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Genetic Diversity among Single, Double, And Triple Cross Hybrids in Beit Alpha Cucumber (Cucumis sativus)

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ABSTRACT

Genetic variation was investigated in fifteen single, eighteen three-way, and nine double hybrid crosses and their parents along with nine elite commercial cucumber hybrids. Six lines (NS5, NS9, NS17, NS29, NS31, and NS33) were used to develop these cross hybrids. Based on AFLP and SSR analysis, the studied cucumber genotypes have a narrow genetic base. The results also showed genetic differentiation compared to commercial varieties. Out of 1352 bands produced from 12 AFLP primer pairs, only 47 were polymorphic. Similarly, out of 26 SSR primers, only one primer was polymorphic. The highest heterozygosity index (0.6044), polymorphic information content value (0.5392), and marker Index (0.6044) were observed with M-CTT_E-AAG AFLP primer combination, while the highest effective multiplex ratio (2.8246) and resolving power (4.8421) were observed with M-CTC_E-AGC AFLP primer combination. Genetic distance estimation revealed clear distinctiveness of the studied genotypes with the control varieties. The cluster analysis by UPGMA showed two main clusters covering 84% of the studied genotypes grouped. This research shows that double and three-way hybrid crosses can be used in cucumber hybrid breeding.

Keywords: cucumber, genetic variation, AFLP, SSR, cluster analysis

INTRODUCTION

Genetic diversity data play an important role in cucumber breeding programs and for cucumber germplasm management. Genetic diversity in cucumber was investigated among cucumber types and between accessions from the same type of pickling cucumber, Dutch type cucumber, open-pollinated cucumber, and sliced cucumber (Fazio et al., Olfati et al., 2012, Mliki et al., 2003, Horejsi and Staub, 1999, Hu et al., 2011). Cucumber has a narrow gene base, which may limit the development of new cucumber varieties by cross-breeding (Fan et al. 2006).

Innark et al. (2013) evaluated the genetic diversity of 38 cucumber accessions collected from 9 countries using eight phenotypic traits and 20 SSR genetic markers. Three main clusters corresponding to cucumber country of origin were formed. Also, they reported that data from genetic diversity analysis could be used to select the best

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parental lines for plant breeding programs and genetic improvement in cucumbers.

Genetic relationships among cucumber germplasm were assessed by AFLP markers and showed effective, repeatable, and dependable markers for polymorphism analysis (Wang et al. 2008). Also, using AFLP markers for genetic similarity estimation in cucumber gave a better discriminating power over morphological similarity estimation. (Olfati et al. 2012). Genetic diversity among seventeen Jordanian snake melon (Cucumis melo var. flexuosus) landraces using eight AFLP primer combinations produced 403 bands among which 219 (54%) were polymorphic with the polymorphic information content ranging from 92.1% to 95.5% (Akash et al.2000). Three-way and double hybrid crosses of Beit Alpha cucumber (Cucumis sativus L.) showed superior genotypes over their parents (Altamari et al.2019). Yilmaz et al., (2012) compared the genetic relationship between melon single, three-way, and double hybrids and their parental lines using SSR markers, the similarity rate was between 0.54 – 1.00. Also, they concluded that comparing hybrids with their parent would provide a better explanation of the similarity rate as one parent clustered with its single and triple hybrid at one branch. The aim of this study was:

to estimate the level of genetic diversity among the inbred lines of cucumber and heterozygosity of the allelic loci of these inbreds using SSR and AFLP markers.

Material and methods:

The study was conducted during the 2015 growing season under greenhouse conditions at National Seed Production (NSP) Company station in Jordan valley. The lab work was done at Hamdi Mango Center for Scientific Research, The University of Jordan, Amman-Jordan.

Six inbred lines were obtained from NSP Company and they were used to develop cross hybrids. These lines are NS5, NS9, NS17, NS29, NS31, and NS33. Transplants from each parent were planted at the NSP station in Amman under plastic house conditions. These inbred lines were crossed to produce fifteen single, 18 three-way, and nine double hybrid crosses.

Male flowers were induced by silver thiosulfate. Pollination was done manually in the early morning for 7 days. Thirty-five days after pollination the mature fruits were harvested and seeds were extracted and dried.

Seeds from six parents, fifteen single hybrids, nine double hybrids, 18 three-way hybrids, and nine elite commercial cucumber hybrids as controls,(namely “Falcon star” and “MulitStar” from Rijk Zwaan, “Neddal” from Nunhems, “Fadia” from Enza Zaden, “Leader” from Daehnfeldt, “Safeer” from Yuksel, “Rami” from Fito, “Nassem” from NSP and “Karol” from HM Clause) were transplanted in the 1st of September 2015 under greenhouse conditions at NSP Company station in Jordan valley (Table1).

Table 1: Six parents, fifteen single hybrids, nine double hybrids, eighteen three-way hybrids, and nine elite commercial cucumber hybrids were used in this study.

1	NS 9X NS 17	**20**	(NS 31) X (NS 9X NS 17)	**39**	Karol F1
2	(NS 29) X (NS 9X NS 17)	**21**	(NS 33) X (NS 9X NS 17)	**40**	NS 31
3	NS 29X NS 33	**22**	Safeer F1	**41**	(NS 31X NS 33) X (NS 17)
4	NS 29X NS 5	**23**	NS 5	**42**	(2 NS 9X NS 31) X (NS 5)

5	(NS 29X NS 31) X (NS 17)	**24**	(NS 29X NS 33) X (NS 5)	**43**	(NS 33) X (NS 5X NS 17)
6	Leader F1	**25**	NS 33X NS 5	**44**	(NS 29X NS 33)X (NS 5X NS 9)
7	NS 33X NS 17	**26**	(NS 29X NS 31) X (NS 5X9 NS)	**45**	NS 31X NS 33
8	NS 29	**27**	NS 5X NS 17	**46**	(NS 31X NS 33)X (NS 9X NS 17)
9	Falcon Star F1	**28**	(NS 29X NS 33)X (NS 5X NS 17)	**47**	(NS 31)X (NS 5X NS 9)
10	NeddalF1	**29**	(NS 29X NS 33)X (NS 9X NS 17)	**48**	Mulit Star F1
11	FadiaF1	**30**	NS 33	**49**	(NS 33) X (NS 5X NS 9)
12	(NS 31X NS 33) X (NS 5X NS 17)	**31**	Rami F1	**50**	NS 5X NS 9
13	(NS 31X NS 33) X (NS 5X NS 9)	**32**	(NS 29) X (NS 5X NS 9)	**51**	NS 9
14	(NS 31) X (NS 5X NS 17)	**33**	NS 29X NS 31	**52**	NS 33X NS 9
15	(NS 29X NS 33) X (NS 9)	**34**	(NS 29X NS 31)X(NS 9X NS 17)	**53**	(NS 31X NS 33) X (NS 5)
16	NS 17	**35**	(NS 31X NS 33) X (NS 9)	**54**	(NS 29X NS 33) X (NS 17)
17	NS 31X NS 17	**36**	NS 29X NS 17	**55**	NS 29X NS 9
18	NS 31X NS 9	**37**	(NS 29X NS 31)X(NS 5X NS 17)	**56**	NS 31X NS 5
19	(NS 29X NS 31) X (NS 9)	**38**	Naseem F1	**57**	(NS 29) X (NS 5X NS 17)

The experiment was planned as an incomplete randomized block design with four plastic houses. Each plastic house was divided horizontally into 59 incomplete blocks. Each incomplete block contained six rows divided vertically; each row contained two planting lines. A total of 24 plants were planted in each incomplete block, spacing of 1.5 meters between rows and 0.4 meters between plants in a row were used. Plants material along with control hybrids were planted in 57 incomplete blocks. The first incomplete block and the last incomplete block were considered as borders.

DNA extraction

Leaves from 24 seedlings at three-week-old were collected from the controls, parents, single, three-way, and double hybrid crosses. A total of 57 leaf samples were kept at -80 ◦C until use. The DNA extraction procedure was applied on leaf samples using the CTAB technique (Torres et al., 1993).

SSR amplification and PCR reaction.

In this study 26 SSR primers (Table 2) (Fazio et al., 2002, Staub et al., 2005, and Nam et al., 2005) were used. PCR amplification reaction solution was carried out in 10µl with 1X buffer, 0.2mM dNTPs, 1.5mM MgCl2, forward primer (with M13 tail) 0.5µM, 1.5µM reverse primer (fluorescent), 1.5µM of M13 (fluorescent), 1 unit of Taq polymers and 22-30 ng of DNA per reaction. The mixture was transferred to C 1000TM Thermal Cycler (Bio-RAD). The PCR reaction was run using a touchdown program 94°C for 5 min, then consisted of subsequent 20 cycles by which the annealing temperature was lowered from 65°C by 0.7°C per cycle while denaturation degree was kept at 94°C for 45 seconds and extended at 72°C for 60 seconds. Continuously after, a set of 20 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds and 72°C for 60 seconds, then extension at 72°C for 10 min were applied.

Table 2: SSR primers and their sequences used in this study

Marker type	**Primer 5' to 3'**
CSWCT02B Forward	TTCTGCATACCCTCTCCT
CSWCT02B Reverse	CACACTTCCAGATGGTTG
CSWCT16B Forward	CTTATGGTCGGAGAAG
CSWCT16B Reverse	CTCAGATAACCCAAAATA
CSWCT25[2] Forward	AAAGAAATTAAGTCAATCAAACCG
CSWCT25[2] Reverse	CCCACCAATAGTAAAATTATACAT
CSWCT28 Forward	GAATTCAAAAGCATTTCAAAACTA
CSWCT28 Reverse	GAATTCAATTGGGTTTTTGAACCC
CSWCT30 Forward	CATGAATCTCAAGTCTTAAACCC
CSWCT30 Reverse	AAAGGATTGAGAAAGAAATTAAGG
CSWCTT08 Forward	GATATAAGCGTTGTGAGGATATGC
CSWCTT08 Reverse	CGTGCTCTATGAAGTAAATTAGTA
CSWGAAA02 Forward	AGGGCGTGTGAAAATTTGATATAA
CSWGAAA02 Reverse	TTCGAGAGTGGAGGGCACTTTCGT
CSWGAAT01 Forward	GTCGGCTTGTGAAGAGAGATTGTG
CSWGAAT01 Reverse	GTGGGCACTGGTCAGGCGTTGAGA
CSWGATT01C Forward	TATTGAAACAGAAATTAACATTGG
CSWGATT01C Reverse	TCTTATCCACATTCCATTAAGAAG
CSWGCA01 Forward	AGTGATGGTGCAGGGCTATCTTAT
CSWGCA01 Reverse	TTGTCTTCCCTCCTCTTCTCGTCT
CSWTA05 Forward	GCATGAGCTCGAGCTGGTGTAGTG
CSWTA05 Reverse	CGCCTGTTTTCATTTTGATTGGTT
CSWTA08B Forward	TTGCATTAATGCTATAAACTTACC
CSWTA08B Reverse	GAAATTAATATTTAGGCATTG
CSWTA09 Forward	CTACAAAACCTCTCATTCCTTATT
CSWTA09 Reverse	TCTACTTTTAAATTTAGCACAACT
CSWTAAA01 Forward	CAATGCCTCAATCTGATAGGAATG
CSWTAAA01 Reverse	ACTGGCTCTCTACATATTGTGAGG
AJ18SCAR Forward	GGCTAGGTGGTATGGGGATGACAT
AJ18SCAR Reverse	GGCTAGGTGGGCTTAAGTTCTTTC
AW14SCAR Forward	GGTTCTGCTCTTCATTCATTTTCA
AW14SCAR Reverse	GGTTCTGCTCTAAATAACCAAAAA
BC523SCAR Forward	ACAGGCAGACCCGACGAGGGGCAG

BC523SCAR Reverse	ACAGGCAGACAAGAGTTTGAGGAT
CS-L18-3SCAR Forward	CTCTTTCAATCATCTTTCTTCTCT
CS-L18-3SCAR Reverse	ATCATAACAATGATATATTTTACG
J5SCAR Forward	CTCCATGGGGTGCACGTTAACGTT
J5SCAR Reverse	CTCCATGGGGCAGCTAAACAGCGG
CMGA165 Forward	CTTGTTTCGAGACTATGGTG
CMGA165 Reverse	TTCAACTACAGCAAGGTCAGC
NR2 Forward	CTGAAAGCAGTTTGTGTCGA
NR2 Reverse	AAAGAAGGAAGAGGCTGAGA
NR60 Forward	AAGCACTTAAATGAGAATCG
NR60 Reverse	AATAGTAGCCTGTTATATCC
CsACS1G (F gene) Forward	CAA CCA GCT TTA GAA CAA GC
CsACS1G (F gene) Reverse	ACT TCA ATC TTC GGA TAG CG
AJ6SCAR Forward	GAT GGC AGT CTG ATA ACT ATG TGA
AJ6SCAR Reverse	GAT GGC AGT CGG GAA GGT CAG TTG
M8SCAR Forward	TCT GTT CCC CAT ACA AGA ATT AAA
M8SCAR Reverse	TCT GTT CCC CAT GAT GTA GAC TTC
CSWCTT14 Forward	AAAATATGAAACCCATGGACATGA
CSWCTT14 Reverse	GATTAAATATTGGGAATTGCTAA

AFLP analysis

AFLP analysis was performed using 12 primer combinations (Table 3). The AFLP protocol was used based on the method of Vos, et al. (1995) with modification done by Akash and Kang, (2009). The protocol included four major steps as the following: restriction digestion of genomic DNA, ligation of adaptors, pre-amplification, and selective AFLP amplification.

Table 3: Adapters and primers used for ligation, pre-amplification, and selective amplification for the AFLP procedure.

Name of Primer/Adapter	**Sequence (5'-3')**
Ligation	
Eco RI adapter	CTCGTAGACTGCGTACC AATTGGTACGCAGTC
*Mse*I adapter	GACGATGAGTCCTGAG TACTCAGGACTCAT
Preamplification	
Eco RI primers E-A	GACTGCGTACCAATTCA
*Mse*I primers M-C	GATGAGTCCTGAGTAAC
Selective amplification primers	
E-AAC labeled (IRDye 700)	GACTGCGTACCAATTCAAC
E-AAG labeled (IRDye 700)	GACTGCGTACCAATTCAAG
E-AGC labeled (IRDye 800)	GACTGCGTACCAATTCAGC
E-AGG labeled (IRDye 800)	GACTGCGTACCAATTCAGG
M-CTT	GATGAGTCCTGAGTAACTT
M-CAG	GATGAGTCCTGAGTAACAG

M-CTA	GATGAGTCCTGAGTAACTA
M-CAA	GATGAGTCCTGAGTAACAA
M-CTC	GATGAGTCCTGAGTAACTC

Gel analysis of AFLP and SSR reaction products

The AFLP and SSR products were analyzed using a Li-Cor DNA analyzer (Li-Cor, Bioscience, Lincoln, NE). Before loading the gel, the AFLP and SSR products were denatured for 5 min at 95ºC at C 1000TM Thermal Cycler and then quickly cooled on ice. Subsequently, 0.8µl of each denatured sample and 0.8µl of the DNA ladder (50-700bp) (Li-Cor, Bioscience, Lincoln, NE) were loaded in 59 wells. The DNA ladder was loaded at the first well and the last well band size determination. This location for the ladder was chosen to adjust for uneven band migration on the gel (necessary for SAGA software) and to facilitate different gel comparisons. Gel electrophoresis was run for three hours to resolve fragments up to about 700bp. The gel images were analyzed and the observed bands were scored by Saga Generation 2 software with GT and MX modules client version 3.1.0 build 315 (Li-Cor, Bioscience, Lincoln, NE) and reviewed manually After band scoring, the data were translated to a numerical data in which the presence (1) and the absence (0) on Excel® spreadsheet program.

Genetic analysis

The band's numerical data for the 57 genotypes were analyzed as 1 for the present, 0 for absent, and 9 for miss bands. The polymorphism information about AFLP and SSR markers that determines their application was calculated for each primer combination using Heterozygosity index (H), Polymorphic Information Content (PIC), Effective multiplex ratio (E), Marker Index (MI), Discriminating power (D) and resolving power (R). These polymorphism analyses were estimated using the iMEC online program (Amiryousefi et al., 2018). Cluster representation of the data was constructed using the Unweighted Pair Group Method, arithmetic Average (UPGMA) where the similarity between genotypes was estimated according to Dice, (1945). The presence and absence matrix was entered into NTSYSpc 2.0 software (Rohlf, 1998).

Results and discussion

Genetic diversity using SSR and AFLP primers:

Assessment of AFLP and SSR primers:

Twenty-six SSR primers and 12 AFLP primer combinations were used to simulate differences between all experimented genotypes. These primers gave reliable and reproducible polymorphism; SSR primers showed a low level of polymorphic with 1/26. From the 26 experimented SSRs, only CSWCT252 gave one polymorphic loci (Figure 1) also 24 SSRs gave a monomorphic allele and AJ6SCAR gave no amplification. Innark et al., (2013) evaluated genetic diversity in cucumber using microsatellite markers, and only 20 SSR out of 300 SSR markers were successfully amplified in the experimented plant material. Low polymorphism level was obtained using SSR markers on single, triple, and double-cross melon hybrids (Yilmaz et al., 2012).

Similar to SSR, AFLP primer combinations generated a total of 1305 monomorphic and 47 polymorphic bands (Table 4). The generated band sizes ranged from 50 to 714bp (Figure 2). The number of amplified DNA fragments per primer ranged from 64 bands (primer combination 12 (CAA and AGC)) to 149 bands (primer combination 5 (CAG and AAC)) and the percentage of polymorphism ranged from 0% (primer combination 7-8 (CTA and AAC/AGC)) to13% (primer combination 1 (CTC and AAC)). A very low polymorphism with 3.6% was recorded among all the AFLP combinations. A low level of polymorphism (21.7%) was reported in a cucumber genetic study using 24 AFLP combinations (Wang et al., 2008). However, the used 24 AFLP marker

combinations were selected from about 50 pairs of AFLP primers. Conversely, high levels of polymorphism using AFLP primer pairs were reported in watermelon (93.4%) (Hwang et al., 2011) and melon (93.25%) (Danesh et al. 2015). For the AFLP marker, the primer combination (M-CTT_E-AAG) scored the highest polymorphic information content (PIC = 0.5392), heterozygosity index (H = 0.6044), and marker index (MI = 0.6044) which determine the general usefulness of molecular markers (Table 5). However, the primer combination (M-CAA_E-AAC) scored the lowest PIC value (0.0), E ratio (0.0) and MI (0.0). On average, the ten analyzed AFLP primer combinations scored a PIC value of 0.3047 while the cswct252 SSR marker scored 0.0948. in another hand, the highest effective multiplex ratio (E = 2.8246) and resolving power (R= 4.8421) were observed with the primer combination (M-CTC_E-AGC), and the lowest E of 0.0351 was observed with the primer combination (M-CAA_E-AGC) with an average E value of 1.0 per AFLP primer combination (Table 5). According to our results, AFLP was more informative than the cswct252 SSR marker (E= 0.0526) mainly due to its higher effective multiplex ratio.

Table 4: AFLP primer combinations and their monomorphic and polymorphic bands tested on the 57 cucumber genotypes.

Combination number	Primer combination		Total number of bands		Polymorphism %
	MseI	*EcoRI*	Monomorphic	Polymorphic	
1	CTC	AAC	100	13	13
2	CTC	AGC	108	7	6.5
3	CTT	AAG	122	7	5.7
4	CTT	AGG	115	7	6.1
5	CAG	AAC	149	2	1.3
6	CAG	AGC	110	3	2.7
7	CTA	AAC	140	0	0
8	CTA	AGC	106	0	0
9	CTT	AAC	106	3	2.8
10	CTT	AGC	83	2	2.4
11	CAA	AAC	102	1	1
12	CAA	AGC	64	2	3.1
Total			1305	47	3.6

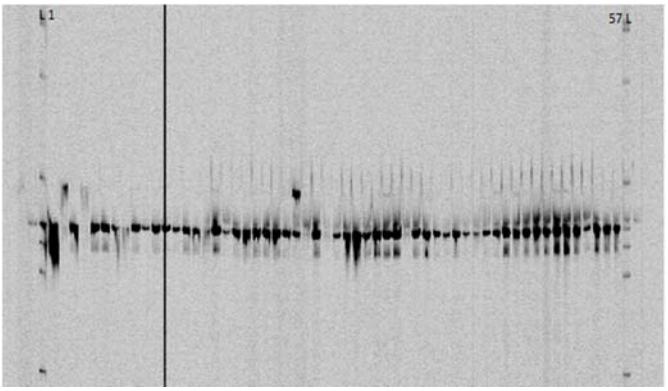

Figure 1: SSR profile obtained from the 57 cucumber genotypes using CSWCT2 primer. Lanes from 1-57 represent the experimented germplasm. Lane L indicated the DNA size marker

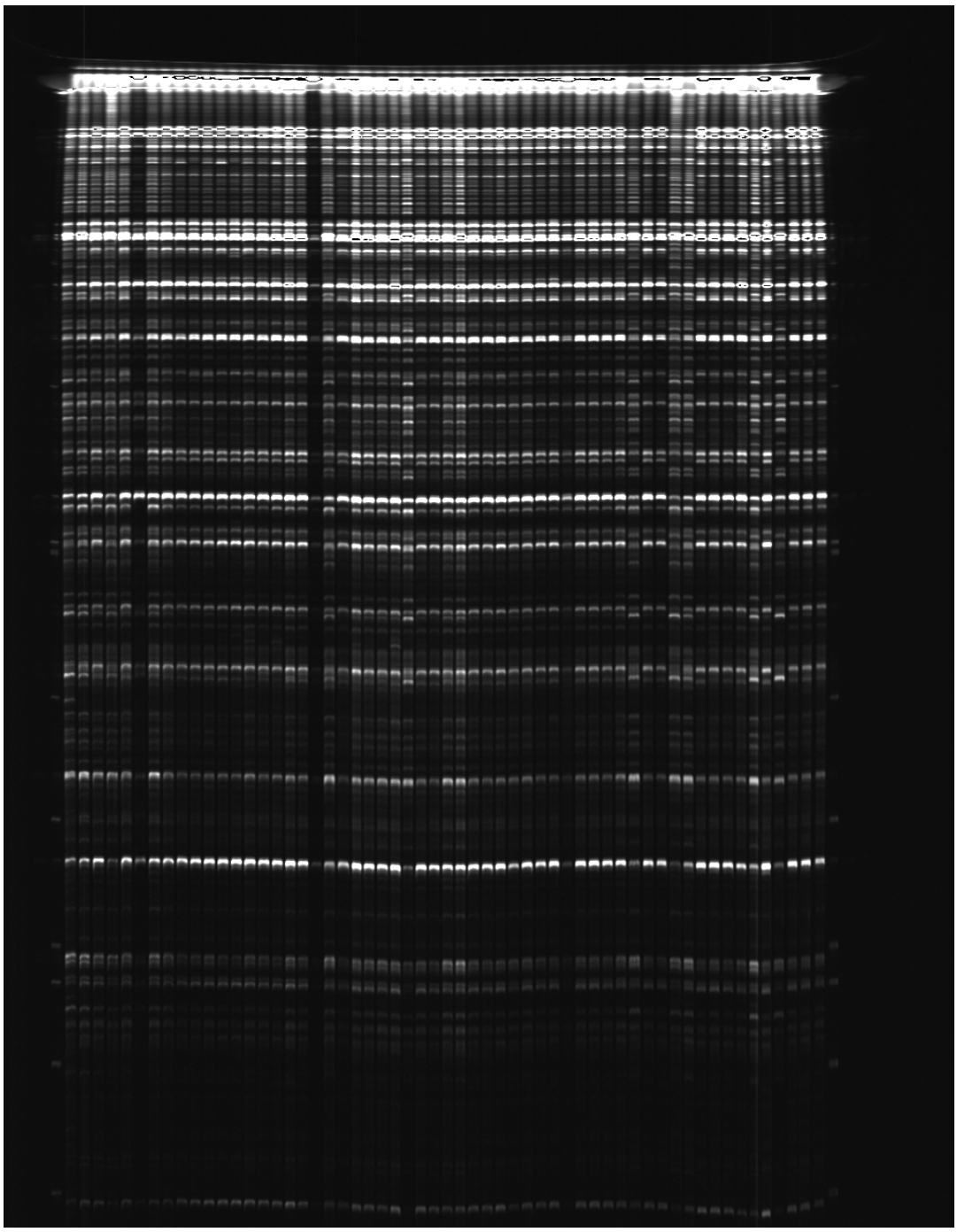

Figure 2: AFLP profiles of the 57 cucumber genotypes analyzed with Li-Cor analyzer using the E-AGC/M-CAA selective primer combination.

Table 5 Polymorphism statistics for the ten amplified AFLP primer combinations and cswct252 SSR marker were estimated using the iMEC online program (Amiryousefi *et al*., 2018).

DNA marker	Primer/combination	Heterozygocity index (H)	Polymorphic Information Content (PIC)	Effective multiplex ratio (E)	Marker Index (MI)	Discriminating power (D)	Resolving power (R)
AFLP	M-CTC_E-AAC	0.4492	0.3629	1	0.4492	0.3177	.
	M-CTC_E-AGC	0.4983	0.3741	2.8246	0.0041	0.7791	4.8421
	M-CTT_E-AAG	0.6044	0.5392	1	0.6044	0.1708	.
	M-CTT_E-AGG	0.5401	0.4365	1	0.5401	0.3193	.
	M-CAA_E-AAC	0	0	1	0	0	0
	M-CAA_E-AGC	0.0677	0.0654	0.0351	0	0.9994	0.0702
	M-CTT_E-AAC	0.1787	0.1705	1	0.1787	0.1817	.
	M-CTT_E-AGC	0.1625	0.1538	1	0.1625	0.1654	.
	M-CAG_E-AAC	0.2413	0.2122	0.1404	0.0006	0.9825	0.2807
	M-CAG_E-AGC	0.5	0.375	1	0.0044	0.7522	1.6842
	Average	0.3047	0.2572	1	0.2155	0.4351	1.2983
SSR	cswct252	0.0997	0.0948	0.0526	0.0001	0.9981	0.1053

Genetic similarities among cucumber genotypes:

Only polymorphic alleles obtained from 26 SSR and 12 AFLP primers were analyzed using NTSYSpc Version 2.0 software (Rohlf, 1998). Dice similarity coefficient (Dice, 1945) which is a matching coefficient for binary data, was used to cluster genotypes with the unweighted pair group method with arithmetic average (UPGMA). Dice similarity coefficient ranged from a high of 0.98 to a low of 0.48. The highest genetic similarity percentage (0.98) was observed between NS17 (NS31X NS17) and NS18 (NS31X NS9); also the lowest similarity coefficient (0.32) was recorded between the NS8 with NS29 and NS51with NS9.

Dendrogram based on AFLP and SSR data discriminated between genotypes (Figure 3). However, 48 genotypes clustered in two major clusters B and D. These groups contained 4 sub-groups and represented 8 parents, 16 three-way cross hybrids, 8 single crosses, and 8 double-crosses. Also, the control varieties integrated with the studied genotypes in clusters B, C, and D. narrow cucumber gene pool was reported in cucumber and the accessions from the similar region were clustered together (Innark et al., 2013). Also, the genetic distance between Mediterranean cucumbers varieties bred in Netherland was ranged between 0.06 and 013 and confirmed the close relationship (Staub et al., 2005). An average of 0.936 Nei's similarity index calculated from 8 AFLP informative primers was reported from 18 local Turkish genotypes (Karakurt et al., 2020). Yilmaz et al., (2012) compared the genetic relationship between melon single, three-way, and double hybrids and their parental lines using SSR markers and found that the similarity rate was between 0.54 – 1.00. They also were concluded that comparing hybrids with their parents would provide a better explanation of the similarity rate, as one parent clustered with its single and triple hybrid at one branch. While in corn, a value of 0.95 genetic distance (GD) was reported in double hybrid crosses when single crosses were obtained from different seeds companies (Balestre et al., 2008). However, a lower value of 0.65 GD was observed when single crosses were obtained from the same seed company.

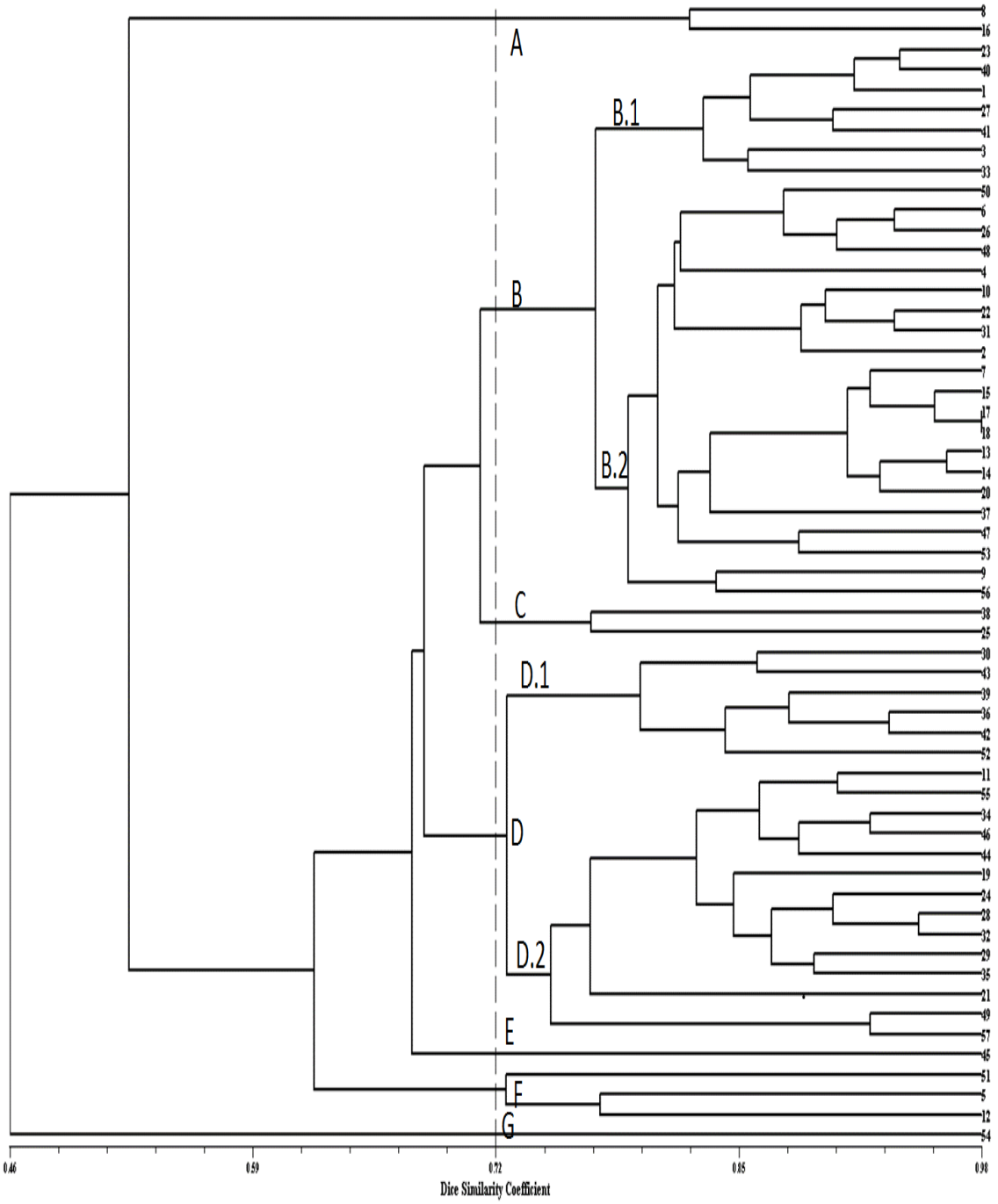

Figure 3: UPMGA dendrogram for 57 cucumber genotypes determined using genetic similarity data obtained from 12 AFLP primers combination and 26 SSR primers analysis using NTSYpc 2.0 software. Numbers from 1 to 57 are referred to as genotypes listed in Table 1.

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المنتجة من هُجن احادية و ثناية و ثلاثية (Beit Alpha) دراسة ألإختلافات الوراثية لأصناف الخيار

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ملخص

تم تحقيق الاختلافات الوراثية لاصناف الخيار في 15 هجين احادي ,18 هجين ثلاثي و 9 هجن وابائهم جنبا الى جنب مع 9 هجن تجارية. وتم استخدام ستة خطوط (NS5, NS9 , NS 17 , NS29 , NS 31 , NS33) لتطوير هذه الهجن. وبناء على تحليل ال AFLP و SSR فقد كانت الانماط الجينية للخيار المدروسة لها قاعدة وراثية ديقة, كما اظهرت النتائج تمايزا وراثيا مقارنة مع الاصناف التجارية. ومن بين ال1352 نطاقا تم انتاجها من 12 زوجا اوليا من الAFLP, كان 47 فقط منها متعددة الاشكال. بالمثل, من اصل 26 SSR الاولي, كان واحدا فقط متعدد الاشكال. ولوحظت اعلى مؤشرات التغاير الزايجوتي (0.6044) ومحتوى المعلومات متعددة الاشكال (0.5392), ومؤشر العلامة (0.6044) مع المزيج الاولي M–CTT_E–AAG AFLP, بينما لوحظت اعلى نسبة مضاعفة فعالة (E2.8246) وقدرة حل (R=4.8421) مع المزيج الاولي M–CTT_E–AAG AFLP. وكشف تقدير المسافة الجينية عن تميز واضح للانماط الجينية المدروسة مع الاصناف الضابطة. اظهر التحليل العنقودي بواسطة UPGMAوجود مجموعتين رئيسيتين تغطيان 84% من مجموعات الطرز الوراثية المدروسة, ويظهر هذا البحث انه يمكن استخدام التهجينات المزدوجة والثلاثية الهجينة في تربية الخيار الهجين.

الكلمات الدالة: خيار, التباين الوراثي, AFLP, SRR, التحليل العنقودي.