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Genetic Variation in Bell Peppers (*Capsicum annuum* L.) Collected from Iraqi Local Markets by Using AFLP Marker

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Abstract**:** *Genetic variation was studied in 20 local and imported samples of bell pepper fruits (Capsicum annuum L.), which collected from Iraqi local markets by using amplified fragment length polymorphism (AFLP) marker. Three selective primer combinations were used in all samples. The first selective primer combination (E_{ACG}/M_{AGG}) produced 4 bands for the studied bell pepper fruits with 3 monomorphic bands in each sample, except the orange and yellow Jordanian bell pepper samples, which produced 4 bands one of them polymorphic band with 25% ratio and 21.43% primer efficiency with 100% discriminatory power. The second selective primer combination (E_{AAG}/M_{AAT}) produced 4 monomorphic bands in each sample with no polymorphic bands with 28.57% primer efficiency and 0% discriminatory power, whereas the third selective primer combination (E_{ACA}/M_{CTG}) produced 7 monomorphic bands in each sample, except the Iraqi/Balad green bell pepper, which produced 3 monomorphic bands with no polymorphic bands and the primer efficiency is 50% with 0% discriminatory power. The results of dendrogram of the studied samples by using Jaccard coefficient for genetic similarity was distributed the samples into 3 main groups. The first group was included the red and orange Jordanian bell pepper fruits samples, whereas the second group included the Iraqi/Balad green bell pepper and the third group included the rest samples. The results of this distribution revealed a high similarity between red and orange Jordanian bell pepper and the other bell pepper samples, except Iraqi/Balad green bell pepper with 0.94 values. This value is the higher, whereas the similarity with the Iraqi/Balad green bell pepper is 0.68 values. The genetic distance between studied samples revealed the highest values between red and orange Jordanian bell pepper and the Iraqi/Balad green bell pepper with 2.236 values.*

Keywords: *AFLP, Bell pepper, Capsicum annuum L., Genetic variation*

1. Introduction

Pepper belongs to the family Solanaceae and it is included 27-30 species, however only five species are domesticated which are *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* [1], [2]. It is believed that these five domesticated species are derived from three distinct genetic species while *C. pubescens* and *C. baccatum* derived from two independent species, and *C. annuum*, *C. chinense* and *C. frutescens* derived from species which is may be descended from an independent or non-independent type; also it is believed that South America is the main source of pepper species [3], [4]. The pepper *C. annuum* is one of the most important of other five species and includes a hot genre Pungent (Hot) and cold type sweet (mide). The hot pepper contains alkaloid compounds which are responsible for the hot taste called Capsaicinoids; this has many medical benefits as well as its importance in the pharmaceutical industry. Capsaicinoids are not found in the sweet pepper; however it's being an important source of nutrients such as carotenoids, polyphenols and vitamins [5]. The classification of this genus of pepper is difficult sometimes when dependents on its morpho-agronomic characteristics due to the effecting the morphological characteristics by environmental factors [2], and also there is no accuracy in the distinguishing between closely genetic types [6]. The pepper *Capsicum annuum* L. is a type of vegetables with different types, with high nutritional value. Furthermore, it is an important source of vitamins A, C, and E, many antioxidant agents, which used as a natural flavor and colorful

for foods, as well as has multiple medical benefits including anti-inflammatory, anti-allergic anti-allergenic and anti-carcinogenesis agent. It was found that using the mature red peppers reduced the risk of cancer [5], [7], [8]. The genome of *Capsicum* pepper consists of 12 pairs of chromosomes with the size haploid chromosomal group estimated 3.3-3.6G base pair [9], [10]. The information about genetic variations help to increase the efficiency, development and improvement of the species in pepper, as many farmers turned to crops that with high production qualities and desired properties rather than the traditional cultivars [11], [12], although the peppers is more vegetables farmed, there is no sufficient genetically and molecular studies in comparison to other crops, due to the difficulties to find a good genetic findings; DNA markers are good markers to study the pepper because it's highly conserved, especially the cultivated pepper which is less in variation than wild types, as well as the sweet pepper with a large fruit shows little variation compared to the diversity of the small fruit in chili [13]. The genetic diversity and evolution have been studied and diagnosis of classes of the pepper as well using the phenotypic chromosomes [4], [14], analysis of allozymes [15] and electrophoresis of soluble proteins [17], [18], [19], [20], [21] and isozyme analysis [22]. The analysis observed that the diversity in the isozyme was low in the varieties of *C. annuum* [18], thus the analysis of isozyme is using in the breeding programs of pepper, especially when combing different types of *Capsicum* in such programs. Amplified fragment length polymorphism (AFLP) technique is dependent on the detection of DNA fragments that cutting with restriction enzymes through amplification using Polymerase chain reaction (PCR) [23]. This technique has been applied to the analysis of diversity between varieties in various fruits and types of walnut trees, also this technique showed a high significant levels in the detection of DNA polymorphism as well as one of the most techniques promising fingerprinting studies, genetic mapping and studies the genetic variations. Moreover, the benefits of this technique is be given a large number of amplified DNA fragments in each PCR reaction [24]. This technique has been used in many genetic variations in the pepper studies [6], [11], [25], [26], [27], [28], [29]. The study aimed to determine the possibility to detect polymorphism in the fruits of the large sweet pepper (bell pepper) that locally grown and imported from other countries by using AFLP technique in the detection of genetic variation in the studied samples and to study the genetic relationship between the studied fruits of pepper using Unweight Pair Group Method with Arithmetic Mean (UPGMA) analysis.

2. Materials and Methods

2.1. Plant Materials

Twenty samples of bell pepper fruits were collected from markets in Baghdad-Iraq. Many samples were imported from other countries and no information was available if these samples were cultivated or genetically modified (Table 1).

TABLE I: Samples of bell pepper (*Capsicum annuum* L.) with their Country of Origin

Sample No.	Country of pepper origin	Pepper color
1	Jordan	Orange
2	Jordan	Red
3	Jordan	Yellow
4	Jordan	Green
5	Spain	Green
6	Iraq/Balad	Green
7	Iraq/Yousifia	Green
8	China	Red
9	Iraq/Souwyera	Green
10	China	Yellow
11	China	Orange
12	Spain	Green
13	Iran	Red
14	Iran	Orange
15	Iran	Yellow
16	Italy	Orange
17	Italy	Yellow
18	Spain	Red
19	Spain	Red
20	Iran	Green

2.2. DNA Extraction

DNA from bell pepper was extracts by using Kang and Yang [30] method with some modification. About 1gm from each sample was dried by using liquid nitrogen. Each sample was transferred to 1.5 ml microfuge tube and homogenized in 100 µl of DNA extraction buffer (100mM Tris-HCl pH7.5, 50mM EDTA pH7.5, 500mM NaCl and 10mM β-mercaptoethanol pH7.5) using a hand-operated homogenizer with a plastic pestle for 15~20 sec. After an initial homogenization, another 300 µl of DNA extraction buffer were added and homogenized with the same homogenizer for 15~20 sec. Then 40 µl of 20% SDS were added and vortex for 30 s. The samples were incubated at 65°C for 15 min for cell lysis. Ten microliter from stock solution (10mg\ml) of DNase-free RNase A was added and incubation was done under 37ºC for 60 min. For protein removal a 200 mg of Proteinase K enzyme was added and the mixture was incubated at 50ºC for 60 min. An equal volume of phenol\chloroform\isoamyl alcohol (25:24:1) was added to the samples, mixed by vortex for 30 sec, and then centrifuged at 13,000 g for 3 min at 4°C. The supernatant was transferred to a fresh tube and extracted one more time with phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged at 13,000 g for 10 min at 4°C. The supernatant was transferred to a fresh tube and extracted with 500 µl of chloroform and then centrifuged at 13,000 g for 10 min at 4°C. The supernatant was transferred to a fresh tube, and a 1\10 of the total volume of the sample 3M NaOAc was added. A double volume of 95% ethanol was added to each sample, mixed well, and the samples were incubated at -20°C for 30 min. The samples were centrifuged at 13,000 g for 30 min at 4°C. The pellet was washed with 70% ethanol and centrifuged at 13,000 g for 10 min at 4°C. The pellet was dried for 10 min, and resuspended in 100 µl TE buffer (1 mM Tris-HCl, 0.1mM EDTA pH8). The concentration and purity were determined by using Nanodrop (Act Gene, USA). The extract DNA was stored at -20ºC.

2.3. AFLP Primers and Adapters

Primers and adapters for genetic variation study by AFLP marker in bell pepper fruits were used according on [23], [31], and synthesized at Alpha DNA Company (Canada) (Table 2).

TABLE II: The Nucleotide Sequence of Primers and Adapters used in Characterization of 20 Bell Peppers Samples by AFLP Markers

Primer	Sequence (5´ → 3´)
*Eco*R1 adaptors	5´-CTCGTAGACTGCGTACC-3´ 3´-CATCTGACGCATGGTTAA-5´
*Mse*I adaptors	5´-GACGATGAGTCCTGAG-3´ 3´-TACTCAGGACTCAT-5´
*Eco*R1 primer+A (E_A)	5´-GACTGCGTACCAATTC+A-3´
*Mse*I primer+C (M_C)	5´-GATGAGTCCTGAGTAA+C-3´
E_{ACG}	5´- GACTGCGTACCAATTC +ACG-3´
E_{AAG}	5´- GACTGCGTACCAATTC +AAG-3´
E_{ACA}	5´- GACTGCGTACCAATTC +ACA-3´
M_{AGG}	5´-GATGAGTCCTGAGTAA+AGG-3´
M_{AAT}	5´-GATGAGTCCTGAGTAA+AAT-3´
M_{CTG}	5´-GATGAGTCCTGAGTAA+CTG-3´

2.4. AFLP Reactions

Genetic variation in bell pepper was studied according to Vose *et al*. [23] with some modifications. Approximately 250 ng (18 µl) of high molecular DNA was digested in 1.5 ml microfuge tube with 1.25 U\µl *Eco*R1 (Promega, USA) and 1.25 U\µl MesI (BioLab, UK) restriction enzymes in 5X restriction reaction buffer (50 mM Tris-HCl pH7.5, 50mM Mg-acetate, 250 mM K-acetate) with a final volume of 25 µl. The reaction was done by incubation at 37ºC for 2 hrs. Then the reaction mixture was incubated 70ºC for 15 min for restriction enzymes inactivation. Two different adaptors, for *Eco*RI and *Mse*I, were ligated to the ends of the genomic restriction fragments. The digested and ligated template DNA was pre-amplified using EcoRI+1 and *Mse*I+1 primer in a total volume of 50 µl containing 24 µl of Adapter/ligation solution (0.4 mM Adenosine triphosphate, 10 mM Tris-HCl, 10 mM Mg-acetate, 50 mM K-acetate) and 1 µl T4 DNA ligase buffer (1 U\µl T4 DNA ligase, 10 mM Tris-HCl pH 7.5, 1 mM Dithiothreitol, 50 mM KCl, 0.1 mM EDTA, 50% Glycerol) to each previous

reaction (25 µl). The reaction was done by incubation at 20ºC for 2 hrs. The completeness of the digestion was checked on a 1.5% agarose gel. Ligation mixture was diluted 10 times in TE for the pre-selective amplification with *Eco*R1+A (E_A) and *Mes*I+C (M_c). Pre-selective amplification was done in 0.2 ml microfuge tube by adding 12.5 µl 2X PCR Go Taq® Green Master Mix (Promega, USA), 20 µl (2.5 pmol) *Eco*R1+A primer, 20 µl (2.5 pmol) *Mes*I+C primer, 5 µl ligation mixture in a final volume of 60 µl. The reaction mixers were placed in thermal cycler (Esco, Singapore), and the amplification profile was 30 sec at 94°C incubation, followed by 20 cycles of 30 sec at 94°C for denaturation, 60 sec at 56°C for annealing and 60 sec at 72°C for extension. Quality of the pre-selective amplification was checked on a 1.5 % agarose gel and were then diluted four-fold with TE buffer prior to the selective amplification step. Selective amplification was performed in a 50 µl final volume containing 5 µl of pre-amplification products, 12.5 µl 2X PCR Go Taq® Green Master Mix, 20 µl (5 pmol) E_{ACG} primer, 20 µl (5 pmol) M_{AGG} primer. Another three primer combinations E_{AAG}/M_{AAT} and E_{ACA}/M_{CTG} were also used for selective amplifications. The reaction mixers were placed in thermal cycler (Esco, Singapore), and the amplification profile was 3 min at 94°C, 12 cycles of 30 sec at 94°C, 30 sec at 65°C with decreasing for 0.7°C after each cycle down to 55.9°C and 60 sec at 72°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55.9°C and 60 sec at 72°C. Amplification products were separated on a 6% polyacrylamide gel (45 gm (7.5 M) Urea, 15 ml (40%) Acrylamide:Methylenbisacrylamid (19:1), in 10 ml 10X TBE, final volume up to 100 ml with ddH_2O). For 10 ml of this solution 10 µl (10%) Ammonium persulphate and 30 µl Tetramethylethylene diamine (TEMED) was added. Ten micrliter of each amplification product was loaded and equal volume (10 ul) of formamide dye (98% formamide, 10mM EDTA pH 8.0, and 0.005% bromophenol blue and 0.005% xylene cyanol as tracking dyes). Then denatured at 94ºC for 5 min and placed immediately on ice. About 5 µl was loaded onto 6% polyacrylamide denaturation gel. The DNA 100 bp ladder (Promega, USA) was also loaded on the polyacrylamide gel. The gel electrophoresis was performed by using 75V for 3-4 hrs and stained with ethidium bromide (Promega, USA) for 15 min. The gel was documented with gel documentation system (Biocom, USA).

2.5. Band Scoring and Data Analysis

The resulted amplified bands by using AFLP marker were converted to two-dimensional matrix. The results between the studied samples were analyzed by using Past software ver. 1.92 [32]. The percentage of polymorphic bands, primer efficiency (number of monomorphic bands\total number of bands x 100) and the discriminatory power (number of polymorphic bands\total number of bands resulted from all the primer combinations x 100) were calculated. For diversity analysis, bands were scored manually as present (1) or absent (0). Only the clearest and strongest bands were recorded and used for the analysis. A square symmetric matrix of similarity was then obtained with the Jaccard's similarity coefficient. The average similarity matrix was used to generate a tree for cluster analyses by UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Euclidean coefficient was used for genetic distance study.

3. Results

3.1. Amplification of DNA in Pepper Fruit Samples by Using AFLP Marker

The genetic variation was studied by using AFLP marker for three selective primer combinations E_{ACG}/M_{AGG}, E_{AAG}/M_{AAT} and E_{ACA}/M_{CTG}. These combinations were produced 15 bands in all the studied samples. The first selective primer combination (E_{ACG}/M_{AGG}) produced 4 bands for the studied bell pepper fruits with 3 monomorphic bands in each sample, except the orange and yellow Jordanian bell pepper samples, which produced 4 bands one of them polymorphic band with 25% ratio and 21.43% primer efficiency with 100% discriminatory power. The second selective primer combination (E_{AAG}/M_{AAT}) produced 4 monomorphic bands in each sample with no polymorphic bands with 28.57% primer efficiency and 0% discriminatory power, whereas the third selective primer combination (E_{ACA}/M_{CTG}) produced 7 monomorphic bands in each sample, except the Iraqi/Balad green bell pepper, which produced 3 monomorphic bands with no polymorphic bands and the primer efficiency is 50% with 0% discriminatory power (Table 3, Fig. 1).

TABLE III: Selective Primer Combinations, Number of Polymorphic Amplicons and Polymorphic Information Content in AFLP Analysis of Bell Pepper

Combination	Mb	Pb	Pb (%)	Pe	Dp
E_{ACG}/M_{AGG}	3	1	25	21.43	100
E_{AAG}/M_{AAT}	4	0	0	28.58	0
E_{ACA}/M_{CTG}	7	0	0	50	0
Total	14	1	-	100	100

Mb: Monomorphic bands, Pb: Polymorphic bands, Pe: Primer efficiency, Dp: Discriminatory power

The decreasing or lack of genetic variation is due to the nature of the auto- pollination of plant classes and also to the genetic basis, as it is known that the plant species with auto- pollination (inoculation) such as tomato *Lycopersicon esculentum* (belonging to the family Solanaceae) showed the low rate in the morphological diversity in own genome [33], [34], [35]. Kochieva and Ryzhova [13] revealed that the genetic variation was low in the fruits of pepper, they observed that the morphological diversity rate was16.5% when using 9 pairs of primers for AFLP technique. Additionally, the low diversity rate was confirmed by [36] when testing samples of Italian peppers and Colombian pepper as well; the low rate of variation suggests a substantial convergence of varieties, as the wild type, which was accession of it, is a limited genepool [28]. Moreover, [29] explained that the low genetic variation in Italian pepper due to the continuous selection for certain types according to the standards prescribed by the breeder. The various geographic regions may contribute to the presence of genetic variation of hereditary between the types of pepper that harvested from several countries while, the genetic variation was less between the types of Ethiopian chili pepper with long fruits as showed in the study of Geleta *et al*. [11] when using AFLP technique. AFLP technique has many limitations, including in the case that the similarity in nucleotide sequences less than 90%, the selective amplification that used in AFLP technique may contribute in some of the pieces due to the large variation in nucleotide sequences in different samples [37]. Aktas *et al*. [6] found that the percentage of polymorphism was 26% and the highest number of bands was up to 60 bands appeared after using selective amplification of $E_{ACG}\backslash M_{AGT}$, and the lowest number of bands was up to 29 bands after using selective amplification $E_{AAG}\backslash M_{AAT}$, and the number of bands was different to the results of the present study after using the same selective amplification as described above, and this is due to the difference in the samples of pepper in both studies in terms of their genotype and the region of collection. Moreover, the reason is related to methods to produce PCR products, as the current study dependent on ethidium bromide dye, which its accuracy less than silver nitrate dye and radioactive labeling. Aktas *et al*. [6] observed that the difficulty in comparing its results with the results of previous studies is due to the different genotypes of the peppers samples, as well as the different primers of AFLP; the low rate of genetic variation is resulting from the excessive artificial selection of agricultures of pepper, as is the case of pepper cultivated in Turkish Alata Institute. Most varieties of pepper in Turkey are open pollinated and some varieties local landraces are still grown in multiple small farms, according to the consumer's requirements, thus many breeders have switched from growing traditional varieties (types) to the cultivation of commercial pepper with high quality and desired properties [38]. Like this low polymorphism in the studied varieties resulting from limited gene pool of pepper with large fruits, as well as reflects the conservative nature of the pepper genome domesticated due to auto-pollination (inoculation) [39]. The genetic variation of the Indian and Taiwan pepper was a close in a study using 8 pairs of AFLP primers for 59 genotypes of pepper; 56 of them was *C. annuum* and only three was *C. baccatum*. These primers produced 414 bands, 389 of it was monomorphic and dendrogram put *C. baccatum* in one group while other types were in a separated group included 9 different clusters. It was found by Krishnamurthy *et al*. [40] that the reason for this finding was to the founder effect and humeral pollination that causes including several genotypes (collected from several geographic locations) in one group [41], [42].

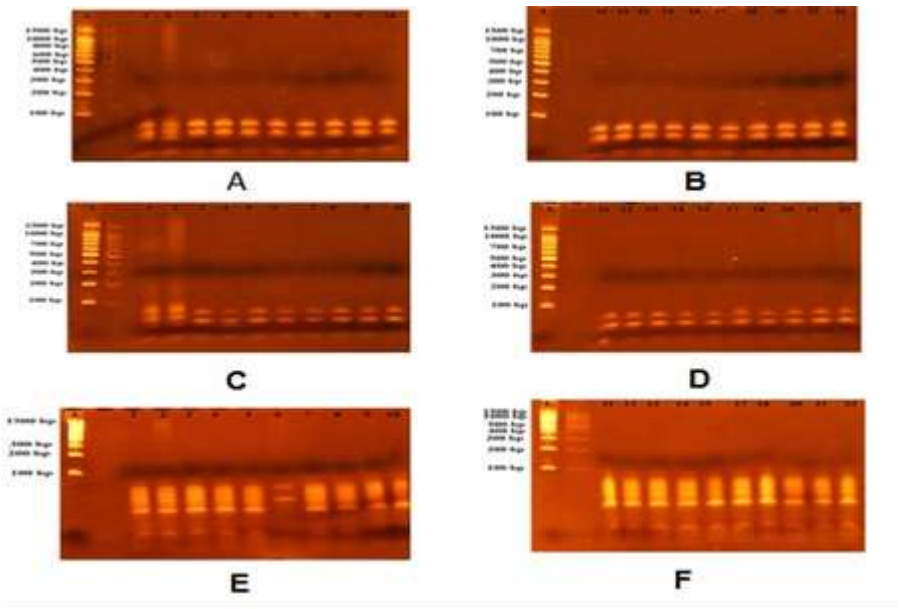

Fig. 1: Fingerprinting of 20 samples of bell pepper by AFLP marker using three primer combinations. The PCR products were electrophoresed on 6% denaturing polyacrylamide gel, 75 Volt for 3-4 hrs. A and B selective primer combination EACG/MAGG, C and D selective primer combination EAAG/MAAT, E and F selective primer combination EACA/MCTG. Numbers refer to bell pepper samples. 1: Jordanian/orange, 2: Jordanian/red, 3: Jordanian/yellow, 4: Jordanian/green, 5: Spanish/green, 6: Iraqi/Balad green, 7: Iraqi/Yousifia green, 8: Chines/red, 9: Iraqi/Souwyera green, 10: Chines/yellow, 11: Chines/orange, 12: Spanish long/green, 13: Iranian/red, 14: Iranian/orange, 15: Iranian/yellow, 17: Italy/orange, 18: Italy/yellow, 20: Spanish/red, 21: Spanish long/red, 22: Iranian/green. L: DNA 100 bp ladder.

3.2. Cluster Analyses of the Bell Peppers Depending on AFLP Marker

Genetic dendrogram was painted depending on the results which obtained from selective primer combinations results in the studied bell pepper fruits using Jaccard genetic similarity coefficient. The results of UPGMA dendrogram of the studied samples by using Jaccard coefficient for genetic similarity was distributed the studied samples into 3 main groups. The first group was included the red and orange Jordanian bell pepper fruits samples, whereas the second group included the Iraqi/Balad green bell pepper and the third group included the rest samples (Fig. 2). This diagram revealed a high similarity between the Jordanian/red and Jordanian/orange bell pepper (first group) with 0.94% in comparisons with other samples of bell pepper (third group), while the similarities with the Iraqi/Balad green bell pepper (second group) was 0.68%. However, other samples in the third group showed high similarity with 1%. The present study is first in Iraq that deals with genetic variation in local and imported bell peppers samples by using AFLP marker. Furthermore, due to the absence of the official sources that confirms the country origin of bell pepper samples, it was difficult to compare them with the same fruits in their origin countries, and that requires prospective studies like DNA sequencing of the generated bands which obtained in this study to compare them with the whole genome sequences of bell pepper published in international websites such as http://genome.ucsc.edu and www.ncbi.nlm.nih.gov to detect the origin country of the imported fruits of bell peppers.

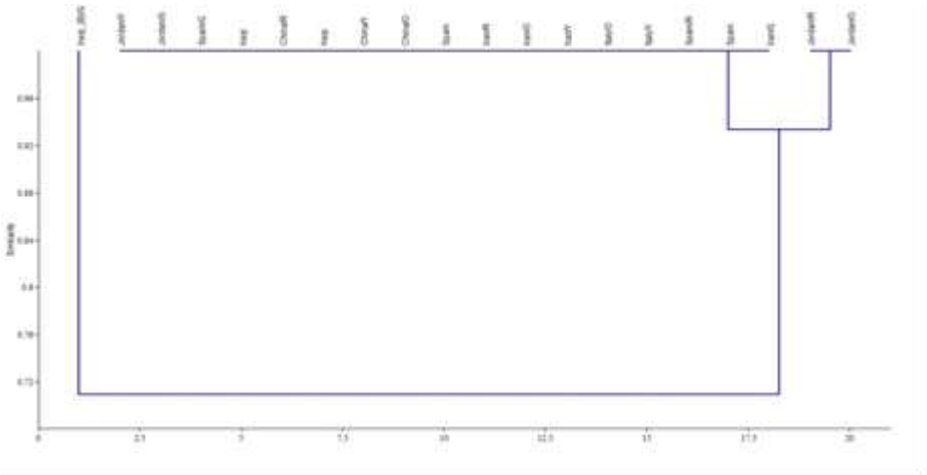

Fig. 2: UPGMA dendrogram of 20 samples of bell pepper constructed based on AFLP marker data generated from three selective primer combinations. High similarity with 1% between almost bell pepper samples (third group), while the similarity between the Jordanian/red and Jordanian/orange bell pepper (first group) was 0.94%. The similarity of Iraqi/Balad green bell pepper (second group) was 0.68% when compared with the other samples. Samples from left to right: Iraq_(B)/G: Iraqi/Balad green, Jordan/Y: Jordanian/yellow, Jordan/G: Jordanian/green, Spain/G: Spanish/green, Iraqi: Iraqi/Yousifia green, China/R: Chines/red, Iraqi: Iraqi/Souwyera green, China/Y: Chines/yellow, China/O: Chines/orange, Spain: Spanish long/green, Iran/R: Iranian/red, Iranian/O: Iranian/orange, Iranian/Y: Iranian/yellow, Italy/O: Italy/orange, Italy/Y: Italy/yellow, Spain/R: Spanish/red, Spain: Spanish long/red, Iran/G: Iranian/green, Jordan/R: Jordanian/red, Jordan/O: Jordanian/orange.

Tam *et al*. [43] made a comparison between AFLP, SSR and sequence specific amplification polymorphism (SSAP) techniques in the detection of genetic variation in 35 lines of the fruits in bell and conical (cone) and chili pepper. The percentage of variation was very low, it was 8.03% when using 9 selective amplification of AFLP primers, and produced 1432 bands, 115 bands was polymorphic, and this illustrates the limited genetic distance in the fruits of bell pepper, and also the dendrogram separated this type from the another most diverse group which included the conical of and chili pepper. While after comparing the results observed from 3 techniques, SSAP technique has been shown as the best technique to identify genetic variation, followed by SSR technique which was higher than four to nine times more AFLP technique. Bell and long divided into two groups according to the map of the genetic dendrogram and the study included thirty-two accessions using five selective primer combinations of AFLP marker and produced 255 polymorphic bands, and this reflects the breeder's efforts in the development and selection of phenotype in the fruits of pepper [44]. In another study included 45 varieties of local and imported pepper used to estimate the genetic variation between them, in which eight selective amplification used for primers of AFLP technique, resulted in 956 polymorphic bands, and the tree of genetic dendrogram formed different groups with weak differentiation, indicating a low degree of genetic variation that indicated a strong genetic relationship between these species of pepper, as the higher production of pepper in the open land and greenhouses directly depends on a range of varieties without renewal, and this result is consistent with the results of [6], [25].

3.3. Genetic Distance between the Bell Pepper Samples Depending on the Results of AFLP Marker

The genetic distance between the bell pepper samples were calculated by using Euclidean coefficient. The results revealed the highest values between red and orange Jordanian bell pepper and the Iraqi/Balad green bell pepper with 2.236 values, while the lowest genetic distance founded between many bell pepper samples with zero value. These results confirmed the fact that the least similarity has the largest genetic variation. The modern breeding of plant led to a significant decreases in the genetic variation of modern varieties [45], and this also explained by Paran *et al*. [25] when they have a low percentage of the polymorphic varieties (13%) after using of 10 AFLP primers according to the dendrogram of this study; the large fruit of bell sweet pepper with low genetic variation separated compared to the variation of small fruit with high variation this is similar to the results [43], [46], [47] as well.

TABLE IV: Genetic Distances among 20 Samples of Bell Pepper Depending on the Results of AFLP Marker

Samples	Jordan/O	Jordan/R	Jordan/Y	Jordan/G	Spain/G	Iraqi (B)/G	Iraqi (Y)/G	China/R	Iraqi (S)/G	China/Y	China/O	Spain (L)/G	Iran/R	Iran/O	Iran/Y	Italy/O	Italy/Y	Spain/R	Spain (L)/R	Iran/G
Jordan/O	0																			
Jordan/R	0	0																		
Jordan/Y	1	1	0							Highest genetic distances values										
Jordan/G	1	1	0	0																
Spain/G	1	1	0	0	0															
Iraqi (B)/G	2.236	2.236	2	2	2	0														
Iraqi (Y)/G	1	1	0	0	0	2	0													
China/R	1	1	0	0	0	2	0	0												
Iraqi (S)/G	1	1	0	0	0	2	0	0	0											
China/Y	1	1	0	0	0	2	0	0	0	0										
China/O	1	1	0	0	0	2	0	0	0	0	0									
Spain	1	1	0	0	0	2	0	0	0	0	0	0								
Iran/R	1	1	0	0	0	2	0	0	0	0	0	0	0							
Iran/O	1	1	0	0	0	2	0	0	0	0	0	0	0	0						
Iran/Y	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0					
Italy/O	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0				
Italy/Y	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0			
Spain/R	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0		
Spain (L)/R	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
Iran/G	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Jordan/O: Jordanian/orange, Jordan/R: Jordanian/red, Jordan/Y: Jordanian/yellow, Jordan/G: Jordanian/green, Spain/G: Spanish/green, Iraq (B)/G: Iraqi/Balad green, Iraqi (Y): Iraqi/Yousifia green, China/R: Chines/red, Iraqi (S): Iraqi/Souwyera green, China/O: Chines/orange, China/Y: Chines/yellow, Spain (L)/G: Spanish long/green, Iran/R: Iranian/red, Iranian/O: Iranian/orange, Iranian/Y: Iranian/yellow, Italy/O: Italy/orange, Italy/Y: Italy/yellow, Spain/R: Spanish/red, Spain (L)/R: Spanish long/red, Iran/G: Iranian/green

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