

INVESTIGATE GENETIC RELATION AMONG WATERMELON CULTIVARS USING MOLECULAR DNA MARKERS

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ABSTRACT

This study was aimed to investigate genetic relation among 21 selective of watermelon seeds which collected from different commercial companies for different origins and produced at different years by using 12 primers for Inter Simple Sequence Repeat (ISSR). The results showed that the selective of Crimson sweet had genotypes highest genetic relation between (K1 and K2) it was found less than the genetic similarity between (K1 and K4) , (K2 and K4) .The four selective were divided two groups A and B. The results showed that the selective a Charleston gray had the highest genetic relation between (CH4 and CH6) while it found less than the genetic relation between (CH5 and CH9). The ten selectives were divided two cluster A and B, The groups A was divided three sub clusters .The results revealed that the highest genetic relation between the selective of the variety Sugar baby (S1 and S3). While found less genetic relation between the (S1 and S5) and divided the selectives of Sugar baby into two main cluster A and B, The cluster B was divided two sub cluster. The result found that the highest genetic relation among the 21 selective it was between (CH4 and CH6), while found less than of genetic similarity among (CH8 and S5) (CH8 and S2), (S2 and K1) ,(S2 and K2) ,(S2 and CH9), (K1 and S5) (S5 and CH9). The 21 selectives was divided two main clusters A and B the group A was divided to three sub clusters while the group B was divided two sub clusters. It could be concluded that the genetic relation it is very important in the hybridization programs to it prove a large genetic base to benefit of plant breeding programs.

Keywords: crimson sweet, sugar baby, charleston gray, genetic diversity.

الياس و الجبوري

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الكشف عن القرابة الوراثية لبعض اصناف الرقي باستخدام مؤشرات الدنا الجزيئية

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المستخلص

درس التماثل الوراثي بين (21) منتخب من بذور الرقي التي تعود الى شركات تجارية و مناشئ مختلفة وفي سنين انتاج مختلفة للبذور بأستعمال (12) بادناً لتقانة التكرارات الترادفية البسيطة البينية (ISSR). تبين ان منتخبات الصنف *Crimson sweet* اعطت اعلى نسبة من التشابه الوراثي كانت ما بين المنتخبين (K1 و K2) في حين وجدت اقل نسبة من التشابه الوراثي كانت ما بين المنتخبين (K1 و K4) و (K2 و K4) وقد توزعت المنتخبات الاربعة الى عنقودين رئيسيين A و B. اظهرت النتائج ان منتخبات الصنف *Charleston gray* اعطت اعلى نسبة من التشابه الوراثي كانت ما بين المنتخبين (CH4 و CH6) في حين وجدت اقل نسبة من التشابه الوراثي كانت ما بين المنتخبين (CH5 و CH9) وقد توزعت المنتخبات العشرة الى عنقودين رئيسيين A و B قسم فيها العنقود A الى ثلاث عناقيد ثانوية. وجد ان اعلى نسبة من التشابه الوراثي في الصنف *Sugar baby* كانت ما بين المنتخبين (S1 و S3) في حين وجد اقل نسبة من التشابه الوراثي كانت ما بين المنتخبين (S1 و S5) وقسمت المنتخبات السبعة للصنف *Sugar baby* الى مجموعتين رئيسيتين A و B قسم فيها العنقود B الى عنقودين ثانوية. وجد ان اعلى نسبة من التشابه الوراثي بين 21 منتخب كانت ما بين المنتخبين (CH4 و CH6) في حين وجدت اقل نسبة من التشابه الوراثي كانت ما بين المنتخبين (CH8 و S2) وبين (S2 و S5) و (K1 و S2) و (K2 و S2) و (S2 و CH9) وبين (S5 و K1) و (S5 و CH9) وقسمت المنتخبات الواحد والعشرون الى مجموعتين رئيسيتين A و B قسم فيها العنقود A الى ثلاث عناقيد ثانوية في حين قسم العنقود B الى عنقودين ثانوية. يمكن الاستنتاج أن تحديد درجة القرابة الوراثية تعد ذات اهمية بالغة في برامج التهجين لانها تؤمن قاعدة وراثية كبيرة للافادة منها في برامج التربية وتحسين النبات.

الكلمات المفتاحية: كرمسن سويت، شكربيبي، جارسلتون كرى، البعد الوراثي .

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INTRODUCTION

Genetic resources are the basic material in breeding and genetic improvement programs, the first step of the plant breeding, since no breeding and improvement program could be carried out without references of genetic diversity within the plant species to be improved, the more diversing of the genetic sources, the successful and faster the goal of the breeding program, which will be to develop genotype with high productivity, desirable qualitative and quantitative characteristics as well as adapter to environmental stresses (20). The heterogeneity of genetic resources could be studied using molecular markers as adopted in the study of molecular taxonomy and evolutionary studies and in the construction of genetic maps are also today one of the important tools for the study of genetic diversity and have proven their ability to effectively detect genetic diversity within and between species and identify similarities (4, 12). The use of adaptive technology of ISSR (Inter Simple Sequence Repeats) as primers single length (16-18 bp) and author of nuclear frequent and sometimes with 2- 4 nuclear, both in 3' or 5' ends (4, 9, 21). It is based on PCR (Polymerase Chain Reaction) and works to amplify sites between (100-3000 bp) sensitive, converging and oppositely present (39). The ISSR technology increases the possibility of giving stable and results, as well as it requires a small amount of DNA and its ability to detect high percentages of polymorphism more than RAPD technology because of the length of the primer used and faster than AFLP technology, and the sufficiency of SSR technology itself and its ability to detect sequences Dominant nucleotides in heritability, as well as not needing prior information about the studied genetic sequence, are suitable for the study of ethno genetics, assessment of genetic diversity, identification of varieties and genetic mapping (4, 5, 6, 7, 8, 10, 14, 15, 17, 23, 25, 26, 27, 37). The red watermelon (*Citrus lanatus*) considered one of the important vegetable crops in the world (28), and Iraq as well as its production for the year 2020 reached (20,853) tons (19). Increase the success of breeding programs phenotype is closely related to the

genetic diversity of the organism, as molecular markers allow the selection of indirect traits at the seedling stage, which facilitates the selection of difficult traits (34). Wang et al. (35) found that when using 8 ISSR primers on watermelon, it gave 198 alleles, of which 120 alleles showed genetic distance with a percentage of 60.6%. Abdel-Ghani and Mahadeen (1) studied eight cultivars of Snake melon selected from eight Jordanian geographical locations using RAPD technology, and found that the highest genetic distance ratio was between Irbid, Ajloun and Karak 1 of 0.23, while the proportion of genetic similarity was 0.07 between Jerash and Amman, and divided the genetic tree of the eight sites into two major clusters, the first cluster included Irbid, Jerash and Amman, while the second cluster included Madeb 1, Karak 2, Madib 2, Ajloun and Karak 1. Alsohim and Motawei (2) used 10 ISSR primers to find genetic diversity and ascertain the presence of the DREB gene among 6 watermelon cultivars sun shade (USA), Black diamond (USA), Charleston (USA), and Fashion (USA). Holland), Charleston gray (origin USA), Crimson Sweet (origin USA) and wild cultivar originating from Saudi Arabia), which gave the lowest genetic similarity between black diamond and wild variety of 0.53 and the highest genetic similarity between sun shade and Charleston gray and between sun shade and Charleston gray and between Charleston gray and Crimson Sweet with a value of 0.95. The genetic tree was divided into two main clusters; the first cluster included 6 cultivars, while the wild cultivar was isolated in a second cluster independent of the rest of the previously mentioned cultivars. (29) Salsabila et al. used after crossing Maduri yellow watermelon with Princess pomegranate red watermelon, to produce orange watermelon hybrids, using 4 PCR-ISSR primers to determine monomorphic and polymorphic to cross orange water melon hybrids with several types of water melon to produce quaternary hybrids, the prefixes gave 36 bands, of which 17 were polymorphic, while 19 were monomorphic. Jena and Chand (16) reported that the increased polymorphism between primers used is due to the amplification of

genomic regions with high genetic variance between samples. Used 11 ISSR technology primers to find genetic diversity among 38 types of red watermelon, as 89 different bands, the studied species were divided into 4 groups, the study showed that there is no correlation between the results of molecular indicators with phenotypes, and this is due to diversity, high genetics and the influence of environmental factors (32). Elias (13) found that the largest genetic distance between Charleston Gray and Sugar Baby cultivars was 0.349, and the lowest genetic distance between Crimson Sweet and Sugar Baby was 0.149 after using 12 primers belonging to ISSR technology to show genetic diversity among 21 genotypes belonging to three cultivars, from red watermelon they are Crimson Sweet, Sugar Baby and Charleston Gray. Studied the genetic similarity of 25 genotypes of Citroides (Citron watermelon) and Lanatus (Dessert watermelon) in order to transfer disease resistance from Citroides to Lanatus, which were selected from five different geographical locations using RAPD and ISSR techniques, ISSR had the highest polymorphism of 95.4% , the genotypes of Citron watermelon in Saudi Arabia were similar to those in Turkey, while the highest genetic variance was found between the commercial and local genotypes of Dessert watermelon in Turkey (24). This study was aimed to investigate genetic relation among different watermelon genotypes.

MATERIALS AND METHODS

Twenty one genotypes seed were planted of three varieties of watermelon related to different commercial companies from different international origins and in different years of seeds production (Table 1). The seeds were sown anvil filled with algae and transplanted to the greenhouse, when true leaves emerged. Samples were taken due to DNA isolation;

some genotypes (S2, S4, S5, S7, K3, and K4) were selected from the seeds immediately after removing the outer shell due to the delay in germination and the absence of true leaves from them.

Molecular Study

The molecular study was carried out in the Research and Development Unit- Central Laboratory and Research Department - Veterinary Department- Ministry of Agriculture. By using the Inter-Simple Sequence Repeats (ISSR) technology using 21 genotypes which it belongs to three original varieties Crimson sweet, Charleston gray and Sugar baby of watermelon. This technology included the following procedures

A- DNA was isolated using attached isolation steps. Purification of the extracted DNA with concentration for the selected varieties was calculated by using a Scan drop device. The concentration of DNA was measured by the wavelength (260-280 nm), and the ratio between the reading of the wavelength 260 nm to 280 nm (OD_{280}/OD_{260}) which help to evaluate the purity of DNA This ratio is between 1.8 - 2.00 for pure DNA (30).

B- Applying ISSR technology:

To 12 primers obtained from Bioneer Company were used. Table 2 shows the nucleotide sequence and annealing temperature of the primers used in the study. The polymerase chain reaction (PCR) was carried out according to (36) with some modifications. The final reaction volume was 25 μ l using 2 X Master mixes obtained from (TOP TAG master MIX KIT, germany Qiagen). The reaction consists of 2 μ l of the initiator at a concentration of 30 picomoles, 12.5 μ l of master mix, 5.5 μ l of distilled water and 5 μ l of DNA, and the reaction was carried out in a thermo cycler according to the following conditions (Table 3)

Table 1. International origins, selected companies and years of production of seeds for 21 selected from three varieties of water melon

Variety	Symbol for each variable	Company	Origin	Year of seed production
Charleston Gray	CH1	Peto Seed	USA	2005
	CH2	Agree Seed	USA	2013
	CH3	Sun Shine	USA	2009
	CH4	Modesto	USA	2011-2010
	CH5	Monarch Seed	Holland	2013
	CH6	Popvriend Seeds	Holland	2009
	CH7	OHISENS ENKE)) Syngenta	Holland	2008
	CH8	Aspero	Greek	2011
	CH9	Niagara	USA	2011
	CH10	Emerald seeds	USA	2006-2005
Sugar Baby	S1	Premium American) Modesto Vegetable Seeds)	USA	2004
	S2	Peto Seed	USA	2004
	S3	Bakker Brothers	Holland	2003
	S4	Monarch Seeds	Holland	2007
	S5	Monarch Seeds	Holland	2007
	S6	Popvriend Seeds	Holland	2004
	S7	Royal sluis	USA	2003
Crimson Sweet	K1	Argeto	Turkey	2011
	K2	United gentics	USA	2009
	K3	Modesto seeds	USA	2004
	K4	Popvriend Seeds	Holland	2008

Table 2. The nucleotide sequence of primers and the annealing temperature of each primer

Primers	Nucleotide sequence 3' - 5'	Annealing temperature
ISSR ₁	(AG)8C	59
ISSR ₂	GAGG(GA)5GG	59
ISSR ₃	(GA)8T	53
ISSR ₄	(CA)8G	54
ISSR ₅	(AC)8C	60
ISSR ₆	(AG)8YT	59
ISSR ₇	(GA)8YT	54
ISSR ₈	(CT)8RG	54
ISSR ₉	(ATG)6	54
ISSR ₁₀	GGAGAGGAGAGGAGA	54
ISSR ₁₁	AGTCGTAGT(AC)7	60
ISSR ₁₂	(TC)8AGA	54

Table 3. Reaction conditions in a thermocycler

Reaction	Number of cycles	Time.	Temp.
DNA Denaturation	1	3min	94C °
40cycles each of which included the following stages			
Denaturation	1	30 sec	94C °
Anneling	1	1min	According to the annealing temperature of each primer and as shown in Table 2
Extension	1	1 min	72 C °
The reaction was completed at 72 C° for 10 minutes, and then the reaction was terminated at 4 C°. The samples were then stored at 4 C° in the refrigerator			

Electrophoresis

The products of the PCR-ISSR reaction were electrophoresis using a horizontal relay device on agarose gel with a concentration of 1.5% dissolved in TAE Buffer 1X buffer solution produced by Cleaver.U.K. (30). In addition, 3

µl of ethidium bromide dye produced by Invetrogen, at a concentration of 10 mg/ml, was added. Then 8 µl of DNA samples were loaded an agarose gel with the addition of 2 µl of loading buffer bromophenol blue 1x. A DNA marker (DNA Lader 100 bp) from

Promega was injected to determine the size and molecular weight of the bands resulting from electrophoresis of 100 V, to separate the DNA bands resulting from amplification and then tested the gel by exposing it to ultraviolet light to see the bands of DNA and photographed the gel using a special camera, the Gel documentation system.

Analysis of the results of the ISSR

Each ISSR gel was manually read by converting its results into characterization tables based on the presence or absence of DNA bands in the measured samples by placing 1 in the presence of the band and 0 in its absence. To the equation of (22) using a computer according to the statistical program (SPSS) version 10, as shows in the following equation (31):

$$G. D. = 1 - (2 n_{xy} / n_x + n_y)$$

G.D. = Genetic Distance

n_{xy} = represents the number of bands in common between the two models x and y that represent either of selected plants.

n_x = the total number of bands in the x model.=

n_y = total number of bands in the y model.

The data was used to build a sub-cluster for the genotype within the same cultivar as well as for all the selections included in the three cultivars to estimate the genetic distance between them based on the un-weighted pair group method for the arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Twelve primers were used, which distinguished by their ability to detect genetic variations among different watermelon genotypes, as the genetic relation among genotypes of one variety was calculated, as well as among all genotypes that related to the three varieties based on the equation of (22). The results showed the similarity and dissimilarity among genotypes under this study, as shows below:

1. Crimson Sweet: The results in Table 4 show that the highest percentage of genetic similarity corresponding to the lowest genetic distance of 0.056 was between the two genotypes K1 and K2, as they formed a small group despite the difference in the producing company, origin and year of seed production, which indicates that the two companies

produce Seeds with same source, had the lowest percentage of genetic similarity corresponding to the largest genetic distance of 0.840 was found between genotypes K1 and K4, as well as between genotypes K2 and K4, with genetic distance between them was 0.81, which means that the source of the seeds of genotype K4 is genetically different from seeds of genotypes K1 and K2 It was revealed that the seeds of the genotype K3 are genetically distant from the seeds of genotypes K2, K1 and K4, with percentages of 0.473, 0.500 and 0.610, respectively. This genetic differences among genotypes mean that the plant breeders in each company could be used a specific breeding method through which they found a selection with distinctive characteristics and had the same name, which is Crimson Sweet. The genetic similarity between some genotypes could be due to introduction between companies in different sources and their marketing seeds produced from the same source.

Table 4. Genetic distance between 4 genotypes of the cultivar Crimson Sweet using 12 primers

	K1	K2	K3	K4
K1	0	0.056	0.500	0.840
K2	0.056	0	0.473	0.811
K3	0.500	0.473	0	0.610
K4	0.840	0.811	0.610	0

Cluster analysis based on genetic distance values using UPGMA method (Fig. 1) and constructed based on ISSR. Results show that the four genotypes with the same name for the cultivar Crimson Sweet were distributed into two main groups A and B, cluster A included three genotypes K1, K2 and K3, the two genotypes K1 and K2 were more close to each other despite their different origins, and in isolation from K3, which has a similar origin to the K2 group. As for cluster B, it included genotype K4 of Dutch origin, which genetically distanced itself from other genotype in high proportions, as shows in Table 4. It can be conclude that the molecular study showed that genotypes bearing the same cultivar name contain high genetic diversity despite the similar geographical locations of some of them, and that this genetic differences among genotypes indicates that the plant breeders in each company could be used a specific breeding method that led to the

development of a selected by selection had distinctive characteristics and gave it the same name, which is Crimson Sweet. As for the genetic similarity between some selections, it could be due to the cooperation among

companies in different countries and their use and marketing of seeds produced from the same source. The results were similar to what was found by others (18, 33).

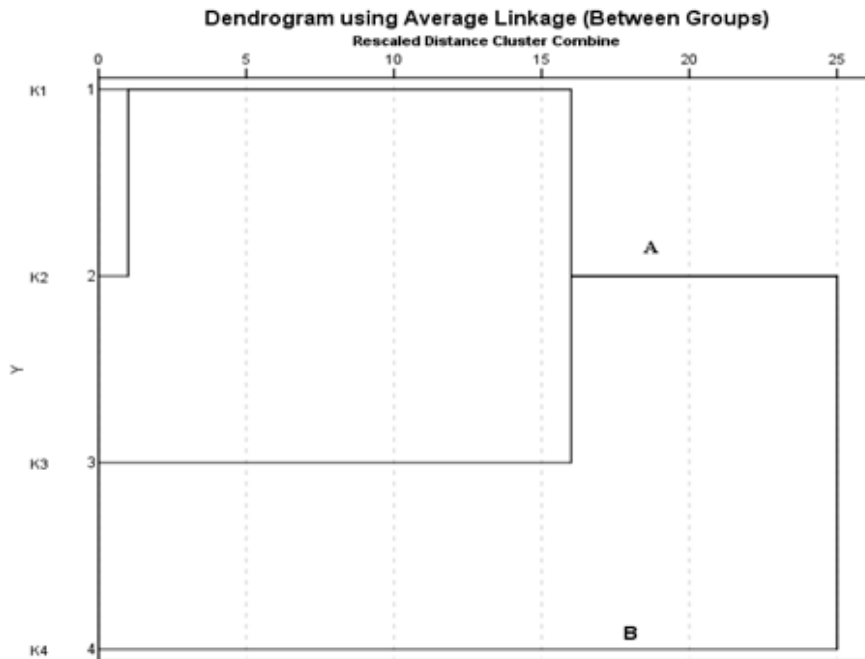


Fig. 1. Cluster analysis between 4 Crimson Sweet groups using 12 primers based on ISSR results

2. Charleston gray: The results in Table 5 show that the highest percentage of genetic similarity corresponding to the lowest genetic distance of 0.008 was between the genotypes CH4 and CH6, as they formed a small group, while the lowest percentage of genetic

similarity corresponding to the largest genetic distance of 0.377 was found, between genotypes CH 5 and CH9.

The cluster analysis based on genotypic distance values using the UPGMA method (Fig. 2).

Table 5. Genetic distance among 10 genotypes of the cultivar Charleston gray using 12 primers

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
CH1	0	0.072	0.086	0.128	0.160	0.136	0.143	0.130	0.303	0.200
CH2	0.072	0	0.100	0.196	0.211	0.204	0.211	0.200	0.310	0.257
CH3	0.086	0.100	0	0.183	0.180	0.190	0.213	0.186	0.348	0.221
CH4	0.128	0.196	0.183	0	0.030	0.008	0.030	0.031	0.365	0.200
CH5	0.160	0.211	0.180	0.030	0	0.022	0.044	0.045	0.377	0.197
CH6	0.136	0.204	0.190	0.008	0.022	0	0.022	0.023	0.352	0.190
CH7	0.143	0.211	0.213	0.030	0.044	0.022	0	0.045	0.358	0.197
CH8	0.130	0.200	0.186	0.031	0.045	0.023	0.045	0	0.333	0.187
CH9	0.303	0.310	0.348	0.365	0.377	0.352	0.358	0.333	0	0.278
CH10	0.200	0.257	0.221	0.200	0.197	0.190	0.197	0.187	0.278	0

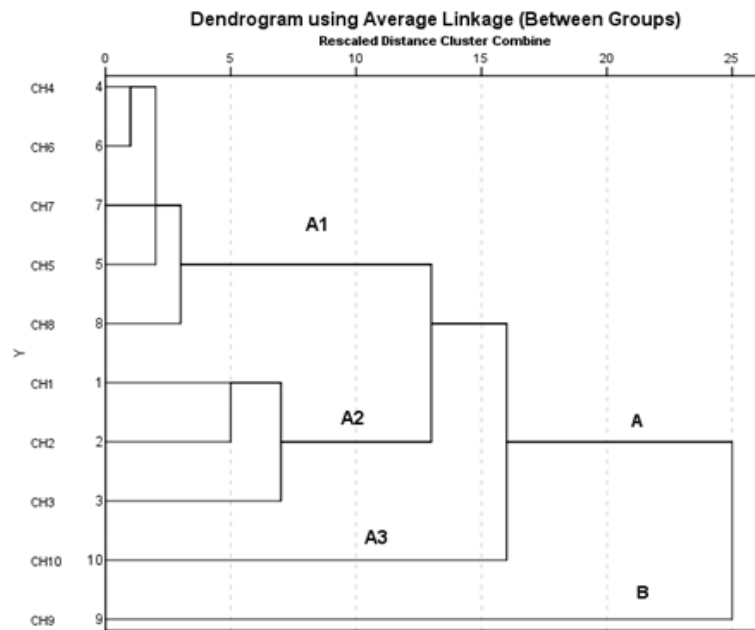


Fig. 2. Cluster analysis cluster among 10 Charleston gray selections using 12 primers based on ISSR analysis results

Established based on the results of ISSR) that the ten genotypes were distributed into two main groups A and B, in which cluster A was divided into three secondary clusters, as the first cluster A1 included five groups CH4, CH6, CH7, CH5 and CH8, find that the genotypes CH4 and CH6 had very high genetic similarity despite their different origins, this could be due to the fact that both genotypes belong to the same parent or the source from which they were produced, and it was found the groups CH7, CH5 and CH8 also with a very high genetic similarity of 0.03 and the second sub-cluster A2 included three groups dating restated to the same origin which, is the United States of America it was that genotypes CH1 and CH2 had lowest distance from each other 0.072 in distance from CH3 and the third sub -cluster, A3 was

included on one genotype CH10, which was the largest genetic distance from the eight groups included in clusters A1 and A2, while cluster B included one genotype CH9. The most distant from the other genotypes, indicates that despite the different origins and companies, ISSR technology showed the genetic distance among those genotypes and the results were similar to what found by (11).

3. Sugar Baby: The results in Table 6 show that the highest percentage of genetic similarity corresponding to the lowest genetic distance between genotypes S1 and S3, which amounted to 0.134, while the lowest percentage of genetic distance corresponding to the largest genetic distance was found between genotypes S1 and S5 which recorded to 0.966.

Table 6. Genetic distance among 7 genotypes of the cultivar Sugar Baby using 12 primers

	S1	S2	S3	S4	S5	S6	S7
S1	0	0.965	0.134	0.701	0.966	0.224	0.791
S2	0.965	0	0.955	0.714	0.200	0.937	0.714
S3	0.134	0.955	0	0.630	0.956	0.301	0.741
S4	0.701	0.714	0.630	0	0.733	0.699	0.333
S5	0.966	0.200	0.956	0.733	0	0.938	0.600
S6	0.224	0.937	0.301	0.699	0.938	0	0.726
S7	0.791	0.714	0.741	0.333	0.600	0.726	0

The cluster analysis dependent on genetic distance values using the UPGMA method show in Fig. 3. Established based on ISSR results that the seven genotypes were divided into two main groups A and B, cluster A included three genotypes S1, S3, and S6. The

origin of genotype S6 with the genotype S3, but it was a distance from the genotypes S1 and S3 by 0.224 and 0.301 respectively, while cluster B was divided into two sub-clusters, as it was included the first sub-cluster B1 genotypes S2 and S5 (0.200), while the second

sub-cluster included B2 genotypes S4 and S7 (0.333). Although the genotypes S4 and S5 belong to the same company, geographic location and year of production, they were distributed in two secondary clusters isolated from each other and with a distance of 0.73 which means that there are two teams of plant breeders belonging to this company working

differently from each other. Or perhaps the company cooperated with another company that has seeds from a different genetic source, or the company produced the seeds in a country with cheap labor and a different environment and genetic source for the other groups.

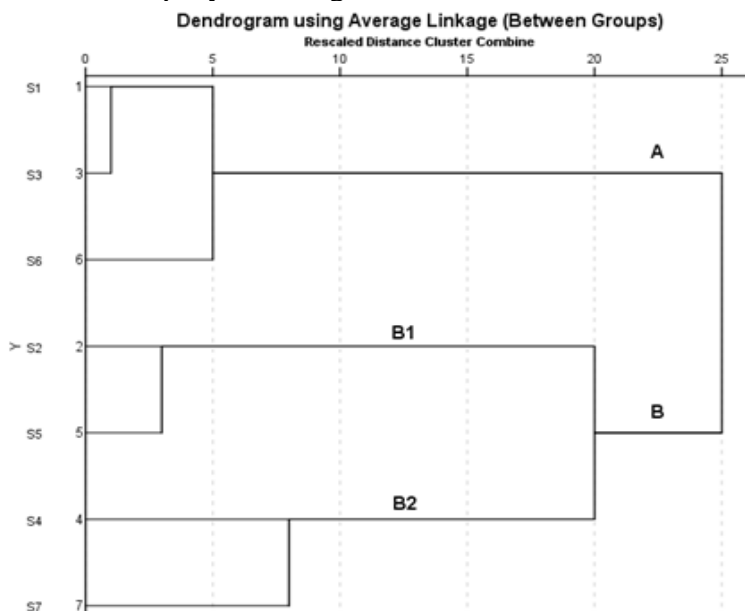


Fig 3. Cluster analysis among 7 Sugar Baby selections using 12 primers based on ISSR results

It conclude from the results showed that it is possible to separate the genotypes by using a small number of ISSR markers to study the distance and genetic diversity and to find a genetic, cellular and geographic relation among the genotypes before they are included in the breeding programs without definitively relying on the information provided by commercial companies. Found by (1, 11, 13).

4. Genetic distance between 21 genotypes of watermelon

The results in Table 7 show the similarities and differences among these genotypes, as it was found that the highest genetic similarity, which corresponds to the lowest genetic

distance, was between the two genotypes CH4 and CH6, as they formed a small group, which indicates that they belong to the same seeds source despite the differences of the company and year of production, while the least genetic similarity that corresponds to the largest genetic distance was found between selections (CH8, S5) and (CH8 and S2), between (S2, K1), (S2, K2) and (S2 and CH9) and between (K1 and S5) and (S5 and CH9) reached (1,000) the reason could be due to the different geographical locations as well as the differences in the original genetic structures that produced these selections.

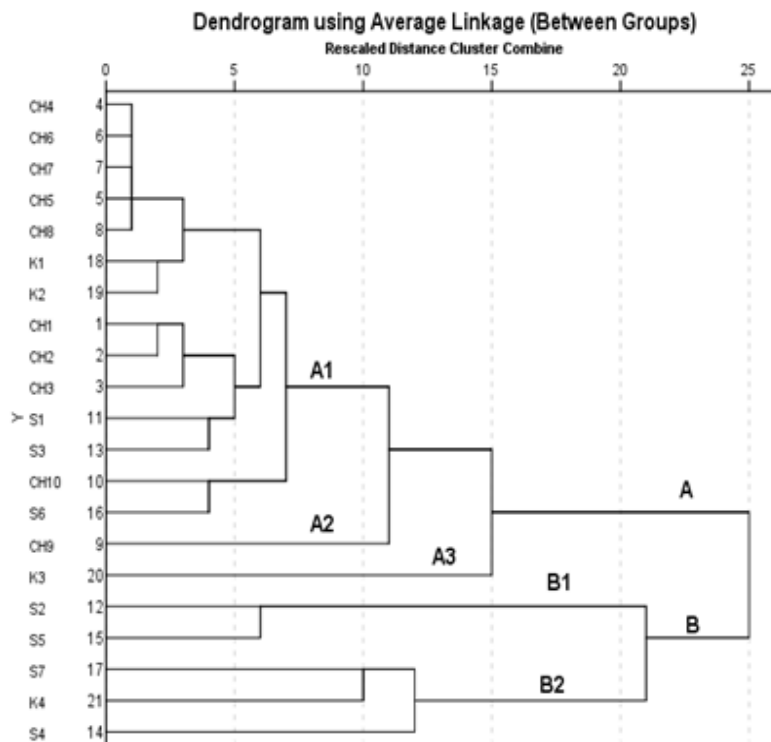


Fig. 4. Cluster analysis based on genetic distance using UPGMA method

Established based on ISSR results that the twenty-one genotypes were divided into two main groups A and B. Cluster A included three sub-clusters. The first sub-cluster A1 included CH4, CH6, CH7, CH5, CH8, K1, K2, CH1, CH2, CH3, S1 and S3. and S6 and CH10, while the second sub-cluster A2 genotype CH9 and the third sub-cluster A3 genotype K3 while the main cluster B was divided into two sub-clusters that included the first sub-cluster B1 on genotypes S2 and S5, while the second sub-cluster B2 included three genotypes which are K4, S7 and S4. It could be concluded that genotypes did not separated according to the

nature of growth and geographical area only, but were separated according to the similarity of genes among species, as determining the degree of genetic distance among different varieties is of great importance in breeding programs because it secures a large genetic base to benefit from in breeding and improvement programs and because determining the geographical dimension or the country of origin or the producing company did not effective in classifying the varieties when used in traditional breeding programs. The results were similar to that of (1, 2, 3, 7, 33, 38).

Table 7. Genetic distance among 21 genotypes of three melon cultivars using 12 primers

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	S1	S2	S3	S4	K1	K2	S5	K3	K4	CH9	S6	S7	CH10
CH1	0	0.072	0.086	0.128	0.160	0.136	0.143	0.130	0.094	0.962	0.140	0.714	0.193	0.168	0.963	0.450	0.810	0.303	0.286	0.778	0.200
CH2	0.072	0	0.100	0.196	0.211	0.204	0.211	0.200	0.168	0.958	0.159	0.690	0.248	0.222	0.959	0.440	0.793	0.310	0.346	0.759	0.257
CH3	0.086	0.100	0	0.183	0.180	0.190	0.213	0.186	0.156	0.964	0.167	0.667	0.248	0.224	0.965	0.446	0.727	0.348	0.304	0.758	0.221
CH4	0.128	0.196	0.183	0	0.030	0.008	0.030	0.031	0.140	0.971	0.259	0.769	0.085	0.078	0.971	0.516	0.821	0.365	0.244	0.821	0.200
CH5	0.160	0.211	0.180	0.030	0	0.022	0.044	0.045	0.171	0.971	0.273	0.750	0.099	0.092	0.972	0.546	0.800	0.377	0.225	0.825	0.197
CH6	0.136	0.204	0.190	0.008	0.022	0	0.022	0.023	0.148	0.971	0.266	0.772	0.077	0.070	0.971	0.521	0.823	0.352	0.234	0.823	0.190
CH7	0.143	0.211	0.213	0.030	0.044	0.022	0	0.045	0.154	0.971	0.273	0.775	0.084	0.092	0.972	0.526	0.850	0.358	0.240	0.825	0.197
CH8	0.130	0.200	0.186	0.031	0.045	0.023	0.045	0	0.143	1.000	0.264	0.789	0.087	0.063	1.000	0.548	0.842	0.333	0.248	0.842	0.187
S1	0.094	0.168	0.156	0.140	0.171	0.148	0.154	0.143	0	0.965	0.134	0.701	0.220	0.197	0.966	0.500	0.821	0.376	0.224	0.791	0.211
S2	0.962	0.958	0.964	0.971	0.971	0.971	0.971	1.000	0.965	0	0.955	0.714	1.000	1.000	0.200	0.871	0.714	1.000	0.937	0.714	0.934
S3	0.140	0.159	0.167	0.259	0.273	0.266	0.273	0.264	0.134	0.955	0	0.630	0.276	0.250	0.956	0.493	0.778	0.375	0.301	0.741	0.307
S4	0.714	0.690	0.667	0.769	0.750	0.772	0.775	0.789	0.701	0.714	0.630	0	0.787	0.784	0.733	0.561	0.417	0.760	0.699	0.333	0.718
K1	0.193	0.248	0.248	0.085	0.099	0.077	0.084	0.087	0.220	1.000	0.276	0.787	0	0.056	1.000	0.500	0.840	0.347	0.226	0.840	0.230
K2	0.168	0.222	0.224	0.078	0.092	0.070	0.092	0.063	0.197	1.000	0.250	0.784	0.056	0	0.969	0.473	0.811	0.340	0.252	0.811	0.207
S5	0.963	0.959	0.965	0.971	0.972	0.971	0.972	1.000	0.966	0.200	0.956	0.733	1.000	0.969	0	0.813	0.600	1.000	0.938	0.600	0.903
K3	0.450	0.440	0.446	0.516	0.546	0.521	0.526	0.548	0.500	0.871	0.493	0.561	0.500	0.473	0.813	0	0.610	0.403	0.467	0.463	0.455
K4	0.810	0.793	0.727	0.821	0.800	0.823	0.850	0.842	0.821	0.714	0.778	0.417	0.840	0.811	0.600	0.610	0	0.840	0.753	0.333	0.746
CH9	0.303	0.310	0.348	0.365	0.377	0.352	0.358	0.333	0.376	1.000	0.375	0.760	0.347	0.340	1.000	0.403	0.840	0	0.313	0.720	0.278
S6	0.286	0.346	0.304	0.244	0.225	0.234	0.240	0.248	0.224	0.937	0.301	0.699	0.226	0.252	0.938	0.467	0.753	0.313	0	0.726	0.133
S7	0.778	0.759	0.758	0.821	0.825	0.823	0.825	0.842	0.791	0.714	0.741	0.333	0.840	0.811	0.600	0.463	0.333	0.720	0.726	0	0.718
CH10	0.200	0.257	0.221	0.200	0.197	0.190	0.197	0.187	0.211	0.934	0.307	0.718	0.230	0.207	0.903	0.455	0.746	0.278	0.133	0.718	0

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