Genetic Diversity of Iraqi Date Palm (*Phoenix dactylifera* L.) by using RAPD Technique

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Abstract

In this study provided all molecular markers of Random amplified polymorphic (RAPD) successfully with the sixty five Iraqi date palm (*Phoenix dactylifera* L.) cultivars, which collected from Hilla city in Iraq, to determine fingerprinting, polymorphic value, and relationships among varieties of date palm cultivars, and also with the same type of cultivars. Data analysis of ten RAPD has been revealed. Number of amplified DNA fragments were (592) bands, polymorphism per all primers were (%64.2), primer efficiency was 0.1, and discriminatory value was (%0.09), which revealed a high percentage similarity about %67 to %100 between cultivars belong to the same variety. There are relationships with twenty four genotypes, divided in to two clusters, clusterI ranged distance from 0.74 to 1.30 represented(Maddany, Ashrasi, Greatli, Smeasmi and sukkary) and clusterII ranged distance from 0.25 to 0.60 which divided into three sub group, there are sub group I represented (Sultana, Khestawi, Breem, Sabb Drrah, Hamrawi, Brban, and Khadrawi), sub groupiesII represented (Zahdi, Tebarzal, Maktom, brahi, Chipchab and Fom Alrman), sub groupies III represented (Usta Umran, Nersi, Najdi, Guntar, Shwethi and Ghanami Ahmer).

Key words: Date palm, RAPD markers, Genetic diversity

الخلاصة

في هذه الدراسة اثبتت جميع مؤشرات التفاعل الثضاعفي لسلسلة الدنا متعدد الاشكال نجاحا مع 65 مستزرع من نخيل التمر العراقي والتي جمعت من مدينة الحلة في العراق, وذلك لتحديد البصمة الوراثية والقيمة المتباينة الاشكال والعلاقة الوراثية بين تلك الانواع وكذلك مع الانواع نفسها المستعملة في الدراسة. وقد اظهر تحليل البيانات لعشرة من المؤشرات الجزيئية المستعملة في هذه الدراسة, مجموع عدد القطع المتضخمة لقطع الدنا هي 592 حزمة ونسبة متعدد الاشكال لكل المؤشرات هي 64.2%, وان قيمة الفعالية لمؤشرات الدنا كانت 0.09%, كذلك اظهرت النسبة المئوية للتشابه الوراثي بين الانواع 67% الى 100% ما بين المستزرعات التي تعود الى نفس المستزرع. العلاقة الوراثية ل24 مستزرع من نخيل الثمر تقسم الى مجموعتين رئيستين, المجموعة الاولى تقع ضمن المدى الوراثي 0.74 الى 1.30 وتمثل (مدني واشرسي ورصاصي وسيسمي وسكري) والمجموعة الثانية تقع ضمن المدى الوراثي من 0.25 الى 0.60 وتقسم الى ثلاثة تحت مجموعات وهي: تحت المجموعة الاولى وتمثل (سلطاني وخستاوي وبريم وسبع ذراع وحمراوي وبربن وخضراوي) , وتحت مجموعة الثانية تمثل (زهدي وتبرزل وبرحي وجبجاب وفوم الرمان) اما تحت مجموعة الثالثة تمثل (اسطة عمران ونيرسي ونجدي وكنطار وشويثي وغنامي احمر).

الكلمات المفتاحية: نخيل التمر، مؤشرات التفاعل الثضاعفي لسلسلة الدنا متعدد الاشكال، تنوع جيني.

Introduction

Date palm (*Phoenix dactylifera* L.*)* is the major fruit crop of arid climate regions. It (2n=2x=36) was considered of great socioeconomic importance in the rabian region (Wrigley, 1995). The number of known date palm cultivars that are distributed all over the world are 5000 of which 600 are found in Iraq. Before 1991, Iraq was the largest

producer of dates in the world (Food and Agriculture Organization of the United Nations, 2008) and had the largest date forest in the world (MacFarquhar, 2003). However, during the Gulf and Iran- Iraq wars, Iraqi number of date palm trees was destroyed. Wars and sanctions imposed on Iraq have negatively affected both the production and natural genetic diversity of the crop in Iraq and inhibited the much- needed impetus to rebuild the date palm industry (Jubrael *et al.,* 2005).

The unique characteristics of date palm can be truly called "tree of life" and is considered as one of the most ancient plant. The rich fruit plays an important role in the nutrition of human population, and also several products are made that generate employment and thus influence socioeconomic aspect of people. Therefore, it is widely acknowledged sustainability value in social, economic and ecological terms. Moreover, this crop has great potential as a source of renewable energy, by producing bio-fuel since its fruits high in carbohydrates 44-88% total sugars (Sudhersan *et al.*, 2009). In spite of the date palm is one of the oldest cultivated fruit trees, but there are a few genetic resources for improving the productivity and development of the dioecious date palm (Methew *et al.*, 2014).

DNA-based markers and its traits in date palm progeny segregation could be used for selection instead of morphological traits. DNA fingerprinting, also known as DNA typing or genetic fingerprinting, uses for identifying individuals by the particular of their DNA. There are many molecular markers applied to identify date palm cultivars, for understanding and analyze the genetic relationships and genetic diversity among date palm varieties. Random Amplified Polymorphic DNAs(RAPDs) are DNA fragments amplified by the polymerase chain reaction using short (usually 10bp) synthetic primers of random sequence. RAPD markers have been applied for identification and DNA fingerprinting of the date palm cultivars, although the related polymorphism was low (Sedra *et al*., 1998; El-Tarras *et al*.,2007) when comparison with other cultivated species (Koller *et al.*,1993; Yang and Quiros, 1993; Farooq *et al.*, 1994; Akkak, 1996). This technique has been applied for cultivar genotyping (Ben-Abdallah *et al*., 2000; Trifi *et al*., 2000) and for analyses of phylogenetic relationships and genetic varieties (Al-Khalifah and Askari, 2003; Al-Moshileh *et al*., 2004; El-Tarras *et al*., 2007). The main aim of the present study is to investigate the suitability of the RAPD and ISSR markers to distinguish some date palm varieties and to detect genetic diversity in natural field populations.

Materials and Methods

Plant Materials

Fifty five date palm females' cultivars and nine males as date superior pollinators collected from Hillah city in Iraq (Fig.1). The young whit leaves collected, up the palm nearby heart of the tree from all genders, which represented number of cultivars per species from different locals. Table 1 illustrated these details.

Table 1: Details of sixty five date palm cultivars were grown in Hilla city.

No.	Cultivar	No. of Cultivar	Code	Gender
1	Bream	3	A	Female
2	Tebarzal	3	B	Female
3	Sabb Drrah	3	C	Female
4	Hamrawi	3	D	Female
5	Brban	3	E	Female
6	Ashrasi	3	F	Female
7	Zahdi	3	G	Female
8	Sultana	3	H	Female
9	Khadrawi	3	I	Female
10	Sukkary	3	J	Female
11	Khestawi	3	K	Female
12	Usta Umran	3	L	Female
13	Guntar	3	M	Female
14	Maktom	3	N	Female
15	Nersi	3	O	Female
16	Maddany	3	P	Female
17	Barhi	2	Q	Female
18	Chipchab	2	R	Female
19	Najdi	2	S	Female
20	Fom Alrman	1	T	Female
21	Shwethi	1	U	Female
22	Greatli	3	V	Male
23	Ghanami Ahmer	3	W	Male
24	Smeasmi	3	X	Male

DNA Extraction

Leaves of date palm cultivars for all species used in this study, which were collected from Hilla city. Leaves (200mg) were grounded to a powder using liquid nitrogen and placed in the microfuge tubes then DNA were extracted by using Mini Kit (Geneaid Biotech. Ltd; Taiwan company), for yield purifying total DNA including genomic DNA, chloroplast and mitochondrial DNA, from plant tissue according to manufacturer manual.

Fig. 1. Sample collection local of date palm in Hillah city, Iraq.

Primers

Ten primers of ten RAPD markers listed in tables(2) mentioned their names and nucleotide sequences of each primer and annealing temperatures.

Table 2: List of (RAPD) primers used in this study.

NO.	Primer	Sequence (5′-3′)	Annealing Temp.
1	Oligo- 203	CAC GGC GAG T	36 C˚
2	Oligo- 33	CCG GCT GGA A	34 C˚
3	Oligo- 29	CCG GCC TTA C	34 C˚
4	Oligo- 345	GCG TGA CCC G	33 C˚
5	Oligo- 349	GGA GCC CCC T	34 C˚
6	Oligo- 213	CAG CGA ACT A	35 C˚
7	Oligo- 214	CAT GTG CTT G	35 C˚
8	Oligo- 42	TTA ACC CGG C	36 C˚
9	Oligo- 342	GAG ATC CCT C	33 C˚
10	Oligo- 211	GAA GCC CGA T	33 C˚

DNA Molecular Size of Markers

Amplicon size was estimated using 100-bp DNA standared (ladder), corporation viogene, (USA). Which used for RAPD analysis, which description a convenient for sizing linear double- stranded DNA fragments from 100-bp to 3-Kbp. They ready- to- use DNA ladder involved of 12 double- stranded, blunt-end fragments with sizes of 3000, 2000, 1500, 1000, 800, 700, 600, 500, 400, 300, 200, and 100 base pairs.

Reaction Mixture (Master Mix)

AccuPower- PCR PreMix. Bioneer Corporation USA is the convenient to perform DNA amplification which have description, 0.2 ml thin- wall 8-strip tubes with attached cap/96 tubes table 3.

Table 3: PCR reaction mixture components.

Component Size (20µl reaction)	Reaction
Top DNA polymerase	1µ
Each: dNTP (dATP, dCTP, dGTP, dTTA)	250 µM
Tries-HCl (pH9.0)	10mM
KCl	30mM
MgCl2	1.5mM
Stabilizer and tracking dye	5 µM

Agarose Gel Electrophoresis

According to Sambrook and Russel (2001) the gel electrophoresis methods were done as the following:

1. Agarose was made in 1% by dissolving 1g of agarose in 10ml of 10x TBE buffer and the volume was completed to 100 of distilled water.
2. A DNA ladder was loaded into the first well. This was used to determine the absolute size of the separated DNA strand by comparing their migration with that of the ladder.
3. 5 µl of genomic DNA sample was added on para film, and mixed with 2 µl of the loading dye, mixed well using the automatic pipette.
4. The lid of the electrophoresis chamber was closed and the current was applied. The gel was run at 70 volts for 45 minutes.
5. DNA bands were visualized by UV illumination at (240, 366 nm) wave length on UV transilluminator, and photographs were taken using digital photographic camera.

PCR Conditions

Amplification

The amplification has been used the experimental protocol of AccuPower® PCR PreMix, according to manufacture instruction as following:

1. 2µl template DNA and 3µl primer (10 pmole/1µl) were added to the AccuPower® PCR PreMix tube.
2. Sterilized deionized distilled water were added to AccuPower® PCR PreMix tubes to yield the final volume of 20µl.
3. All tubes have been mixed with vortex to dissolve the lyophilized blue pellet.
4. All samples were amplified individually by using PCR apparatus and corresponding annealing temperatures mentioned in table (2) with RAPD primers.

Initial denaturation was 94Cº for 4 minutes followed by 30 cycles 65 sample at 94Cº for 1 minutes, 1 mint at annealing temperature and 2 minutes at 72Cº, and a final extension step at 72Cº for 7 minutes. The amplification of ISSR- primers have been used the same experimental protocol corresponding annealing temperatures mentioned in

table (3) with ISSR primers. Initial denaturation was 94Cº for 4 minutes followed by 35 cycles 65 sample at 94Cº for 1 minute, 45 second at annealing temperature and 1.5 mints at 72Cº, and a final extension step at 72Cº for 8 minutes. Samples after amplified DNA with RAPD primers were separated by electrophoresis 1% agarose gels (1, 15- 1, 30 hr., 70v) to yields fine PCR products (Weigand *et al*.,1993). The results of PCR products have been visualized by U.V Transilluminator and then were imaged (Hashemi *et al*.,2009). Amplicons size products were estimated using 100-bp DNA adder 100-3000bp.

Genetic Relationships and Genetic distance

Genetic diversity in the genome DNA, which can yield from application DNA-markers for determination genetic diversity among varieties (Nei and Li 1979). As a following:

1. The results revealed in profile, converting as a data in table for chacterization such as 1 for present bands and 0 for absent. Genetic relationships between selective varieties which converted to characterization data to estimated similarity value by(SIMQUL) similarity for Qualitative Data, by formula Nei and Li:

   Similarity = 2n xy / nx + ny
2. Determination genetic distance between varieties by using formula: Genetic distance = 1- (2nxy / nx + ny) × 100

   Whereas nxy : Number of bands in x and y

   nx : Number of all bands in x

   ny: Number of all bands in y

Results and Discussion

RAPD- Primers results analysis

In this study implode Random Amplification Polymorphism-DNA as a molecular marker system has been successfully applied in sixty five date palm cultivars, which collected from Hella city. RAPD markers have been used for identification and DNA fingerprinting of the date palm varieties. The results in table (4) have been reveals highest number of amplified bands with the Oligo-203 primer (93), presenting of polymorphism was %100, and presenting of discriminatory value was (0.13). The highest of primer efficiency was (0.2) represented with Oligo-214 primer, when compared with the other RAPD primers which used in this study, while the lowest number of amplified was (24) bands represented with Oligo-214 primer and the lowest of prestige of polymorphisms (43) with Oligo-42 primer, whereas the lowest value of primer efficiency were (0.07) with Oligo-33 primer and Oligo-349 primers, and the lowest presenting of discriminatory value was (0.060) with Oligo-42 primers. Whereas the highest of polymorphic DNA bands generated in primer Oligo 203 was 7 bands for identification and genetic diversity testing in date palm. The lowest value can be observed in primer Oligo 42 was 3 bands.

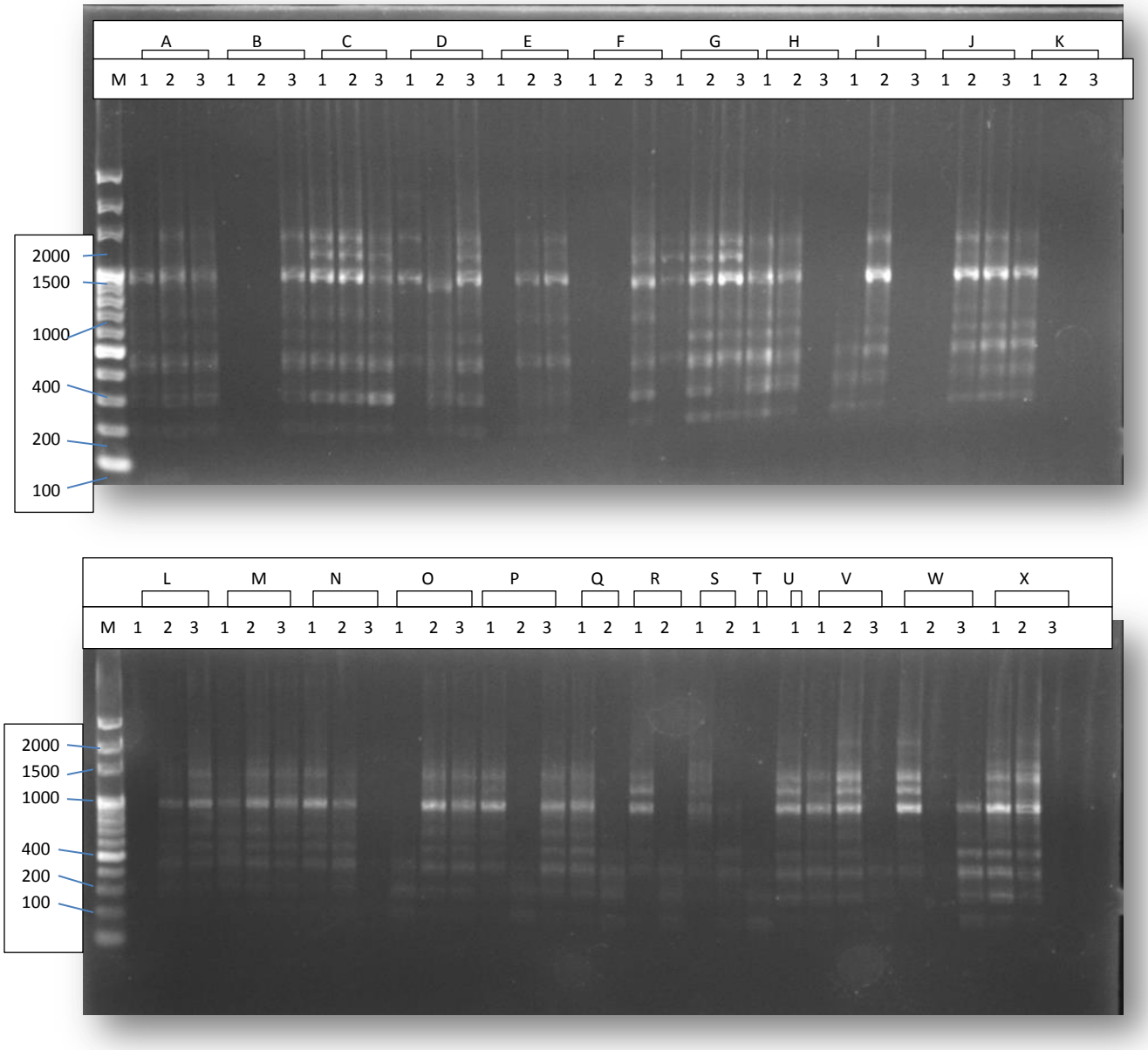

Figure 2: The amplification obtained with primer Oligo- 214

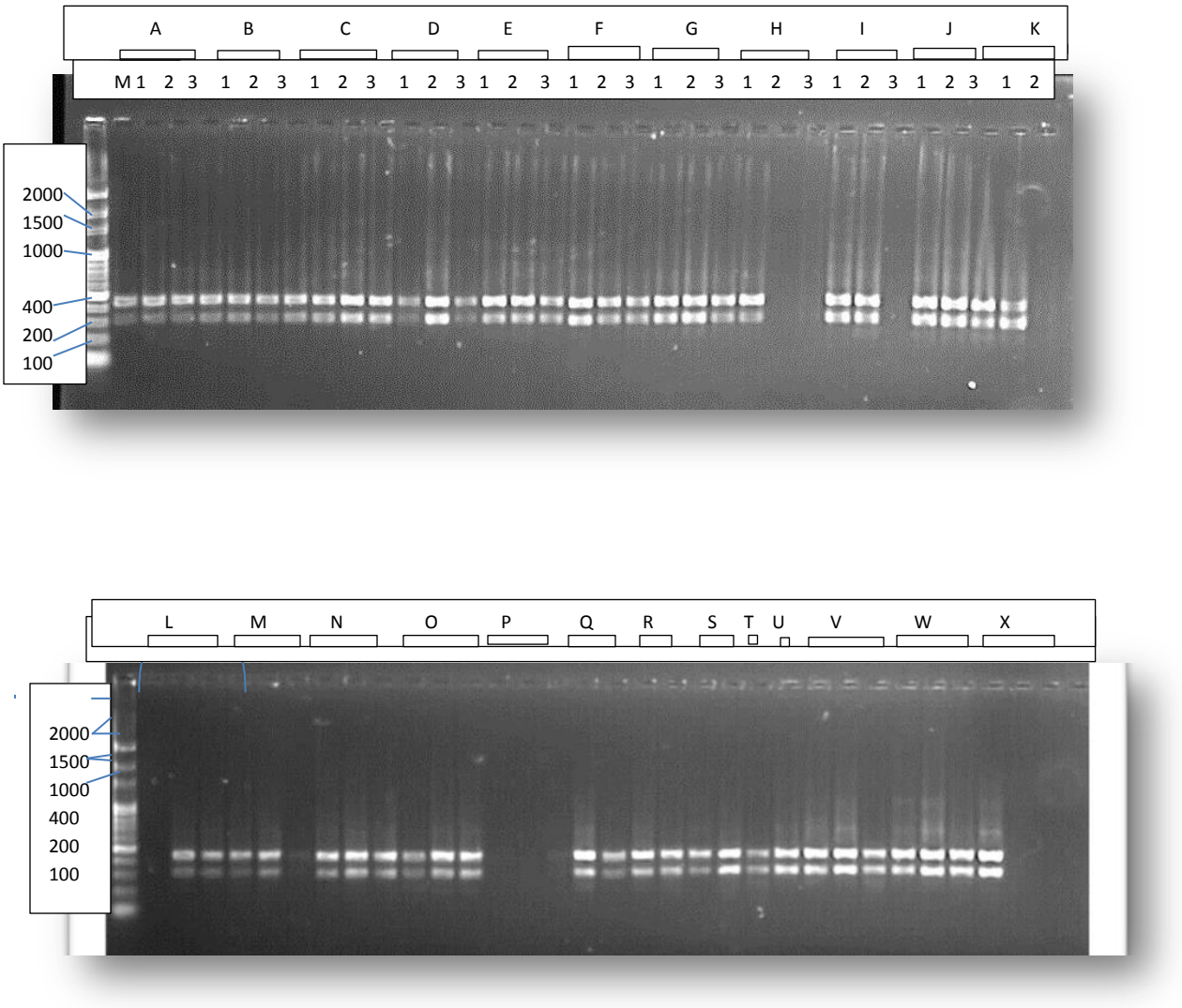

Figure 3: The amplification obtained with primer Oligo-42

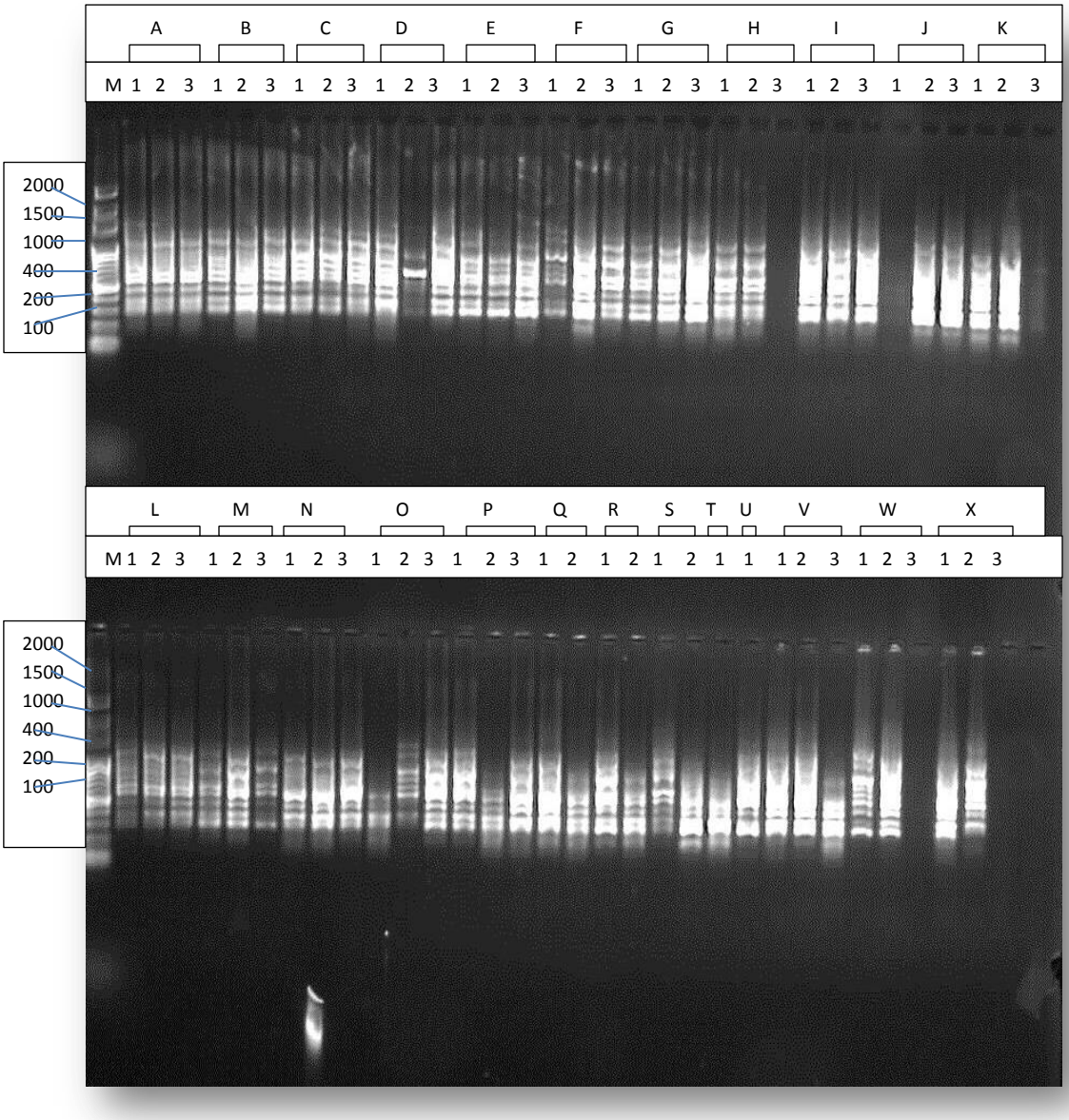

Figure4: The amplification obtained with primer Oligo-33

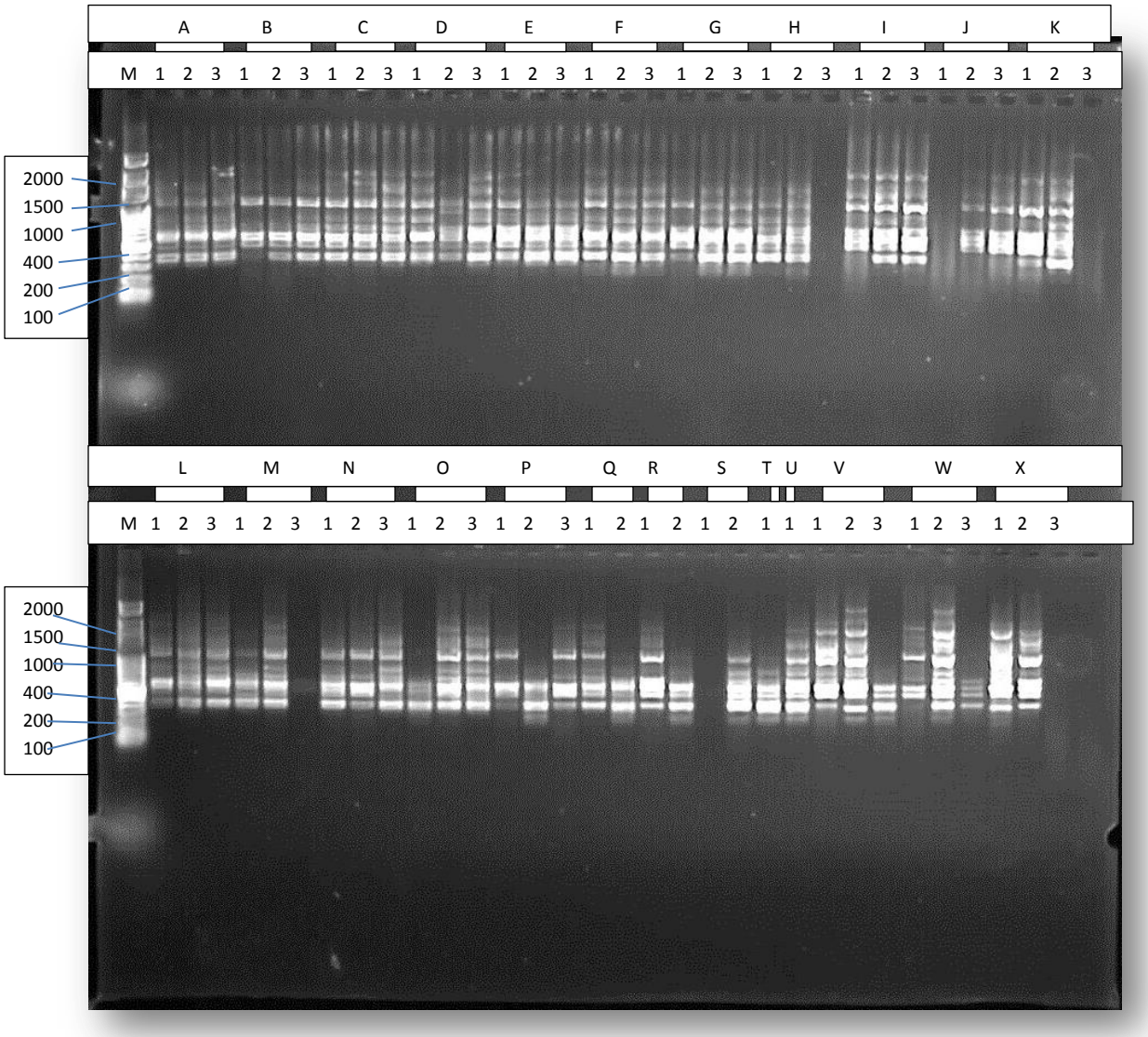

Figure 5: The amplification obtained with primer Oligo- 349

Table 4: the fragment size range (bp), No. of mainbands, No. of amplified bands, no. of monomorphic bands, No. of polymorphic bands, No. of unique bands, polymorphism, primer efficiency and discrimination value of each RAPD primer in this study.

No.	Primer	Fragment Size rang	No. of main (bp)	No. of amplified bands	No. of monomorphic bands	No. of polymorphic bands	No. of unique	polymorphism (%)	primer efficiency bands	discriminator value(%)
1	Oligo-203	200-2000	7	93	0	7	0	%100	0.08	0.13
2	Oligo-33	200-1500	7	90	1	6	0	% 86	0.07	0.11
3	Oligo-29	200-2000	7	55	0	6	1	% 86	0.1	0.11
4	Oligo-345	300-1500	7	43	2	5	0	%71	0.1	0.09
5	Oligo-349	200-1500	7	75	1	5	1	%71	0.07	0.09
6	Oligo-213	200-1500	7	41	1	6	0	%71	0.1	0.11
7	Oligo-214	200-1500	7	24	1	4	2	%57	0.2	0.08
8	Oligo-42	200-1500	7	40	4	3	0	%43	0.08	0.06
9	Oligo-342	200-1500	7	63	2	5	0	%71	0.08	0.09
10	Oligo-211	200-1500	7	68	1	6	0	%86	0.09	0.11
Total No. of bands			70	592	13	53	4	-	-	-
Average bands per primer			7	59.2	1.3	5.3	0.4	-	-	-
Average per primer %			-	-		--	-	64.2	0.1	0.09

The results of this study revealed clearly number of amplified DNA fragments (592)bands provided useful technique for the analysis of genetic diversity of date palm germplasm. In this study the results of the average polymorphism per all primers, were (%64.2) polymorphic, 0.1 primer efficiency, and (%0.09) discriminatory value. Whereas RAPD data showed high polymorphism (%92.4) among cultivars, reported from Hussein *et al*. (2005) and Adawy et al. (2005) yields low RAPD polymorphism on Egyotian date palm cultivars (%25.2 and %18.9), respectively. Moghaieb *et al* (2010) yields high RAPD polymorphism (%60.2).

Genetic similarity of RAPD

Genetic variation in this study was not only a cross of twenty four date palm cultivars, but also within each cultivar. Which was illustrated previously with all RAPD primers in Table1 2. This variation is useful in the relationships between Iraqi date palm cultivars from Hillah city, however, phylogenetic analysis is more suitable for the interpretation of all possible relationships among a large group of genotypes (Lang and Hang, 2007).

Table (6) illustrated genetic distance with 24 genotypes of date palm cultivars.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0	0.67003	0.60606	0.47376	0.589	1.069	0.65461	0.42857	0.53562	1.4142	0.5345	0.78246	0.74232	0.51507	0.84515	0.98975	0.65459	0.62263
2	0.67003	0	0.78244	0.7693	0.68507	1.2454	0.51504	0.91469	1.011	1.5386	0.88062	0.89986	0.84519	0.58902	0.86896	1.2936	0.58902	0.62268
3	0.60606	0.78244	0	0.24745	0.47383	1.0301	0.47379	0.65468	0.83337	1.4568	0.53458	0.90351	1	0.68512	1.0202	1.2122	0.79535	0.71428
4	0.47376	0.7693	0.24745	0	0.40404	0.93678	0.53455	0.53455	0.65509	1.407	0.42863	0.86898	0.96893	0.67005	0.9689	1.1158	0.80806	0.72843
5	0.589	0.68507	0.47383	0.40404	0	0.95829	0.5714	0.72841	0.71468	1.3924	0.6227	0.89211	0.98975	0.63887	0.94756	1.2037	0.80805	0.7624
6	1.069	1.2454	1.0301	0.93678	0.95829	0	0.9794	0.86895	0.96887	0.94762	0.75595	0.94757	1.0202	1.2205	0.74228	0.83299	1.2697	1.1339
7	0.65461	0.51504	0.47379	0.53455	0.5714	0.9794	0	0.75591	0.95892	1.2857	0.58905	0.65465	0.85718	0.67005	0.72843	1.1158	0.72839	0.53452
8	0.42857	0.91469	0.65468	0.53455	0.72841	0.86895	0.75591	0	0.5543	1.3924	0.3194	0.8451	0.69982	0.66998	0.83295	0.71424	0.78235	0.6388
9	0.53562	1.011	0.83337	0.65509	0.71468	0.96887	0.95892	0.5543	0	1.2929	0.67088	0.88095	0.86928	0.74269	0.95857	0.88124	0.89241	0.89275
10	1.4142	1.5386	1.4568	1.407	1.3924	0.94762	1.2857	1.3924	1.2929	0	1.3093	1.1065	1.2536	1.5185	0.84512	1.178	1.5583	1.4357
11	0.5345	0.88062	0.53458	0.42863	0.6227	0.75595	0.58905	0.3194	0.67088	1.3093	0	0.8081	0.84515	0.74226	0.79538	0.75589	0.86888	0.62266
12	0.78246	0.89986	0.90351	0.86898	0.89211	0.94757	0.65465	0.8451	0.88095	1.1065	0.8081	0	0.65465	0.76926	0.55322	0.90348	0.68512	0.65463
13	0.74232	0.84519	1	0.96893	0.98975	1.0202	0.85718	0.69982	0.86928	1.2536	0.84515	0.65465	0	0.6388	0.57148	0.82065	0.57138	0.60608
14	0.51507	0.58902	0.68512	0.67005	0.63887	1.2205	0.67005	0.66998	0.74269	1.5185	0.74226	0.76926	0.6388	0	0.90346	1.1157	0.2856	0.40401
15	0.84515	0.86896	1.0202	0.9689	0.94756	0.74228	0.72843	0.83295	0.95857	0.84512	0.79538	0.55322	0.57148	0.90346	0	0.76932	0.90346	0.75593
16	0.98975	1.2936	1.2122	1.1158	1.2037	0.83299	1.1158	0.71424	0.88124	1.178	0.75589	0.90348	0.82065	1.1157	0.76932	0	1.1693	0.93678
17	0.65459	0.58902	0.79535	0.80806	0.80805	1.2697	0.72839	0.78235	0.89241	1.5583	0.86888	0.68512	0.57138	0.2856	0.90346	1.1693	0	0.4517
18	0.62263	0.62268	0.71428	0.72843	0.7624	1.1339	0.53452	0.6388	0.89275	1.4357	0.62266	0.65463	0.60608	0.40401	0.75593	0.93678	0.4517	0
19	0.60616	0.72847	0.96894	0.89219	0.89215	1.069	0.71432	0.71424	0.83367	1.1952	0.72839	0.60606	0.58902	0.65468	0.51509	0.80808	0.71428	0.51504
20	0.77003	0.59043	0.76967	0.72883	0.63927	1.0202	0.63974	0.83361	0.84515	1.3621	0.79607	0.68545	0.72879	0.49543	0.8084	1.1344	0.53501	0.5724
21	0.62273	0.84515	0.71426	0.63887	0.75589	0.84512	0.69986	0.60606	0.65424	1.1339	0.62268	0.55327	0.60608	0.60606	0.67003	0.89214	0.80606	0.60611
22	0.95832	1.0786	0.89214	0.85715	0.88059	0.86901	0.92585	0.75589	0.86864	1.3777	0.7423	0.86896	0.78248	0.75587	0.92584	0.91477	0.7824	0.72847
23	0.62268	0.89212	0.68509	0.69986	0.90348	0.9794	0.69986	0.53452	0.84549	1.3171	0.62273	0.65468	0.5714	0.69974	0.75593	0.89215	0.63878	0.60606
24	0.86895	1.0594	0.7954	0.80812	0.90348	1.1867	0.9476	0.90351	0.86801	1.3924	0.95833	0.89211	0.85708	0.69978	1.069	1.2857	0.66998	0.85713

19	20	21	22	23	24
0.60616	0.77003	0.62273	0.95832	0.62268	0.86895
0.72847	0.59043	0.84515	1.0786	0.89212	1.0594
0.96894	0.76967	0.71426	0.89214	0.68509	0.7954
0.89219	0.72883	0.63887	0.85715	0.69986	0.80812
0.89215	0.63927	0.75589	0.88059	0.90348	0.90348
1.069	1.0202	0.84512	0.86901	0.9794	1.1867
0.71432	0.63974	0.69986	0.92585	0.69986	0.9476
0.71424	0.83361	0.60606	0.75589	0.53452	0.90351
0.83367	0.84515	0.65424	0.86864	0.84549	0.86801
1.1952	1.3621	1.1339	1.3777	1.3171	1.3924
0.72839	0.79607	0.62268	0.7423	0.62273	0.95833
0.60606	0.68545	0.55327	0.86896	0.65468	0.89211
0.58902	0.72879	0.60608	0.78248	0.5714	0.85708
0.65468	0.49543	0.60606	0.75587	0.69974	0.69978
0.51509	0.8084	0.67003	0.92584	0.75593	1.069
0.80808	1.1344	0.89214	0.91477	0.89215	1.2857
0.71428	0.53501	0.60606	0.7824	0.63878	0.66998
0.51504	0.5724	0.60611	0.72847	0.60606	0.85713
0	0.77001	0.6547	0.95832	0.74234	1.04
0.77001	0	0.5709	0.60562	0.8574	0.75478
0.6547	0.5709	0	0.57143	0.49485	0.49485
0.95832	0.60562	0.57143	0	0.8081	0.72843
0.74234	0.8574	0.49485	0.8081	0	0.6998
1.04	0.75478	0.49485	0.72843	0.6998	0

1: Breem 6: Ashrasi 11: Khestawi 16: Maddny 21: Shwethi
2: Tebarzal 7: Zahdi 12: Usta Umran 17: Barhi 22:Greatli
3: Sabb Drrah 8: Sultana 13: Guntar 18: Chipchab 23:Ghanami Ahmer
4: Hamrawi 9: Khadrawi 14: Maktom 19: Najdi 24:Smeasmi
5: Brban 10: Sukkary 15: Nersi 20: Fom Alrman

The results in table 5 represent the genetic distance among date palm cultivars. In order to calculate the genetic distance between date palm cultivars, by using SIMQUL with (Nie and Li, 2012). The lowest genetic distance was (0.02858) between date palm cultivars Barhi and Maktom. Which means that the presence of similarity in high degree between these cultivars by using RAPD markers, and the highest genetic distance was (1.5583) between Barhi and Sukkary. That means the presence of similarity in low degree between these cultivars. In another study, the genetics distance between 4 cultivars in Saudi Arabia was 0.66 to 0.85 (Abdulla and Gamal, 2010). Principal coordinated analysis (PCA) was also employed to reveal dimension of the distribution of the accessions in a scatter-pot by PAST software version 1.62 (Hammer *et al*., 2001). By using RAPD and ISSR, the similar scenario was detected for date palm of Iraq, Saudi Arabia, Tunisia and Morocco (Sedra *et al*., 1998; Al-Khalifah and Askari, 2003; Zehdi, *et al*., 2004; Munshi and Osman, 2010). Al-Khalifah and Askari (2003), also the study reported that by the exchange of cultivars between the date palm in different plantation areas, clonal propagation of ecotypes, and development of new hybridization by seedling selection and limited sexual reproduction. Haider , *et al.,* (2012) reported that the foreign date palm cultivars recently introduced to Syria groves are closely grouped with indigenous ones. These results can be illustrated by the presence of a common genetic origin among the tested cultivars in spite of their great diversity (Trifi, *et al.,* 2000). Zehdi, *et al.,* (2004) mentioned that since all date palm ecotypes are originated by hybridization, it may be occur that they have a common genetic basis, and cultivars diverged from others by mutational events.

4.8. Phylogentic Tree

Relationships between varieties can be conducted using appropriate programs. Analyses clusters including the tested varieties are apparently related according to variation of date palm. To produced a genetic distance matrix using the formula of Nei and Li (Nei, Li, 1979), which gave the similarity between any two population on the basis of the number of generated bands. The matrix was then computed with the Neighbour program based on the unweighted pair group method with the arithmetic averaging (UPGMA), (Munshi, and Osman.,2010). Whereas computed these product treefile in the installation program (NTSYS-PC) or any Tree view program can be used to draw phylogenetic diagrams (Trifi. *et al*., 2000).

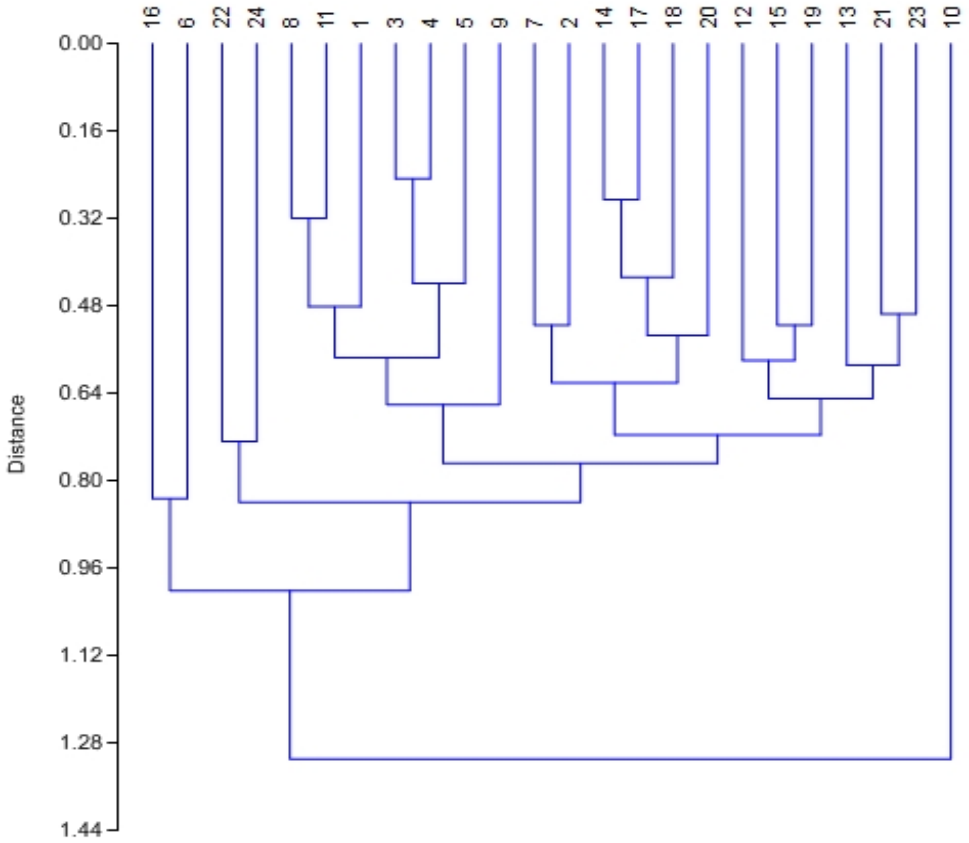

Figure 6: UPGMA dendrogram of Genetic relationships among twenty four date palm cultivars estimated by RAPD primers. With Jaccard's similarity index.

1: Breem 6: Ashrasi 11: Khestawi 16: Maddny 21: Shwethi
2: Tebarzal 7: Zahdi 12: Usta Umran 17: Barhi 22:Greatli
3: Sabb Drrah 8: Sultana 13: Guntar 18: Chipchab 23:Ghanami Ahmer

4: Hamrawi 9: Khadrawi 14: Maktom 19: Najdi 24:Smeasmi
5: Brban 10: Sukkary 15: Nersi 20: Fom Alrman

The results have been revealed in the Figure 6. There are relationships with twenty four genotypes, divided in to two clusters, clusterI ranged distance from 0.74 to 1.30 represented(Maddany, Ashrasi, Greatli, Smeasmi and sukkary) and clusterII ranged distance from 0.25 to 0.60 which divided into three sub group, there are sub group I represented (Sultana, Khestawi, Breem, Sabb Drrah, Hamrawi, Brban, and Khadrawi), sub groupiesII represented (Zahdi, Tebarzal, Maktom, brahi, Chipchab and Fom Alrman), sub groupies III represented (Usta Umran, Nersi, Najdi, Guntar, Shwethi and Ghanami Ahmer).

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