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Fingerprint and Molecular Identification of Some Iraqi Date Palm Cultivars Using Inter-Simple Sequence Repeat (ISSR) Markers.

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Abstract

many of moleculer markers used to determination the fingerprint and diversity in date palm. the ISSR markers are more sutable and effeciency to use for this aim, in this paper we are used a seven ISSR markers to find the fingerprinting and diversity for seven Iraqi date palm, which are not mentioned in auther papers by this teqniq, after the DNA extraction from young leaves with reliable quality and quantity, the genomic DNA and ISSR markers were amplification by PCR Special program, then a DNA fragment amplification cultivers were electrophoresis, then calculating the number of mono- and polymorphic bands, which used to calculat a similarity and genetic distance between cultivar, then it are used to build a phylogenetic tree and cluster analysis.

Introduction

Dates palm is one of the most important fruit in Iraq, the Arabian Gulfand a large part of the Arab world, because it is tolerance of the dry and semi-dry environment, as well as it is represent a big think in Iraqis and Arabs live becouse it is mantion in the Qoran and the Prophet Muhammad peace be upon him, It has great nutritional and economic benefits, fruit may be represent a Integrated food, the other part of the tree can inter in Manufacturing industry useful to humans (Abdulwahid,2011; ,2011) Jaradat).

Date palm tree is dicious plant, Thus, the process of cross-pollination was necessary to give an economic crop, but the resulting plants from the seed whic come from cross-pollination process are non-similar to the parents, therefor there are many cvltivers were predusing from it. The Al Baker,1972, mation there are more the 600 cvltivar can found in Iraq land.(Abdulwahid,2011).

The diversity of dates palm varieties and their spread over large areas was the reason that a large number of these varieties did not have the right to study and diagnosis, some of them are similar in appearance and fruit traits, but there cultivar take a deference name. The Outher cvltivar were similar in names but differ in genetics and appearance. Becouse the phenotyping trate was effected by inveromantal, theres phenotypic trait have made it a weak tool for diagnosis and identification of varieties. Therefor the researcher have descover a new good tool to identification the veraatise depend on the moleculare markers whih have not effected by everomental or age tessue and give a highet polymorphesim (Hammadi et al.,2009) ..

DNA-based molecular markers have differed in power, speed, and cost. These techniques include RAPD, AFLP, ISSR, STR and SNP others. In the current study, ISSR is one of the most powerful techniques adopted by many researchers in distinguishing date palm varieties for strength, ease and cost (Zehdi et al.2004 ; Al-Khateeb and Jubrael,2006 Munshi and Osman,2010)

In order to preserve date palm cultivars scattered in Iraq from loss without knowing its genetic and phenotypic characteristics and to prevent the confusion of labels and the formation of a relational database to resolve disputes over naming and to preserve ownership

of varieties, this research was carried out to document the genetic destance and drow the fingerprint for each cultivars, we are tack a seven uniq cultivare (Aumrani, Jamal aldeen, Stwi, Umalblaliz, Sultini, Azraq Azraq and Asrasi) of date palm which mentioned a few in researchs.

Materials and Methods

The genomic DNA was extracted from the young leaves of seven dates palm trees (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi) using a ready- plant kit to extract the DNA from the leaves. The younger white leaves which near the center of sout tip of date palm trees, were selected for low concentrations of chlorophyll and low fiber. They were transferred to the laboratory and sterilized with ethylene alcohol 70% using medical cotton and then cut into small pieces and grind to powder by liquid nitrogen, then transfer in to vial til used.

Extraction of genomic DNA using peq_LAP kit from the VWR US company, as described in Instruction Book. The genome extract was trsted by electrophoreses with 2% agarose. The purity of the genome produced using Nanodrop was evaluated at a wavelength of 260 and 280 nanometers.

PCR program with ISSR markers:

Seven ISSR primer which used (814, 844, hb09, hb10, hb12, Is02 and IS71). As the instraction book company for reaction mixture, showed that the sample size of the reaction was 25ml containing Green Mix, 12.5 ml, 2 ml of primer, 8 ml genomic DNA and then complete the volume to 25 microliters with free ions distilled water. The PCR program was descriped in table (1) .The PCR product was electrophoreses by 1.5% agarose.

Table (1) The PCR program

T	stage	Temperature	Duration time	no of circle
1	Pre – Denaturation	95	5 min	1
2	Denaturation	95	1 min	40
3	Annealing	36	1 min	
4	Extension	72	1 min	
5	Final extension	72	5 min	1

The agarose gel results were analyzed by photocapt programe package, the expressed of spand, when the band is appare give (1), while the absence band of the was expressed as (0), the Polymorphism , monomorphesim, efficiency, diagnostic power and number of unique pands were calculated and reselt was descased.

Results and discussion

Primer 814

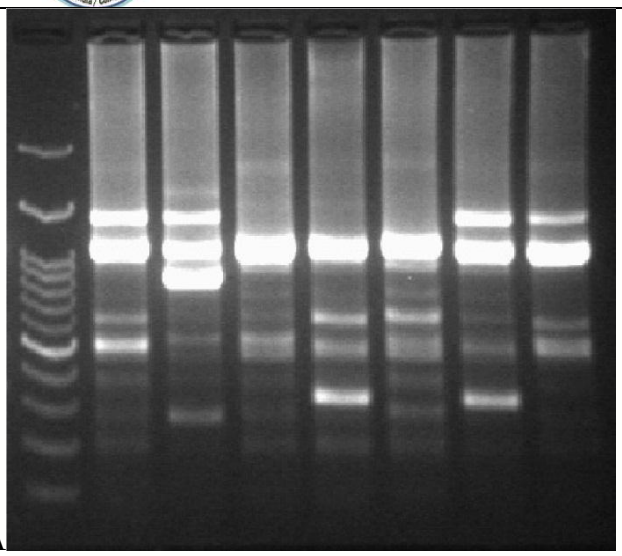
The results showed Fig (1) that the primer 814 gave a 40 total bands, the F3 and F1 cultivars were given a maximum number of bands (7 bands), whereas the f7 cultivar was gaven a minimum bands, the maximum average of band by cultivars equal 5.71 band/cv, the cluster f5 and f3 which contant two cultivars was equal in genetic distance with cluster f6 and f7 wich reach 1.73, this is a nearest destanse between two cultivars, but the long distance between two cultivars were recorded by f2 with f3, this is very Clear in PCA fig. These results were agreed with Yonis et al.(2008) on Egypt date palm when found there were a hight genetic diversity between seven cultivars and found there was 87% of this cultivars were difference between them and The results are consistent in one aspect with the researchers (Hussein, 2005 ; Eissa, 2009; Hamza, 2011 and Haider, 2012).

Primer 844

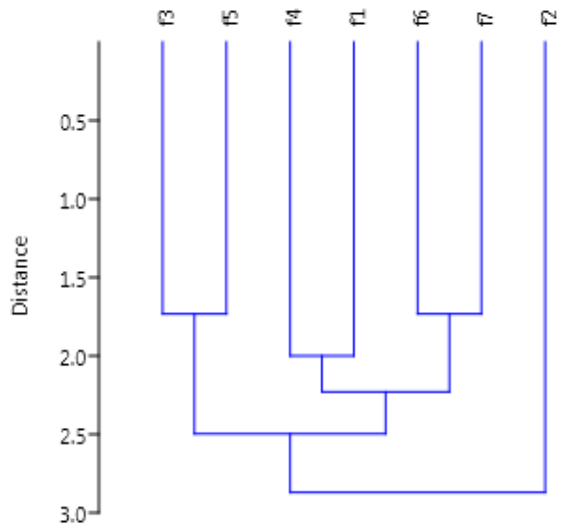
Fig (2) shown the 844 primer gave 51 total bands, the average band per cultivar was 7.28 (band / class). The F2 cultivar was gaven a miximum bands reach 9 bands wereas the f1 cultivar gaven a minimum band (6 bands), all of theses bands was a polymorphic band and non recorded the monomorphic band at this cultivare when tested wich primer 844. The cluster of f1 and f2 have a nearest distance between two cultivars reach 1.73, then the cluster of f3 and f4 cultivar with destance reach 2.00, while the long destance between two cultivars were noted at f7 with f1 and f3 wich recorded 3.60. from principle component analysis fig. noted the long distance between (f5 , f6 and f7) cultivars and (f1, f2, f3 and f4) becouse the Principal component analysis (PCA) is a statistical analysis used an orthogonal transformation to reducing a number of observations of possibly correlated variables in to linearly value variables can represented in two dimantional fig. These results were agreed with Rania et al., (2008) when using this primer on seven Dates palm cultivars in Egypt, the maxmum band is 7 band, but using this primer In Qatar on several date palm cultivars by Ahmed and al Qaradawi (2008) who conclude ther ISSR primer 844 is a good tool to cultivars diagnostics.

ISSR 814

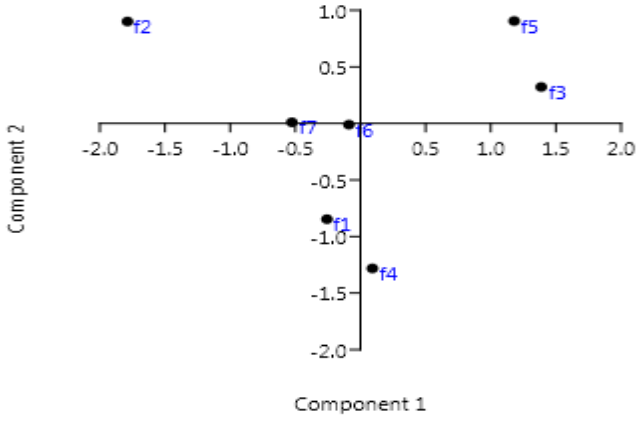
A



B



C



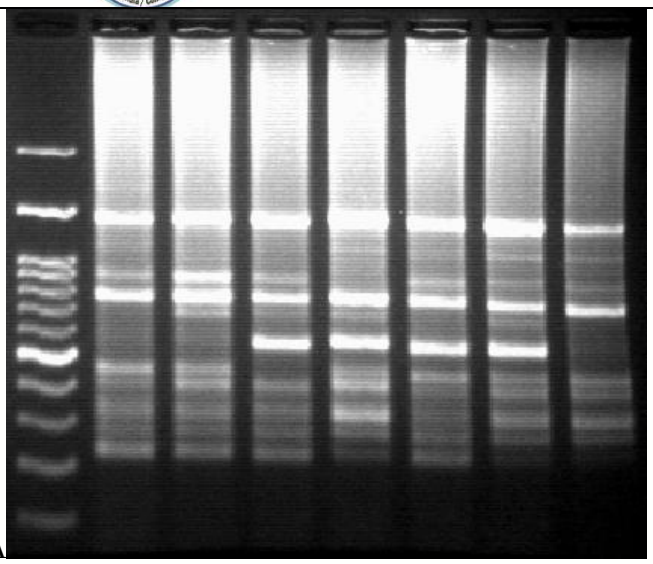
D

	f1	f2	f3	f4	f5	f6	f7
f1	0	2.6457513	2.4494897	2	2.6457513	2.4494897	2.236068
f2	2.6457513	0	3.3166248	3	3.1622777	2.6457513	2.4494897
f3	2.4494897	3.3166248	0	2.4494897	1.7320508	2.4494897	2.6457513
f4	2	3	2.4494897	0	2.6457513	2	2.236068
f5	2.6457513	3.1622777	1.7320508	2.6457513	0	2.236068	2.4494897
f6	2.4494897	2.6457513	2.4494897	2	2.236068	0	1.7320508
f7	2.236068	2.4494897	2.6457513	2.236068	2.4494897	1.7320508	0

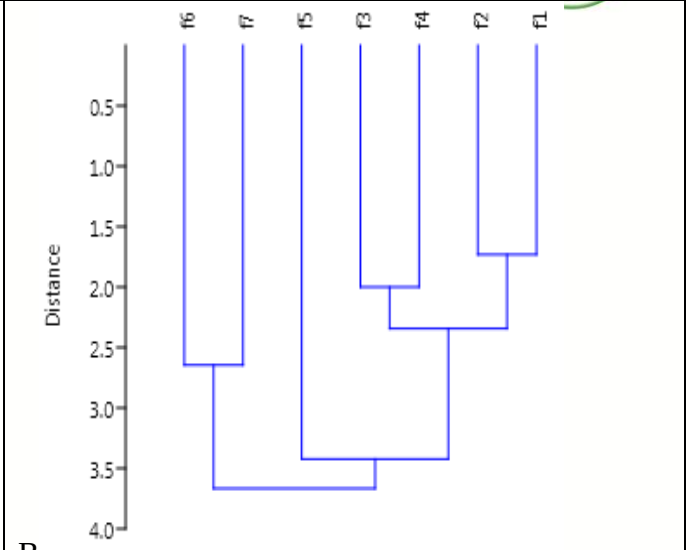
Figure 1. A picture for the PCR product for the primer ISSR 814, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

ISSR844

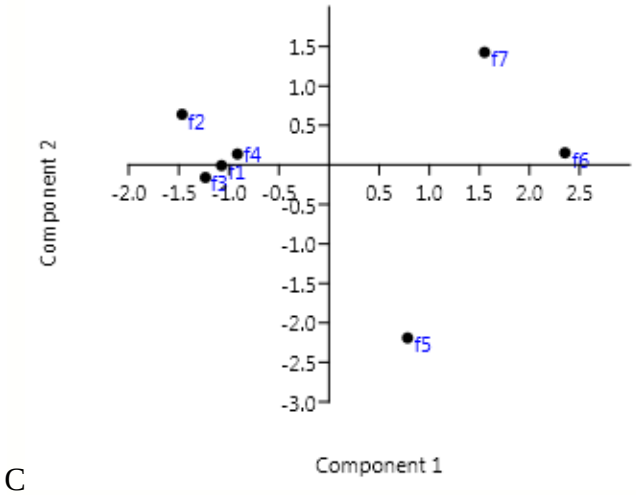
A



B



C



D

	f1	f2	f3	f4	f5	f6	f7
f1	0	1.7320508	2.4494897	2.4494897	3.3166248	3.7416574	3.6055513
f2	1.7320508	0	2.236068	2.236068	3.7416574	4.1231056	3.4641016
f3	2.4494897	2.236068	0	2	3.3166248	4	3.6055513
f4	2.4494897	2.236068	2	0	3.3166248	3.7416574	3.3166248
f5	3.3166248	3.7416574	3.3166248	3.3166248	0	3.3166248	3.7416574
f6	3.7416574	4.1231056	4	3.7416574	3.3166248	0	2.6457513
f7	3.6055513	3.4641016	3.6055513	3.3166248	3.7416574	2.6457513	0

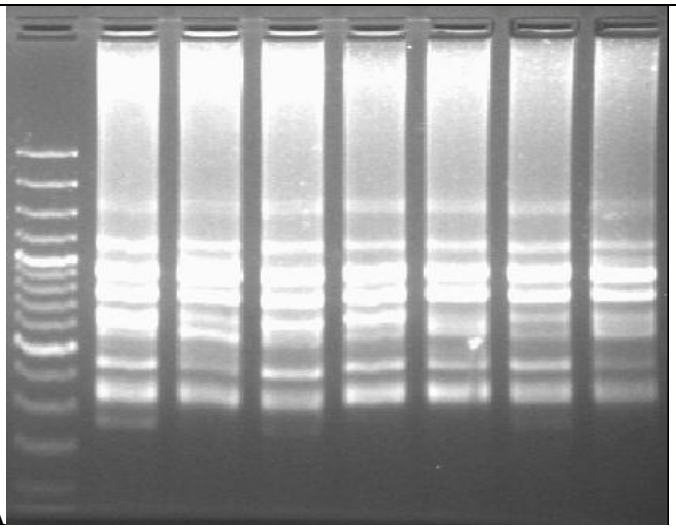
Figure 2. A picture for the PCR product for the primer ISSR 844, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

primers hb9, hb10 and hb12

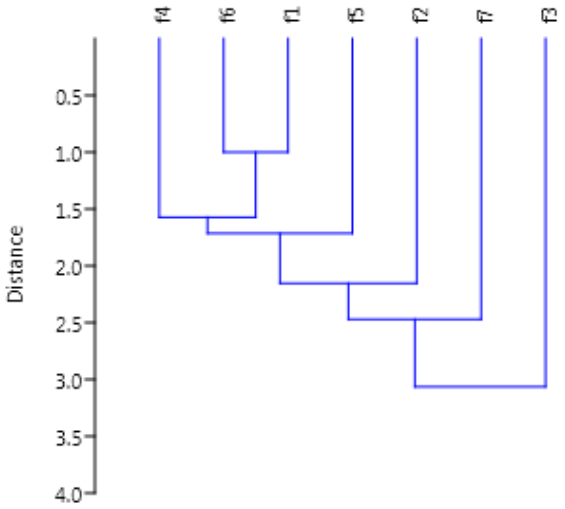
fegares (3,4 and 5) the primers hb9, hb10 and hb12 were recorded the total band 52, 33, and 61 with average 7.42, 4.71 and 8.71 respectively,. The nerast destansed was recorded by f3 and f5 in primer hb10 which mean there are no defference between this cultivare and the primer hb10 didnot recognase the defferance between these cultivars, becose they have a 5 similar bands between 425-1100pb. then the second genetic destance recorded by primer hb9 between f1 and f6, which reach 1.00 then the f6 and f7 in hb12 which reach 1.41. The long destance was recorded between f3 and f7 whene uesed the hb9 reached 3.60, then second long destanse (3.31) were recorded by more on cultivar ander three primers which used. The results are consistent in one aspect with the researchers (Karim et al, 2010 ; Moghaieb, 2010 and Srivashtav, 2014).

ISSRhb09

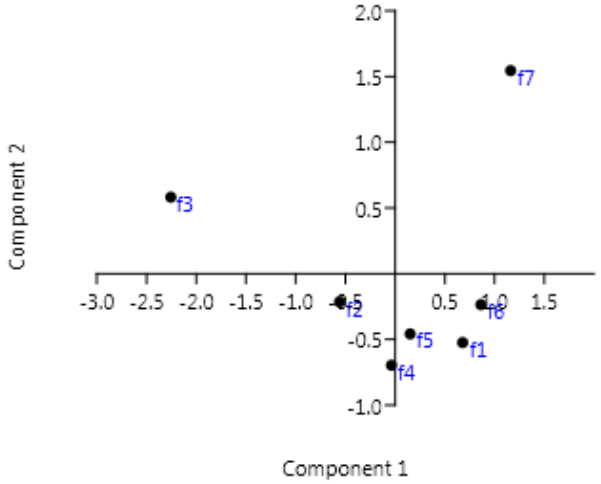
A



B



C

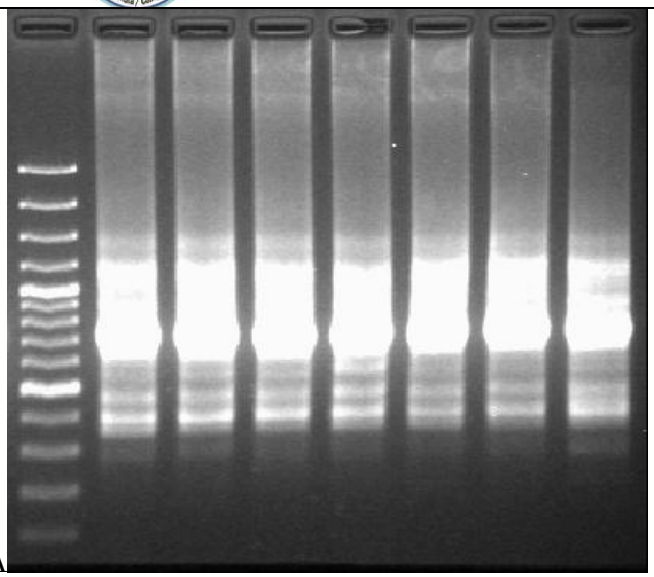


D

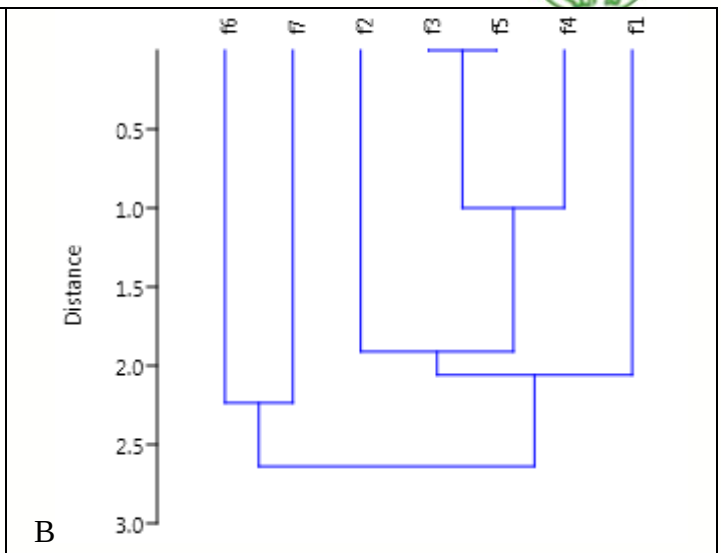
	f1	f2	f3	f4	f5	f6	f7
f1	0	2.236068	3.1622777	1.4142136	1.4142136	1	2.236068
f2	2.236068	0	2.6457513	1.7320508	2.6457513	2	2.8284271
f3	3.1622777	2.6457513	0	2.8284271	2.8284271	3.3166248	3.6055513
f4	1.4142136	1.7320508	2.8284271	0	2	1.7320508	2.6457513
f5	1.4142136	2.6457513	2.8284271	2	0	1.7320508	2.6457513
f6	1	2	3.3166248	1.7320508	1.7320508	0	2
f7	2.236068	2.8284271	3.6055513	2.6457513	2.6457513	2	0

Figure 3. A picture for the PCR product for the primer ISSR 844, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

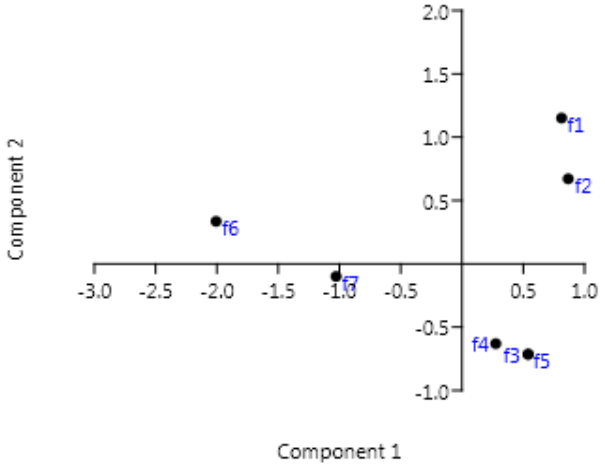
ISSR hb10



A



B



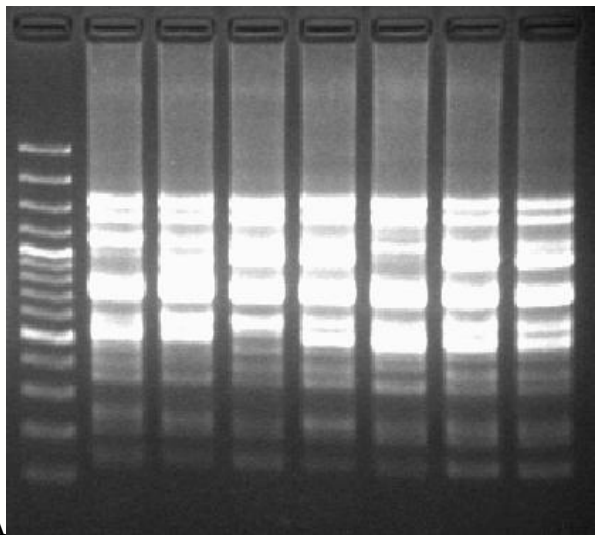
C

D

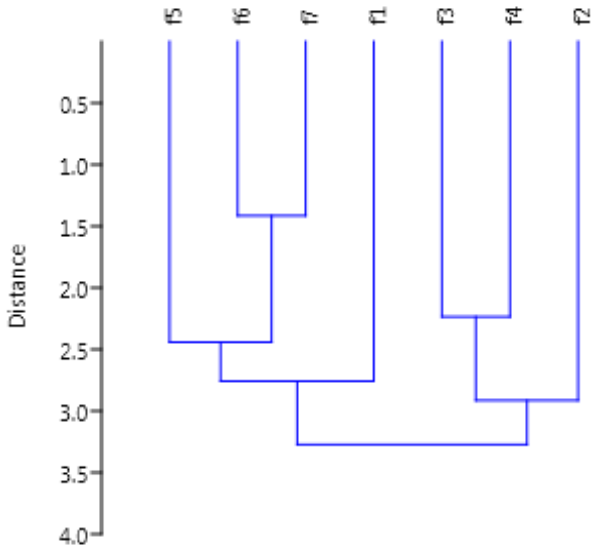
	f1	f2	f3	f4	f5	f6	f7
f1	0	2	2	2.236068	2	3.1622777	2.6457513
f2	2	0	2	1.7320508	2	3.1622777	2.6457513
f3	2	2	0	1	0	2.8284271	2.236068
f4	2.236068	1.7320508	1	0	1	2.6457513	2
f5	2	2	0	1	0	2.8284271	2.236068
f6	3.1622777	3.1622777	2.8284271	2.6457513	2.8284271	0	2.236068
f7	2.6457513	2.6457513	2.236068	2	2.236068	2.236068	0

Figure 4. A picture for the PCR product for the primer ISSR hb10, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

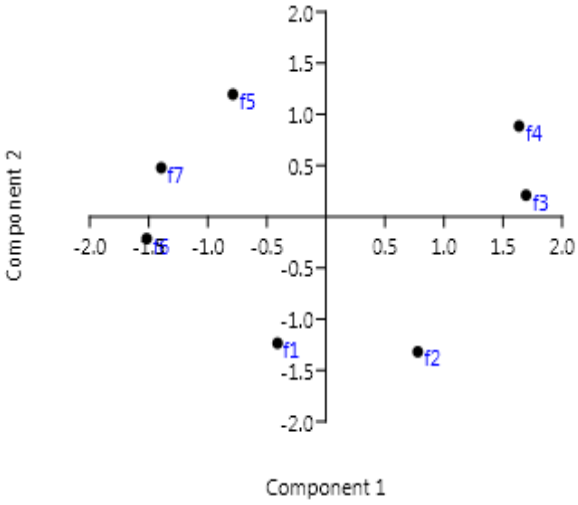
ISSR hb12



A



B



C

	f1	f2	f3	f4	f5	f6	f7
f1	0	2.8284271	3.1622777	3.3166248	3	2.4494897	2.8284271
f2	2.8284271	0	2.8284271	3	3.3166248	3.1622777	3.1622777
f3	3.1622777	2.8284271	0	2.236068	3.3166248	3.4641016	3.4641016
f4	3.3166248	3	2.236068	0	3.1622777	3.6055513	3.3166248
f5	3	3.3166248	3.3166248	3.1622777	0	2.6457513	2.236068
f6	2.4494897	3.1622777	3.4641016	3.6055513	2.6457513	0	1.4142136
f7	2.8284271	3.1622777	3.4641016	3.3166248	2.236068	1.4142136	0

D

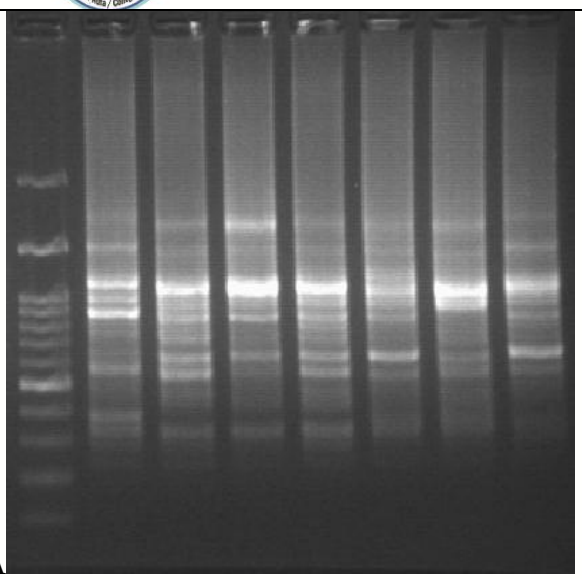
Figure 5. A picture for the PCR product for the primer ISSR hb12, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

Primer IS02

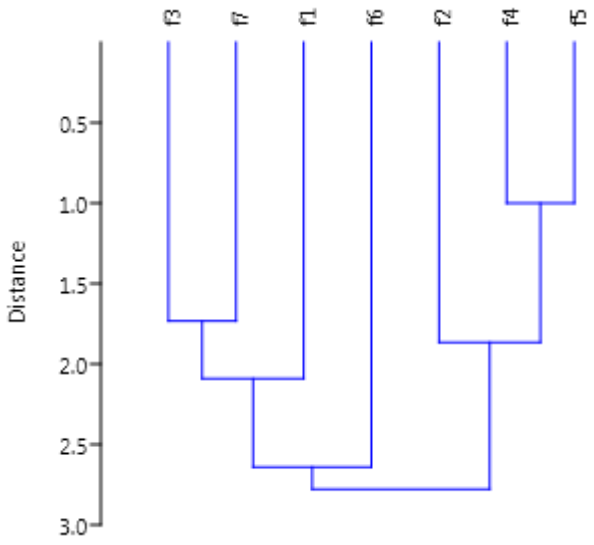
The primer IS02 and IS71 in figer (6 and 7) were gaven total pand 43 and 77 respectively, with averag 6.13 and 11.00 bands/cv. the nearest cultivars in primer IS02 was f4 and f5 which recorded a genetic distance 1.00 , wheareas the nearest distance in primer IS71 between f5 and f7 cultivars wthich recorded 2.00, This is illustrated by the PCA fig. It shows that two Close points are genetically close, and farther points are genetically longer distant. The results are consistent in one aspect with the researchers (Mohamed et al, 2014 ; Ibrahim, 2014 and Elsheikh, 2014).

ISSR IS02

A



B



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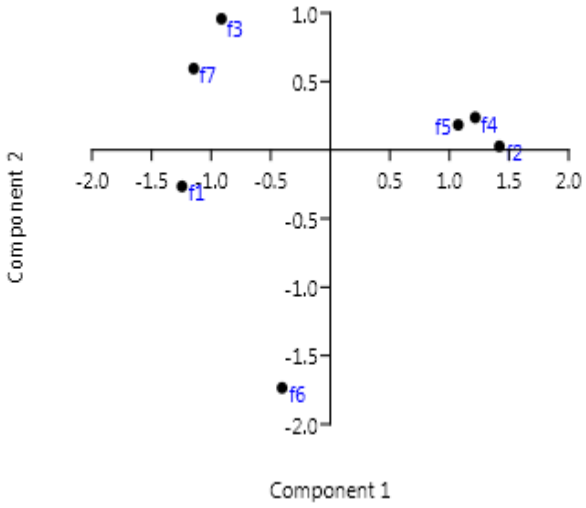
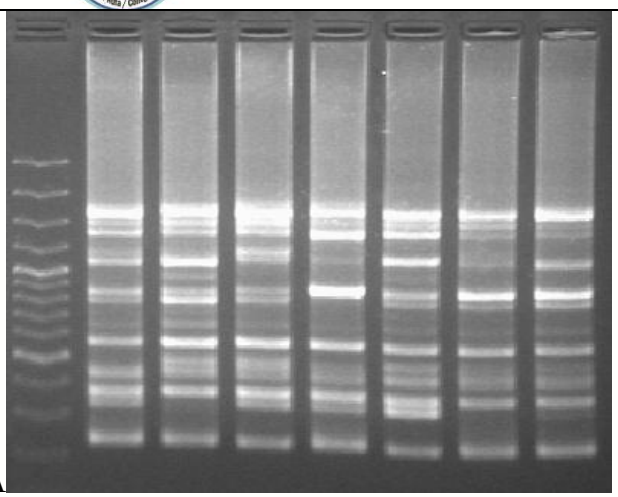


D

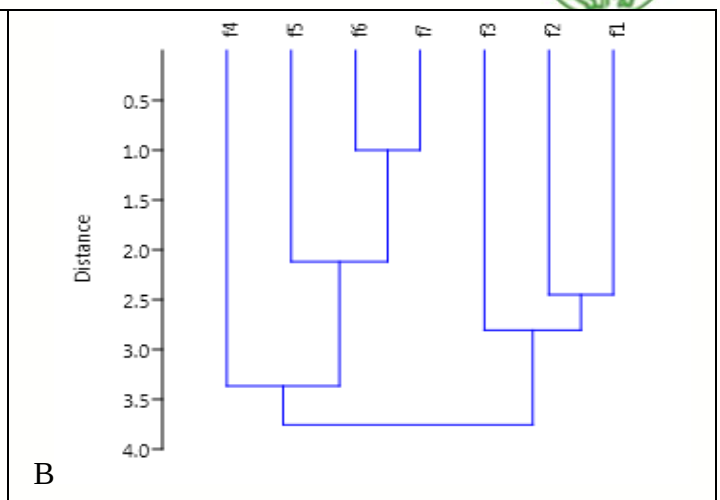
	f1	f2	f3	f4	f5	f6	f7
f1	0	3.1622777	2.4494897	2.8284271	2.6457513	2.4494897	1.7320508
f2	3.1622777	0	2.8284271	2	1.7320508	2.8284271	3
f3	2.4494897	2.8284271	0	2.8284271	2.6457513	2.8284271	1.7320508
f4	2.8284271	2	2.8284271	0	1	2.8284271	2.6457513
f5	2.6457513	1.7320508	2.6457513	1	0	2.6457513	2.4494897
f6	2.4494897	2.8284271	2.8284271	2.8284271	2.6457513	0	2.6457513
f7	1.7320508	3	1.7320508	2.6457513	2.4494897	2.6457513	0

Figure 6. A picture for the PCR product for the primer ISSR IS02, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

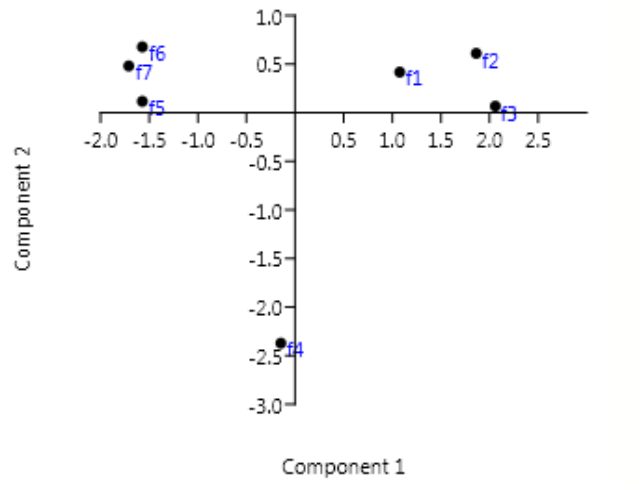
Primer IS71



A



B



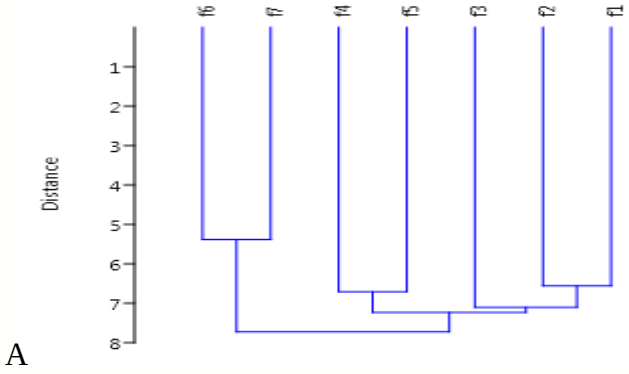
C

D

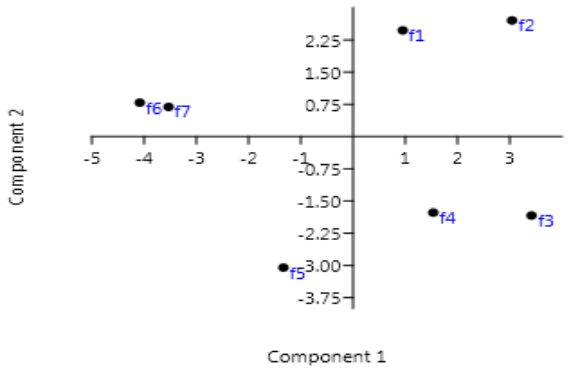
	f1	f2	f3	f4	f5	f6	f7
f1	0	2.4494897	3.1622777	3.4641016	3.6055513	3.4641016	3.3166248
f2	2.4494897	0	2.4494897	3.7416574	3.8729833	3.7416574	3.8729833
f3	3.1622777	2.4494897	0	3.7416574	4.1231056	4	4.1231056
f4	3.4641016	3.7416574	3.7416574	0	3.3166248	3.4641016	3.3166248
f5	3.6055513	3.8729833	4.1231056	3.3166248	0	2.236068	2
f6	3.4641016	3.7416574	4	3.4641016	2.236068	0	1
f7	3.3166248	3.8729833	4.1231056	3.3166248	2	1	0

Figure 7. A picture for the PCR product for the primer ISSR IS71, B - The phylogenetic tree c - Principle component analysis D - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

Figure (8) and table (2) shown the cultivares were devided in to two clusters, the firest clusters was contant f6 and f7 cultivars, whereas the second cluster was contant three sub clusters, the f1 and f2 were a nearest cultivars in one sub cluster, a genetic distance reach 6.55 then the f4 and f5 in ather cluster reach 6.70, but the f3 cultivar take the indivitual cluster. the table (1) shows that the total number of bands produced from the PCR product for the seven primers of the ISSR techneq is (357) bands, the hb12 recorded a highest noumber of band reach 61, whereas the primer 814 recorded a loweres number of band reach 40 band, the primer 844 was recorded a highest polymorphic pand percent (100%) and good discriminaton power. the bater uniq bands were recorded by primer 844 reach 8 bands with 17.02 uniq percent. The results of the paper carry out in Syria on 23 date palm cultivars using the ISSR markers, showed that the phylogenetic tree devided in two clusters, first group (Khastawi ,Deglet Noor, and Maktoom) at genetic distance reach 0.30, and the second group included the other cultivars, the researcher addition the ISSR markers is a good tool to find diversity and fingerprint between date palm cultivars (Haideret al.2012).



A



B

C

	f1	f2	f3	f4	f5	f6	f7
f1	0	6.5574385	7.2111026	6.9282032	7.2801099	7.4161985	7.2111026
f2	6.5574385	0	7	6.8556546	8	8.3666003	8.1853528
f3	7.2111026	7	0	6.78233	7.5498344	8.7749644	8.3666003
f4	6.9282032	6.8556546	6.78233	0	6.7082039	7.8102497	7.4833148
f5	7.2801099	8	7.5498344	6.7082039	0	6.78233	6.8556546
f6	7.4161985	8.3666003	8.7749644	7.8102497	6.78233	0	5.3851648
f7	7.2111026	8.1853528	8.3666003	7.4833148	6.8556546	5.3851648	0

Figure 8. A - The phylogenetic tree B - Principle component analysis C - The table of genetic distance between the cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

Table (2) characteristic of seven ISSR primers on seven date palm cultivars left to right (f1:Aumrani, f2:Jamal aldeen, f3:Stwi, f4:Umalblaliz, f5:Sultini, f6:Azraq Azraq and f7:Asrasi)

No	Peimer Name	Total band	Monomorphic bands	Monomorphic band percent	Polymorphic bands	Polymorphic bands percent	Unique band	Unique Band percent	Efficient of primer	Discrimination power
1	814	40	2	0.04	45	95.74	6	12.76	11.20	14.24
2	844	51	0	0	47	100	8	17.02	14.28	14.87
3	Hb09	52	2	0.04	45	95.74	4	8.51	14.56	14.24
4	Hb10	33	1	0.02	46	97.87	5	10.63	9.24	14.55
5	Hb12	61	3	0.06	44	93.61	6	12.76	17.08	13.92
6	Is02	43	2	0.04	45	95.74	6	12.76	12.04	14.24
7	IS71	77	3	0.06	44	93.61	6	12.76	21.56	13.92
	total	357	13		316				100	

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