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ORIGINAL ARTICLE

FINDING THE FINGERPRINT AND GENETIC VARIANCE FOR A NUMBER OF PHENOTYPICALLY SIMILAR DATE PALM CULTIVARS

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Abstract: The study was conducted in order to determine the genetic fingerprint for a number of phenotypically similar date palm cultivars *Phoenix dactylifera* L. The study included eighteen cultivars (each two cultivars are similar vegetatively, but differ in the color of the fruit), And it has the same name used in the palm groves. which are scattered in several regions of southern and central Iraq. Six of SSR primers were used in this study. The results showed the presence of polymorphic alleles between cultivars and the same alleles among some of the studied strains. The number of alleles reached 150 and the number of alleles ranged between 1 and 4 alleles in the genetic locus. The results of the molecular analysis of the primers showed that the Halawi red strain gave nine alleles of different molecular weights than the Halawi yellow strain, while the Barhi red strain gave four alleles different from the Barhi yellow strain and the Shouethi yellow strain gave five different alleles in molecular weight from the Shouethi red strain. Five alleles appeared in the Maktum red strain, but this alleles did not appear in the Maktum yellow strain. As for as the Jawzi red strain, it gave three alleles similar to the Jawzi yellow strain out of a total of eight alleles for each of them. The results of the primers also showed that the alleles produced by the cultivars Brim yellow strain and the Brim white strain were identical, which means that they are one cultivar, while the Brim red strain showed a difference in all alleles resulting from it with the yellow strain and white strain, as well as the same result gave Khadrawi Basrah strain and the Khadrawi Karbala same alleles in the number and molecular weight, while the Khadrawi Maysan was differed from them.

***Key words*:** Date palm, Fingerprint, SSR, Similarity cultivars.

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1. Introduction

The date palm, *Phoenix dactylifera* L., is considered one of the main fruit trees in the Arabian Peninsula and an important source of food. It is a holy tree at the Arab people. It belongs to the Arecaceae family and it is one of the most important plant families for human benefit after the Graminaceae family, because it contains important economic types. One of the most widespread plants of this family in Iraq is the date palm, as it is considered one of the most important fruit trees at the local level due to its suitability for growth in most Iraqi regions and its cultivation extends from Mandali in the east, Tikrit in the north and Al-Faw in the south [Al-Bakr (1972)].

There are about 5,000 cultivars of date palm as well as other seed strains scattered in different parts of the world, but every date producing country has only a small number of the main commercial cultivars that are nurtured and cared for and their dates are exported to all parts of the world [Abul-Soad *et al*. (2017), Al-Hasany *et al*. (2020)].

Previously, farmers relied on choosing cultivars grown from seeds that are characterized by the quality of their fruits and then they are vegetatively propagated by offshoot and in this way the desired cultivars were preserved, while the cultivars multiplication from seeds resulted in a great diversity in cultivars as a result of

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the nature of cross-pollination and genetic isolation [Peyron (2000)]. Many researchers pointed out the difficulty of distinguishing date palm cultivars by their phenotypic characteristics and without the presence of fruits, because the vegetative growth characteristics are affected by the surrounding environmental conditions and service processes [Sedra *et al*. (1998)].

DNA fingerprinting is an effective method for date palm cultivar identification, genetic diversity estimation and phylogenetic tree analysis, and in recent years, the DNA fingerprinting indicator technique has become increasingly important for distinguishing closely related taxa. Molecular indicators such as Simple Sequences Repeate (SSR) have proven to be a very powerful tool in plant diversity analysis because they are not only site-specific but also possess a high degree of polymorphism, fulfilling most of the requirements to achieve accurate analysis of date palm fingerprints [Khanam *et al*. (2012)]. The SSR technique is also considered one of the very important and widespread molecular indicators currently, and it is used in the identification of cultivars, genetic mapping and to detect genetic diversity [Yusuf *et al*. (2015), Noaema *et al*. (2020a)].

The study by Racchi *et al*. (2014) on genetic characterization of 18 date palm cultivars using 16 SSR primers, noted total band was 110 with average of 6.88 bands for each initiator, showed 28 bands are unique and specific to some cultivars. The average polymorphism rate ranged from 81%.

In other study characterization of 12 Tunisian date palm cultivar was carried out using nine primers of SSR that produced 39 amplified bands and the number of bands for each primer ranged from 3 to 5 bands and the amplified band sizes ranged between (117-300) base pairs [Metoui *et al*. (2017)].

The SSR markers used in a study by Jamil *et al*. (2020) and Noaema *et al*. (2020b) succeeded in distinguishing 12 out of 13 Pakistani date palm cultivars, while the Halawi cultivar was identified using a two-step method for DNA-level identification Nuclear.

Due to the presence of a number of date palm strains that are similar in names and have become famous, it was necessary to establish a rule through which these forms of naming could be resolved, especially as they were named after well-known cultivars. This study was conducted to distinguish between similar cultivars in name and to determine the genetic fingerprint. Moreover, finding the genetic relationship to determine the genetic convergence and divergence between the studied strains for improvement and production of new selected cultivars.

2. Materials and Methods

The study was carried out during the 2020 growth season to aim of identifying and diagnosing the genetic fingerprint of some phenotypically similar date palm cultivars spread in several regions of southern and central Iraq using SSR indicators. 18 cultivars (All two cultivars are morphologically similar, and have the same name, but different fruit color), each cultivar has three different strains (Table 1). The work was carried out in the Laboratory of Date palm and Genetic Engineering, Department of Horticulture and Landscaping, College of Agriculture, University of Basra.

The plant samples were collected from the fresh and white leaves close to the shoot tip. The leaves were washed several times with sterile distilled water, to clean them of dust and plankton, then wiped it with a medical cotton dipped in alcohol at a concentration of 70% for sterilization, then it was cut into small pieces (1 cm^2). Using clean, sterile sharp scissors, the pieces placed in ceramic mortar then the Liquid Nitrogen was added to it, the samples were ground well until they turned into a white powder, the powder was kept in a sample container with a volume of 10 milliliters.

DNA was extracted by CTAB method, as mentioned by Doyle (1991) and Aitchitt *et al*. (1993) DNA quality and quantity were estimated using a Nano Drop ND-2000 spectrophotometer (THERMO SCIENTIFIC, USA) at 260nm and purity was checked by the A260/A280 ratio.

Six primers for SSR markers produced by BIONEER were used. Table 2 shows the primers, their sequences, sources, MT temperature for each primer, and GC ratio.

The following SSR-PCR program was used: one cycle of 5 minutes at 95°C for the initial denaturation of the DNA strand, followed by 35 replication cycles including each cycle: 30 seconds at 95°C for the template denaturation and 45 seconds at 49°C, then 57°C for binding of primers to template DNA and 1 minute at 72°C for elongation of bound primers with one final cycle of 72°C for 7 minutes as the final cycle

Table 1: Cultivars under study.

Code	Cultivar name	Cultivar location	Cultivar Coordinates
H1	Halawi yellow	Basra - Abi Khasib	Lat: N 30^0. 47654 Lon: E 47^0. 88210
H2	Halawi red	Basra - Abi Khasib	Lat: N 30^0. 47647 Lon: E 47^0. 88028
B1	Barhi yellow	Basra - Mdina	Lat: N 30^0. 99383 Lon: E 47^0. 35735
B2	Barhi red	Basra - Mdina	Lat: N 30^0. 99383 Lon: E 47^0. 35735
SH1	Shouethi yellow	Basra - katiban	Lat: N 30^0. 71244 Lon: E 47^0. 78305
SH2	Shouethi red	Basra - katiban	Lat: N 30^0. 71244 Lon: E 47^0. 78305
M1	Maktum yellow	Babylon - Tourist	Lat: N 32^0. 39563 Lon: E 44^0. 53092
M2	Maktum red	Babylon - Tourist	Lat: N 32^0. 39701 Lon: E 44^0. 59516
J1	Jawzi yellow	Babylon - Tourist	Lat: N 32^0. 44389 Lon: E 44^0. 47102
J2	Jawzi red	Babylon - Tourist	Lat: N 32^0. 44405 Lon: E 44^0. 47100
CH1	Jabjab Basrah	Basra - Shuaiba	Lat: N 30^0. 42284 Lon: E 47^0. 66652
CH2	Jabjab Karbala	Karbala, Nakheel Hussainiya station	Lat: N 32^0. 68404 Lon: E 44^0. 10017
BR1	Brim yellow	Basra - Abi Khasib	Lat: N 30^0. 47976 Lon: E 47^0. 88764
BR2	Brim red	Basra - Mdina	Lat: N 30^0. 99395 Lon: E 47^0. 35807
BR3	Brim white	Basra - Mdina	Lat: N 30^0. 99383 Lon: E 47^0. 35735
KH1	Khadrawi Basrah	Basra - Al-Faw	Lat: N 30^0. 18651 Lon: E 48^0. 39694
KH2	Khadrawi Maysan	Maysan - Maysan research station	Lat: N 31^0. 49227 Lon: E 47^0. 10456
KH3	Khadrawi Karbala	Karbala, Nakheel Hussainiya station	Lat: N 32^0. 68354 Lon: E 44^0. 10016

of final elongation.

2.1 Data analysis

Using the Gel Analyzer 2010a digital documentation program to allocate and identify the DNA bands resulting from the polymerase chain reaction (PCR), based on the DNA Ladder 100bp volume guide produced by Promega company, then the results were transmitted to the Excel program for statistical processing, extracting the results from them and calculating the number of similar and different alleles [Fazekas *et al*. (2014), Abou-Elwafa (2018)].

The results were analyzed according to the pre-prepared tables using Power Marker version 3.25 software and STRUCTURE version 2.3 software.

3. Results and Discussion

The results showed that primers gave a number

Table 2: Primers characteristics of Technology SSR from the BIONEER company Korea.

Primers	Sequence	Annealing temp (°C)	GC %	Source
PDCAT6	F: AATCAGGGAAACCACAGCCA R : GTTTAAAGCCTTCTCAAGATAGCCTCAG	53	46	Akkak *et al.* (2009)
PDCAT18	F : CCTAAACCTGAATGAATCAAAGCA R : ACTAACATAAGGACAGTGCTATGTGATTG	54	38	Akkak *et al.* (2009)
MPDCIR70	F : CCATTTATCATTCCCTCTCTTG R: CTTGGTAGCTGCGTTTCTTG	51.8	45	Billotte *et al.* (2004)
MPDCIR78	F : CCCCTCATTAGGATTCTAC R: GCACGAGAAGGCTTATAGT	49.3	47	Billotte *et al.* (2004)
DP157	F : TGGACAATGACACCCCTTTT R: GCCCACACAACAACAACCTCTCT	54.6	50	Elmeer *et al.* (2011)
DP175	F: ACACACACACACACACACACACC R : GTGGCTTCTTTTTGGCTGTC	57.6	51	Elmeer *et al.* (2011

of different alleles, the number of total alleles was 150 alleles, with an average of 25 alleles for each primer, and the number of alleles ranged between 1 and 5 alleles in the genetic locus, the polymorphic alleles reached 43, with an average of 7.17 alleles for each primer, the six primers proved to be effective in giving the polymorphism between the strains under study, the percentage of the total morphological polymorphism was 100%, which indicates the large genetic differences between the strains. The average expected heterogeneity (He) was 0.250 and the average (PIC) was 0.691 (Table 3). The alleles differed in number and molecular weights and varied from one primer to another. Some primers were able to identify the known cultivars and distinguish them from the phenotypically similar strains.

3.1 Primer PDCAT6

The results of the primer showed the presence of 20 alleles, and the primer was able to distinguish between Fourteen cultivars that are similar in name.(Fig. 1). The cultivars produced alleles with molecular weights different from the alleles that resulted from their strains. Halawi yellow gave the allele with a size of 170bp, while the Halawi red strain gave a different allele with a size of 190bp. The Barhi yellow cultivar and the Barhi red strain also produced two different alleles with a size of (200bp, 210bp), respectively. The cultivars (Maktum yellow, Jawzi yellow and Jabjab Basra) were distinguished from their strains by the presence of allan in each cultivar, as Maktum gave (170bp, 200bp), while Jawzi and Jabjab Basra were given (190bp, 200bp), while the strains (Maktum red, Jawzi red and Jabjab Karbala) each of them produced one allele (210bp, 220bp, 220bp), respectively.

The results of the same primer showed that the Brim yellow and the Brim white are the same as they produced the same allele at 220bp, while the Brim red strain differed from them, which did not give any allele during DNA amplification. The primer was also able to identify the Khadrawi Basra and Khadrawi Karbala strain, as they matched in the size of the resulting allele 230bp, while the Khadrawi Maysan strain differed from them as it gave the allele of 200bp. The primer was unable to distinguish between Shouethi yellow cultivar and Shouethi red strain, as it gave a similar allele of 190bp.

3.2 Primer PDCAT18

The Primer gave totaling 27 alleles shown in Fig. 2, as he was able to identify most cultivars and their it strains, which were characterized by the presence of alleles of varying molecular sizes, as in the Halawi red strain that gave the 120bp allele that did not appear in the cultivar Halawi yellow, as well as the Barhi red strain that produced the allele. Its size reached 140bp. It was not found in the Barhi yellow cultivar, while the Shouethi yellow cultivar was distinguished from the Shouethi red strain by its allele production of 115bp. The primer was also able to determine the genetic fingerprint of the Maktum yellow cultivar, which gave an allele of 125 bp in size, different from the Maktum red strain, which gave allele reached 150bp, the cultivar Jawzi yellow and the Jawzi red strain identical in production of the allele reached 140bp. The same primer

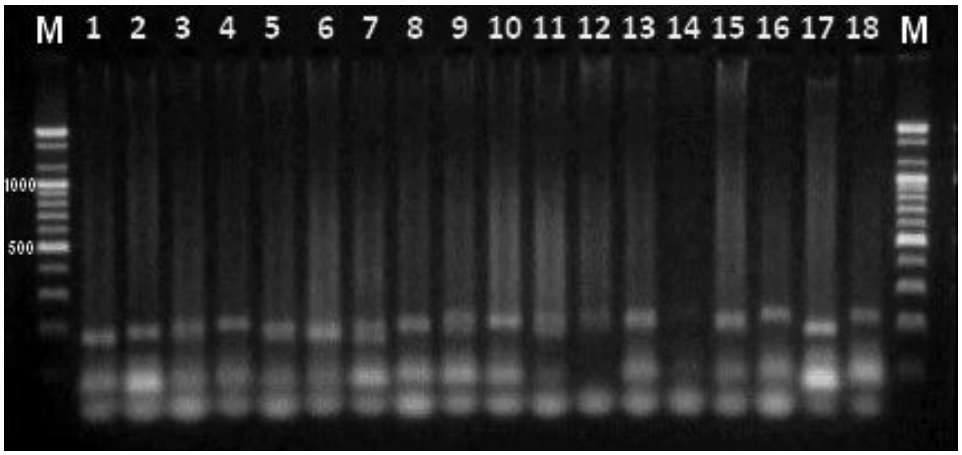

Fig. 1: PCR amplification of *PDCAT6* in 18 date palm cultivars on 2% agarose gel, M = 100bp plus DNA ladder. (1. Halawi yellow 2 Halawi red 3. Barhi yellow 4. Barhi red 5. Shouethi yellow 6 Shouethi red 7. Maktum yellow 8. Maktum red 9. Jawzi yellow 10 Jawzi red 11. Jabjab Basrah 12. Jabjab Karbala 13. Brim yellow 14. Brim red 15. Brim white 16.Khadrawi Basrah 17.Khadrawi Maysan 18.Khadrawi Karbala)

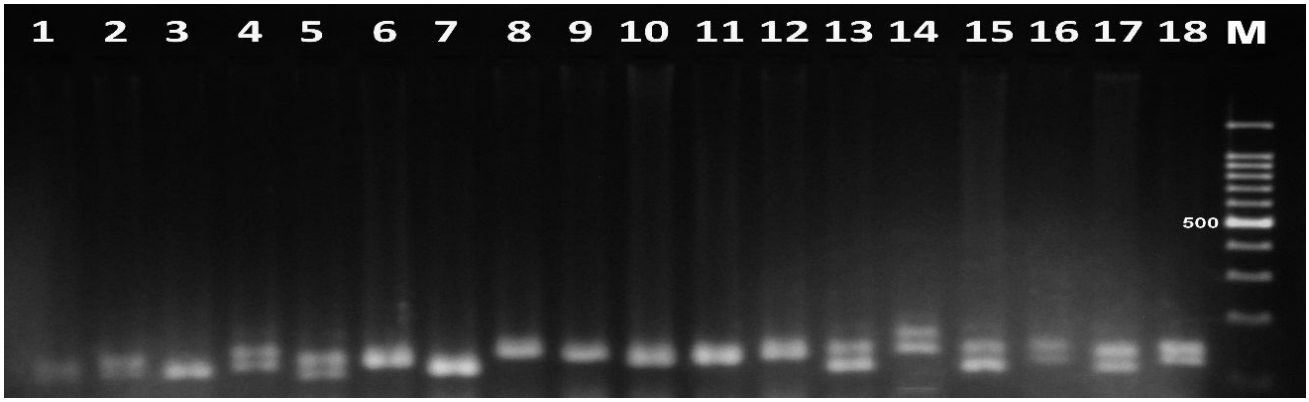

Fig. 2: PCR amplification of *PDCAT18* in 18 date palm cultivars on 2% agarose gel, M = 100bp plus DNA ladder

was also able to distinguish the Jabjab Basra cultivar from the Jabjab Karbala strain, the primer was able to confirm the conformity of the Brim yellow cultivar with the Brim White strain, while the Brim red differed from them by the appearance of a different allel at 180bp. Also, Khadrawi Basra and Khadrawi Karbala were identical in producing the same alleles, while Khadrawi Maysan produced a different allele of 125bp.

3.3 Primer mPdCIR70

The total of alleles resulting from the SSR-PCR reaction due to this primer was 18alleles, with a molecular weight ranging from 170bp to 200bp (Table 3). It was able to identify some cultivars such as Halawi, Shuwathi and Maktum, which produced alleles different from their phenotypically similar strains. The primer was also able to distinguish the Brim red strain by producing an allele with a size of 200bp, while both the Brim yellow and Brim white were identical with the allele of 180bp in size. As for the rest of the cultivars, they were identical to their strains and did not differ from them (Fig. 3).

3.4 Primer mPdCIR78

The primer mPdCIR78 gave alleles reached 18 alleles, and most of them are of similar in molecular weights at the cultivars and strains except for the two cultivars (Jawzi yellow and Jabjab Basra), each one has produced an allele with a size of 170bp different from the allele produced by the slates Jawzi red and Jabjab Karbala which is (200bp, 140bp), respectively. The primer was also able to distinguish the Brim red strain with its allele production of 180bp different from Brim yellow and Brem White, which were similarity in allele production of 150bp.

3.5 Primer DP157

The Primer DP157 succeeded to giving 40 alleles (Fig. 4). It was able to determine the genetic fingerprint of each studied cultivar and distinguish it from the phenotypically similar strains. The Halawi red strain was distinguished by the appear of three alleles, while

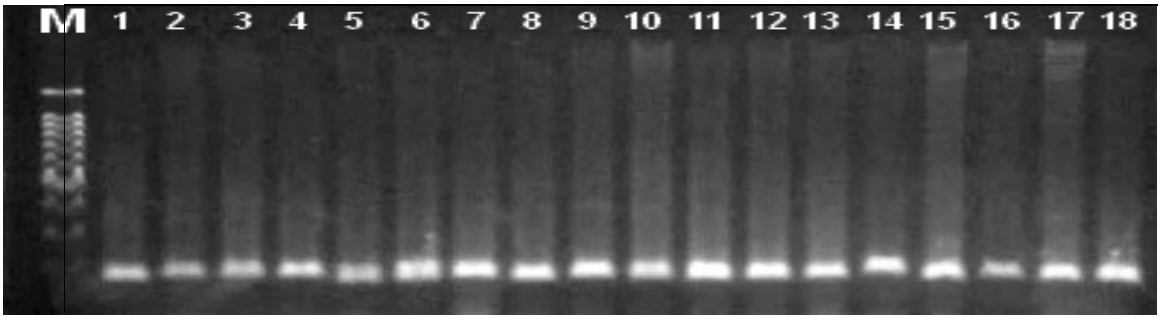

Fig. 3: PCR amplification of *mPdCIR70* in 18 date palm cultivars on 2% agarose gel. M=100bp plus DNA ladder

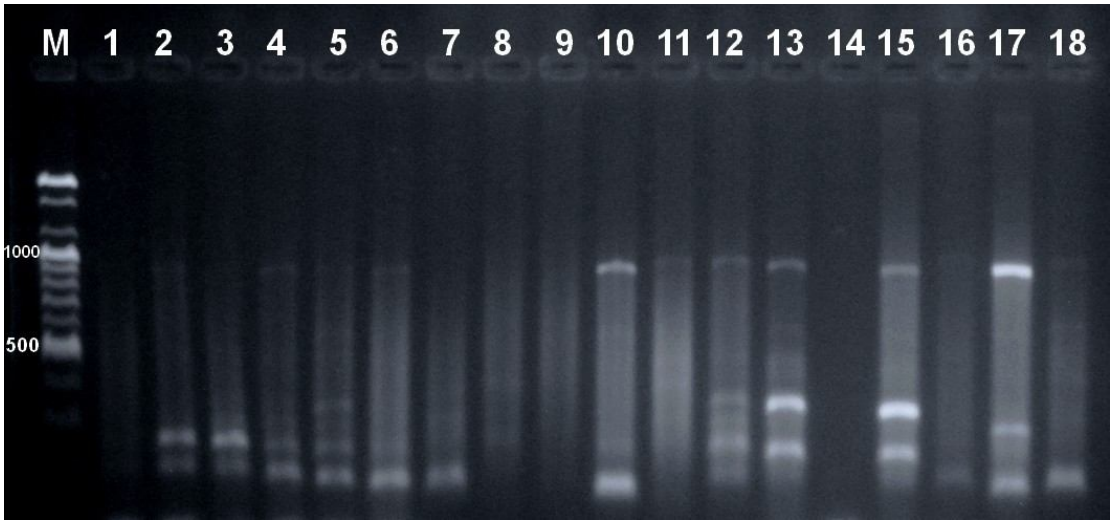

Fig. 4: PCR amplification of *DP157* in 18 date palm cultivars on 2% agarose gel, M = 100bp plus DNA ladder

ther was no allele produced in the Halawi yellow cultivar. The Barhi red strain was also distinguished for Barhi yellow cultivar by allele reached 900bp and the this primer was able to identify the two cultivars (Shouethi yellow and Maktum yellow) and distinguish them from the two strains (Shouethi red and Maktum red) by producing each of them alleles reached (330bp and 200bp), respectively. The primer DP157 was also able to produce alleles with difference molecular weights that distinguish between the Jawzi yellow cultivar and his strain Jawzi red, the Jabjab Basra cultivar and the Jabjab Karbala strain. The results of this primer, there were confirmed in genetic match between the Brim yellow cultivars and the Brim white strain which produced the same three alleles, while no allele appeared in the Brim red strain. Also, a genetic match was obtained between the Khdrawi Basra and the Khdrawi Karbala through their production of two identical alleles (170bp and 1000bp) and did not appear in the Khdrawi Maysan strain.

3.6 Primer DP175

In this Primer, the results show a number of alleles difference in numbers and sizes, and their total was 27 alleles (Fig. 5). These alleles were able to determine the genetic fingerprint of most of the studied cultivars, the Halawi yellow and the Halawi red strains each produced two different alleles (300bp and 120bp), respectively and the Barhi red strain was characterized by the appearance of the 300bp allele that did not appear in the Barhi yellow cultivar. The primer was also able to determine the identity of the Shouethi yellow cultivar by producing two alleles of size (320bp, 170bp) that differed from the alleles produced by the Shouethi red strain. On the other hand, the same allele appeared in the Maktum yellow cultivar and the Maktum red strain, with a size of 330bp. As for the Jawzi yellow cultivar, its identity was identified and distinguished from the Jawzi red strain by the appearance of three alleles differ in molecular weights (300bp, 170bp, 120bp). The results of the primer DP175 confirmed the genetic similarity between the Brim yellow cultivar and the Brim White strain, as well as the conformity of the Basra Khadrawi with the Karbala Khadrawi.

3.7 Genetic relationship and construction of a dendrogram

The dendrogram (Fig. 6) showed the genetic

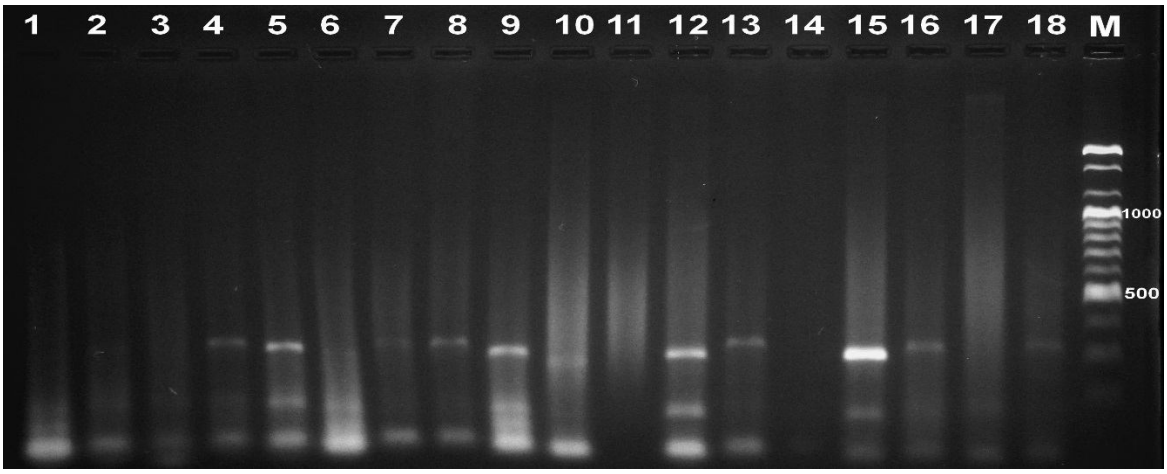

Fig. 5: PCR amplification of *DP175* in 18 date palm cultivars on 2% agarose gel, M = 100bp plus DNA ladder

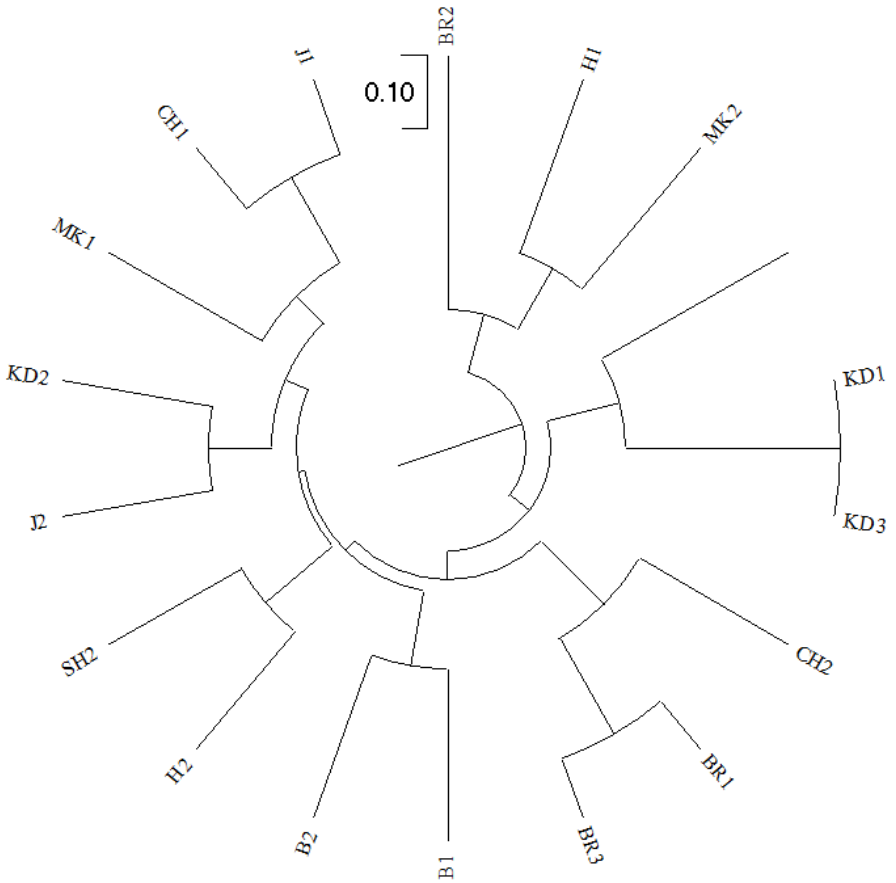

Fig. 6: Dendrogram of 18 date palm cultivars generated with data from 6 SSR primers

relationship between the cultivars and their strains under study based on the results of primer amplification, number of cultivars appeared that matched each other, such as the two cultivars (Khadrawi Basra and Khadrawi Karbala) and the two cultivars (Brim yellow and Brim White) because of their matching in the number and size of alleles. This indicates their genetic affinity, which indicates the existence of a common genetic material between these cultivars [Mathieu-Daudé *et al*. (1997)].

Class stratification of the inputs based on ΔK values computed by STRUCTURE HARVESTER revealed six groups as the most likely number of K (Fig. 7). The optimal number of studied cultivars in these 18 accessions was determined to be six (Kopt = 6, Fig. 7). The overall stratification was in part in agreement with the dendrogram in Fig. 6. Each vertical line represents a single multilocular genotype. Each color represents

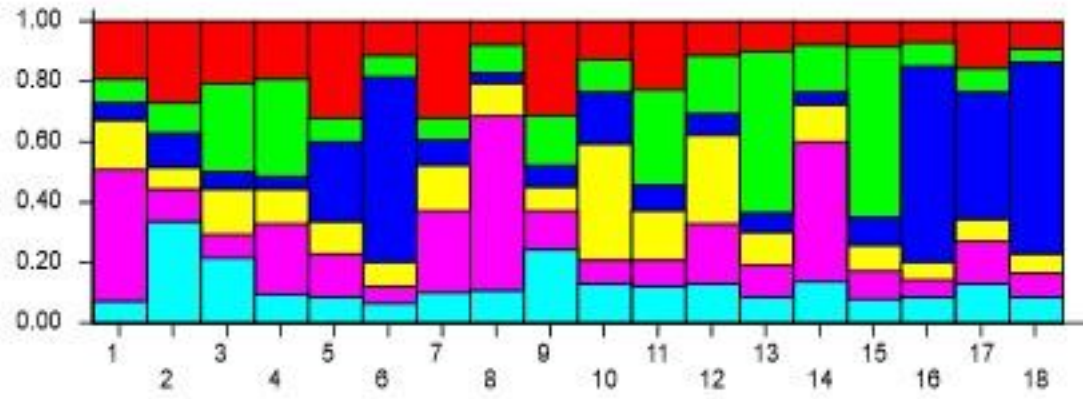

Fig. 7: Model-based cluster construction using Bayesian analysis among 18 date palm cultivars based on allelic variants at 6 SSR loci. Six clusters were defined using the Evanno *et al*. (2005) method

Table 3: Descriptive genetic parameters for 6 microsatellite loci analyzed on 18 date palm cultivars.

Primers	No total bands	No of bands	Polymorphism bands	Major Allele Frequency	Gene Diversity	Expected heterozygosity He	PIC
PDCAT6	20	6	6	0.278	0.819	0.167	0.796
PDCAT18	27	8	8	0.278	0.826	0.500	0.804
MPDCIR70	18	3	3	0.778	0.364	0.000	0.327
MPDCIR78	18	5	5	0.333	0.722	0.000	0.672
DP157	40	13	13	0.278	0.863	0.778	0.851
DP175	27	8	8	0.389	0.739	0.056	0.699
Total	150	43	43	2.334	4.333	1.501	4.148
Mean	25	7.17	7.17	0.389	0.722	0.250	0.691

the most likely strain from which the partial genotype was derived. Long monochromatic individuals are genetically similar, while polychromatic individuals have mixed genotypes from multiple groups.

We conclude from this study that these strains that are phenotypically similar with the well-known cultivars (Khadrawi Basra and Khadrawi Karbala), (Brim yellow and Brim White) are genetically the same, but there was a change in the phenotype due to the environment in which it was grown, as well as the different naming of farmers according to his geographical area. As for the rest of the strains, they are different strains that differ from the genetically known cultivars as a result of genetic mutations, so they must be called by names that differ from the known cultivar.

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