classes 39 and 40 were the most closely related varieties as shown in fig. (8) for fresh water irrigated varieties only, the varieties were distributed in two main groups. The results of fig. (7) were similar

to the previous one from the cluster analysis of all the cultivars irrigated with fresh and salt water. For the cultivars irrigated with salt water, the varieties here retained the same genetic distance as seen in fig. (8), the distribution of the varieties into two main classes.

As for the correlation, it is noted from fig. (9) that the highest correlation between the type 21 and each of 17, 18, 19 and 20 was 0.992 from the analysis for the varieties irrigated with fresh water, and the lowest correlation between the types 1 and 16 by recording the lowest rates Correlation of 0.204. As for fig. (10) for salt water irrigated products, the highest correlation among classes 21, 20 and 19 was reported, amounting was 0.998.

The effect of two NaCl salt stress concentrations (75 mM and 150 mM) on six soybean cultivars (Giza 21, Giza 22, Giza 35, Giza 82, Giza 111 and Crawford) was analysed using ISSR at molecular level, and analysis using four primers showed 36 polymorphic fragments with 91.57% polymorphism out of a total of 39 amplified salinity stress fragments. With 74.83 per cent polymorphism, the six soybean cultivars showed 30 ISSR individual positive fragments for salinity tolerance, while Giza 82 and Giza 35 had the largest number of specific salinity markers at all NaCl concentrations. For all the cultivars tested, NaCl salt stress induced differences in the number of electrophoretic protein bands, while Giza 82 displayed the largest number of new protein bands (3 bands) and Giza 111 according to our findings, ISSR techniques are valuable methods for detecting particular salinity tolerance markers and could be used in soybean breeding programs to pick the most resistant cultivars (Mahgoub *et al*., 2016).

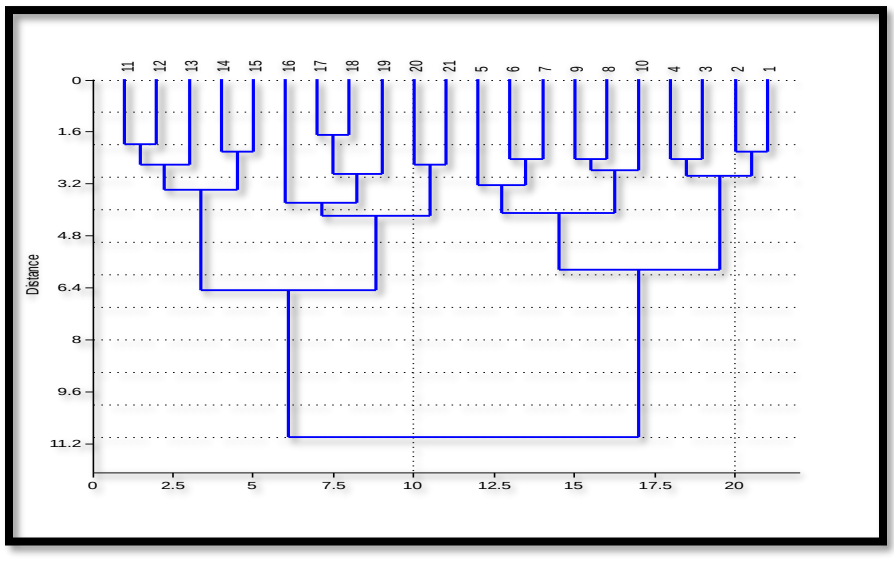

Fig. (7): Cluster analysis of the cultivars irrigated with fresh water.

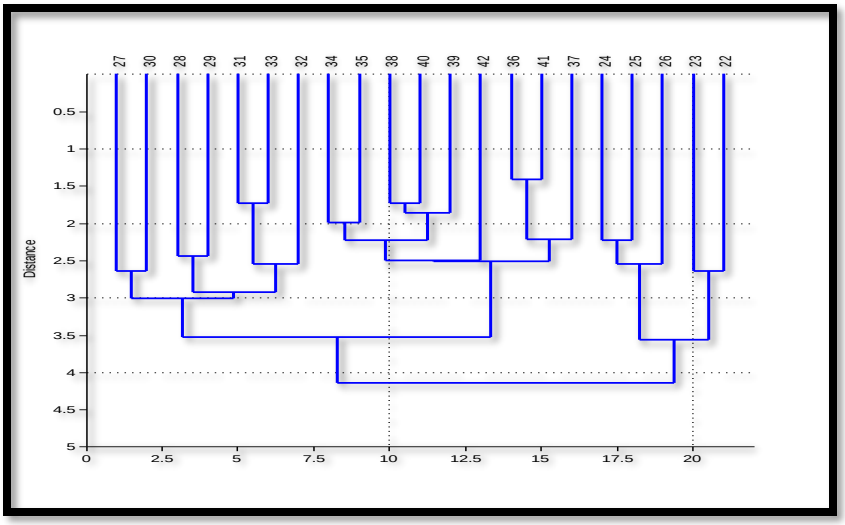

Fig. (8): Cluster analysis of the cultivars irrigated with salt water.

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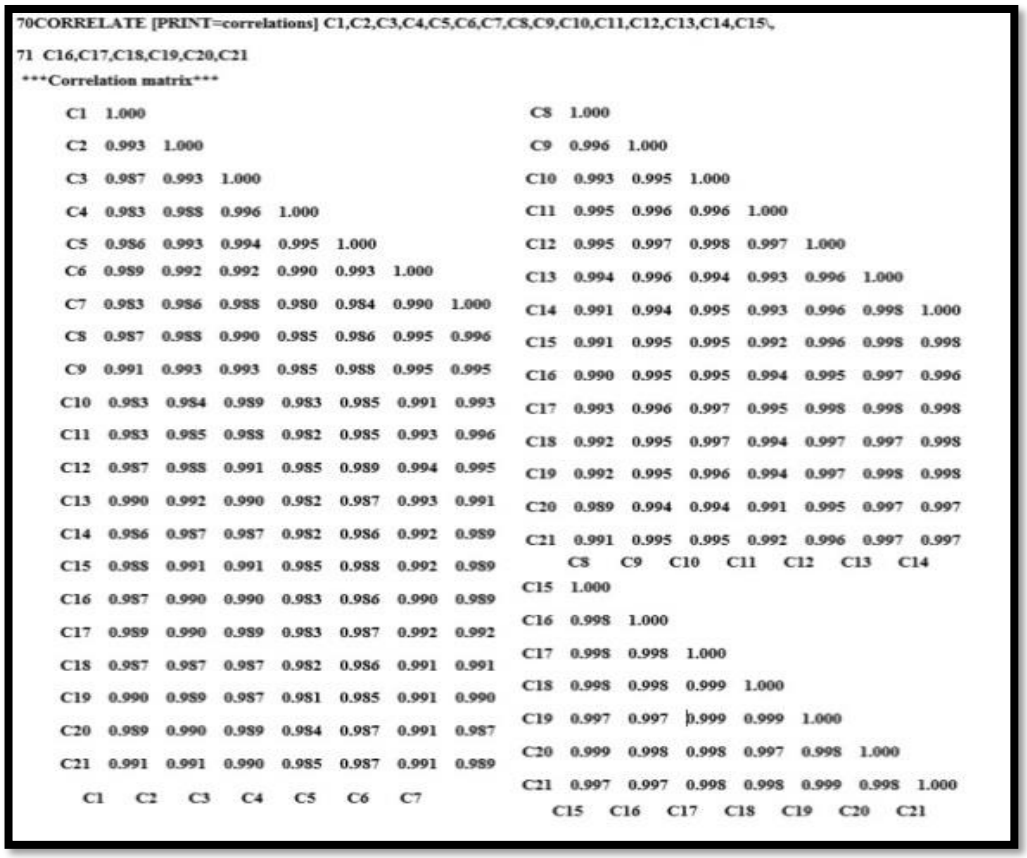
70 CORRELATE [PRINT=correlations] C1,C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15\,
71 C16,C17,C18,C19,C20,C21
Correlation matrix

	C1	C2	C3	C4	C5	C6	C7
C1	1.000						
C2	0.873	1.000					
C3	0.844	0.878	1.000				
C4	0.773	0.836	0.900	1.000			
C5	0.631	0.799	0.876	0.869	1.000		
C6	0.526	0.650	0.817	0.833	0.926	1.000	
C7	0.502	0.644	0.778	0.839	0.858	0.946	1.000
C8	0.483	0.609	0.760	0.868	0.865	0.918	0.965
C9	0.376	0.547	0.696	0.816	0.832	0.909	0.962
C10	0.402	0.549	0.734	0.809	0.827	0.889	0.960
C11	0.414	0.585	0.743	0.818	0.866	0.927	0.975
C12	0.395	0.553	0.727	0.805	0.854	0.895	0.948
C13	0.345	0.549	0.690	0.790	0.855	0.900	0.948
C14	0.323	0.497	0.674	0.777	0.822	0.882	0.934
C15	0.296	0.459	0.641	0.736	0.799	0.862	0.925
C16	0.204	0.419	0.581	0.661	0.765	0.849	0.913
C17	0.218	0.434	0.582	0.696	0.747	0.808	0.894
C18	0.248	0.461	0.616	0.715	0.781	0.841	0.910
C19	0.196	0.391	0.553	0.661	0.726	0.800	0.888
C20	0.165	0.374	0.567	0.645	0.753	0.824	0.883
C21	0.185	0.404	0.575	0.671	0.743	0.809	0.884

	C8	C9	C10	C11	C12	C13	C14
C8	1.000						
C9	0.965	1.000					
C10	0.959	0.975	1.000				
C11	0.969	0.979	0.974	1.000			
C12	0.964	0.961	0.973	0.989	1.000		
C13	0.953	0.973	0.969	0.985	0.983	1.000	
C14	0.951	0.973	0.969	0.979	0.985	0.985	1.000
C15	0.936	0.968	0.972	0.976	0.983	0.980	0.990
C16	0.905	0.946	0.940	0.960	0.958	0.964	0.970
C17	0.903	0.953	0.946	0.954	0.958	0.964	0.980
C18	0.914	0.958	0.956	0.967	0.969	0.974	0.985
C19	0.896	0.942	0.942	0.945	0.954	0.957	0.977
C20	0.890	0.936	0.941	0.948	0.957	0.960	0.975
C21	0.892	0.944	0.943	0.950	0.954	0.962	0.977

	C15	C16	C17	C18	C19	C20	C21
C15	1.000						
C16	0.976	1.000					
C17	0.982	0.979	1.000				
C18	0.987	0.984	0.997	1.000			
C19	0.984	0.982	0.992	0.989	1.000		
C20	0.982	0.987	0.983	0.987	0.986	1.000	
C21	0.981	0.983	0.992	0.992	0.992	0.992	1.000

Fig. (9): The correlation between the varieties irrigated with fresh water.



70CORRELATE [PRINT=correlations] C1,C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15\,
71 C16,C17,C18,C19,C20,C21
Correlation matrix

	C1	C2	C3	C4	C5	C6	C7
C1	1.000						
C2	0.993	1.000					
C3	0.987	0.993	1.000				
C4	0.983	0.988	0.996	1.000			
C5	0.986	0.993	0.994	0.995	1.000		
C6	0.989	0.992	0.992	0.990	0.993	1.000	
C7	0.983	0.986	0.988	0.980	0.984	0.990	1.000
C8	0.987	0.988	0.990	0.985	0.986	0.995	0.996
C9	0.991	0.993	0.993	0.985	0.988	0.995	0.995
C10	0.983	0.984	0.989	0.983	0.985	0.991	0.993
C11	0.983	0.985	0.988	0.982	0.985	0.993	0.996
C12	0.987	0.988	0.991	0.985	0.989	0.994	0.995
C13	0.990	0.992	0.990	0.982	0.987	0.993	0.991
C14	0.986	0.987	0.987	0.982	0.986	0.992	0.989
C15	0.988	0.991	0.991	0.985	0.988	0.992	0.989
C16	0.987	0.990	0.990	0.983	0.986	0.990	0.989
C17	0.989	0.990	0.989	0.983	0.987	0.992	0.992
C18	0.987	0.987	0.987	0.982	0.986	0.991	0.991
C19	0.990	0.989	0.987	0.981	0.985	0.991	0.990
C20	0.989	0.990	0.989	0.984	0.987	0.991	0.987
C21	0.991	0.991	0.990	0.985	0.987	0.991	0.989

	C8	C9	C10	C11	C12	C13	C14
C8	1.000						
C9	0.996	1.000					
C10	0.993	0.995	1.000				
C11	0.995	0.996	0.996	1.000			
C12	0.995	0.997	0.998	0.997	1.000		
C13	0.994	0.996	0.994	0.993	0.996	1.000	
C14	0.991	0.994	0.995	0.993	0.996	0.998	1.000
C15	0.991	0.995	0.995	0.992	0.996	0.998	0.998
C16	0.990	0.995	0.995	0.994	0.995	0.997	0.996
C17	0.993	0.996	0.997	0.995	0.998	0.998	0.998
C18	0.992	0.995	0.997	0.994	0.997	0.997	0.998
C19	0.992	0.995	0.996	0.994	0.997	0.998	0.998
C20	0.989	0.994	0.994	0.991	0.995	0.997	0.997
C21	0.991	0.995	0.995	0.992	0.996	0.997	0.997

	C15	C16	C17	C18	C19	C20	C21
C15	1.000						
C16	0.998	1.000					
C17	0.998	0.998	1.000				
C18	0.998	0.998	0.999	1.000			
C19	0.997	0.997	0.999	0.999	1.000		
C20	0.999	0.998	0.998	0.997	0.998	1.000	
C21	0.997	0.997	0.998	0.998	0.999	0.998	1.000

Fig. (10): The correlation between the varieties irrigated with salt water.

90 ISSR primers were also used by Sestili *et al*. (2008), 39 of which demonstrated polymorphism among the 13 Italian melon accessions. Nine ISSR primers that produced 71 monomorphic bands between Palestinian melon accessions were used by Mallah (2014). While the analysis carried out by Akash *et al*. (2020). The 14 ISSR primers developed 63 bands, among which 27 (43 per cent) were polymorphic, and divided the landraces into two classes ranging from a high among 0.97 to a low of 0.39 per cent similarity. Genetic variation were found among 17 Jordanian snake melons (*C. melo* var. *flexuosus*).

Conclusions

We conclude that the ISSR indicators are important in detecting variations and determining a distinct fingerprint for the varieties under study, but that depends on the number of used primers and the number of varieties, and this in the future may have significance in the field of plant breeding and determine a footprint for promising varieties for breeding and plant improvement programs.

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تحديد البعد الوراثي لاصناف مختلفة من خيار القثاء المحلية والاجنبية باستعمال تقنية التكرارات الترادفية البسيطة الداخلية (ISSR)

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المستخلص: اجريت التجربة في موسم الزراعي الشتوي 2020 في احد المزارع التابعة لشركة الفارس الزراعية في المنطقة الصحراوية محافظة البصرة بهدف دراسة البعد الوراثي لواحد عشرين صنف من اصناف القثاء باستعمال تقنية التكرارات الترادفية البسيطة الداخلية (ISSR). كشف عن التباينات بين القطع المتضاعفة لكل صنف(اعدادها واحجامها الجزيئية) بعد ترحيل نواتج التضاعف للعينات على هلام الاكاروز وتصبيغها ببروميد الاثيديوم. اختيرت خمس بوادئ والتي اظهرت نواتج تضاعف متباينة بين الاصناف قيد الدراسة. اذ انتجت البوادئ الخمسة جميعها 713 حزمة فقد انتج البادئين UBC813 وUBC 815 اعلى عدد حزم بلغ 177 حزمة في حين انتج البادئ UBC 862 اقل عدد جزم بلغ 100 وبلغ عدد الحزم ذات التعدد الشكلي الناتجة من فعل البوادئ 46 حزمة، واظهرت نتائج تضخيم البوادئ حزم فريدة unique بلغ عددها 14 اذ اظهرت منها 5 حزمة للبادئ UBC842 في حين انتج البادئ UBC862 ثلاثة حزم فريدة ولم ينتج البادئ UBC 807 اي حزم فريدة اما التعددية الشكلية فقد سجلت البوادئ 813، 815، 842 و 862 اعلى نسبة مئوية من التباين او التعددية الشكلية وبلغت 100%، اقل نسبة مئوية للتعددية الشكلية فكانت 75% للبادئ UBC 807 وكفاءة البادئ وقدرته التميزية، اذ سجلت اعلى كفاءة في البادئين 813 و815 وبلغت 24.82%واقل كفاءة كانت في البادئ 862 بلغت 14.02% في حين كانت اعلى قدرة تميزية 30.43% في البادئ 842 وسجل البادئ 807 اقل قدرة تميزية 6.52%. وكان للتحليل العنقودي الاثر في تباين الاصناف المدروسة.

الكلمات المفتاحية: نبات خيار القثاء *Cucumis melo* Var. *flexuosus* ، ISSR، البعد الوراثي.