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Study on Identification of Five *Citrus Sinensis* Cultivars Based On Similarity / Diversity in Some Genetic Parameters of Their Molecular DNA Profiles Using IRAP-PCR technique

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Abstract

The present study was carried out on five sweet orange Cvs., namely White Khalili, Red Khalili, Succari, Navel and Mazizi to throw Their some light on genetic relationships and fingerprinting Profiles via 5 IRAP-PCR Primers. These primers characterized by their higher to moderate degrees of successful in amplification potentiality for reproducible, polymorphic fragments, specific markers and Co-dominated, as well as their discriminatory power. Obtained data regarding the genetic analysis of the 5 orange Cvs. based on the 5 IRAP primers detected that 59 fragments were amplified from which 37 bands were polymorphic with 63%. In addition, 29 bands of these poly morphic ones were positive unique and considered as specific markers. So there 5 sweet orange Cvs didn't give identical DNA fingerprint. The number of total and polymorphic amplified fragments across the 5 orange Cvs by each IRAP Primer showed a considerable variation. Since, the F10&B6 was the superior (21 total and 18 polymorphic fragments), with the highest Polymorphism % (86). In addition, the number of Positive unique DNA fragments (specific markers) generated by the F10&B6 primer varied greatly from one cultivar to another. Herein Navel Orange cv. occurred the highest number followed by Red Khalili, both (White Khalili & Succary) and finally Mazizi i.e, exhibited 5,4,3,3 and 2 specific markers, respectively.

Results of similarity matrix showed that the highest genetic Similarity (0.877) was existed between both Mazizi &White Khali. Cvs, while the reverse was detected between White Khal. & Red Khal. (0,716). Beside, the UPĢMA dendrogram classified the 5 orange Cvs into two main groups (A &B) i.e, (A.) includes only Succari while (B) was subdivided into (C& D sub- groups) C includes White Khal. only D included E & Fsub-sub clusters, E includes only Navel orange, while F (Mazizi &. Red Khal.) . So the IRAP- PCR an efficient technique for discriminating between Citrus sinensis cultivars even the closely related ones.

Keywords: Sweet orange, *Citrus sinensis*, DNA Fingerprints, genetic diversity, IRAP markers.

Introduction

Citrus is one of the most important fruits , crop it ranked 1st in the world and Egypt .Its cultivated area reached 456082 Feddans and its total fruit production 4245684 Tons and this represented 58.15 % and 36.34 % of the total cultivated fruits area and production respectively after **Ministry of Agriculture Statics, 2019 years.** The total cultivated area of sweet orange represented 316756 Feddans which produced 306665Tons of the fresh fruits.

The Citrus taxonomy based on morphology and geography are very complicated, controversial and confusing **(Jannati *et al.*, 2009).** This led to major controversy on systematics of species within the Citrus subgenus. Two dissimilar classifications schemes have been developed and adopted; the Swingle system that recognizes 16 species (**Swingle and Reece, 1967)** and the Tanaka taxonomy that superfluously splits and identifies 162 species in the genus (**Tanaka, 1977**). However, advanced studies based on biochemical and morphological traits, suggests that there are only three 'true' species, i.e. citron (*C. medica* L.), mandarin (*C. reticulata* Blanco), and pummelo (*C. maxima* L. Osbeck). Other mentioned cultivated Citrus spp. theorized to be hybrids derived as apodictically perpetuated biotypes (**Barrett & Rhodes, 1976 and Scora, 1988)**. Therefore, use of molecular markers has more advantages than that of morphologically based phenotypic characterization, because molecular markers are generally unaffected by external impact (**Uzun and Yesiloglu, 2012**). (**Asins *et al.*, 1999**) investigated the presence of copia-like retrotransposon in citrus. They found that these elements were quite abundant throughout the citrus genome and very heterogeneous. Polymorphisms based on copia-like elements (RFLPs and IRAPs) have been found distinguishing groups of varieties within Citrus sinensis (**Asins *et al.*, 1999**). Moreover, polymorphisms based on these elements are more abundant than those based on primers of random sequence or simple sequence repeats (**Breto *et al.*, 2001**). (**Wei, 2007**) used IRAP markers to estimate phylogenetic relationship among some Citrus cultivars.

Therefore little is known about the genetic relationship and variability of the Egyptian Citrus species and cultivars. So, the main objective of the present study aimed to assess genetic diversity and relationships of some important Citrus sinensis cultivars through investigating genetic molecular analysis via IRAP-PCR technique.

Materials and Methods

DNA extraction:

Total genomic DNA was isolated from young " recently full expanded" leaves sampled separately

from each of the five Citrus sinensis cultivars under study (White Khalili –lane1, Red Khalili –lane2, Succary –lane3, Washington navel –lane4 and Mazizi-lane5) .Sampled leaves were washed in distilled water then 200mg tissue per every sample were girdled to powder using liquid nitrogen in microphage tubes . Extraction was performed by incubating leaves samples in preheated extraction buffer (2.0 % CTAP, 1.4 M NACL ,0.2 % meraptoethanol, 20.0 Mm EDTA, 100.0 mM Tris –HCL- PH 8) at 60 ºC for at least 40 minutes with gentle agitation , after **Doyle and Doyle,(1987).**The Axyprepmultisowrte Genomic DNA Mini – prep Kit (Axygen Bioscience , USA, Cat) was used for DNA isolation.

IRAP- PCR Analysis:

Based on the previous investigations, five IRAP primers characterized by their efficiency and reproducibility of clear banding were selected Table (1). The reactions were carried out in 20 µl volumes in a tube using five primers. Each reaction tube contained 20 ng templates DNA, 2.5 mM MgCl2, 0.2 mM of dNTPs, and 2 µL of 1xTaq DNA polymerase buffer, 0.3 mM primer and 1 units of Taq DNA polymerase.

Amplification was performed in a DNA thermal cycler (Biorad Thermal Cycler MJ Research, Inc, USA), using the following conditions: 94ºC for 2 min, 35 cycles at 94ºC for 30 s, 59-60ºC for 30 s and 72ºC for 1 min, final extensions at 72ºC for 10 min. PCR products were resolved on 2% agarose gel in 1xTAE buffer. The DNA was stained with 0.5 mg/mL ethidium bromide, visualized and photographed under a UV transilluminator. Electrophoretic profile was visualized under UV radiation and photographed with a UV transilluminator. The sizes of DNA fragments were estimated by comparison with standard ladder (1kb; fermentase, Germany).

Table 1. Sequences of the forward and backward IRAP primers.

Name	Forward primer	Name	Back word Primer
IRAP-F1	**5**-AGGAGGTGAATACCTTAG-**3**	**IRAP-B3**	**5**-ATTCCCATCTGCACCAAT-**3**
IRAP-F4	**5**-TATAGTACCTATTGGGTG-**3**	**IRAP-B6**	**5**-ATATATGGACTTAAGCAAGCA-**3**
IRAP-F5	**5**-ATATATGGACTTAAGCAAGC-**3**	**IRAP-B8**	**5**-CCTCCTTATTGGGAATGATAT-**3**
IRAP-F9	**5**-ATATGGACTTAAGCAAGCCA-**3**	**IRAP-B10**	**5**-GACCCTTTTGAAAACACATG-**3**
IRAP-F10	**5**-GATCAAAAAGTTTGGTTTCAT-**3**	**IRAP-B8**	**5**-CCTCCTTATTGGGAATGATAT-**3**

Statistical analysis:

Presence or absence of each band was scored with one and zero for the used five IRAP primers. Then Zero-one matrix was prepared. The total number of amplified fragments / bands and polymorphic bands for each primer were calculated with using Total lab software and the percent of polymorphism was estimated. Polymorphism information content (PIC) was calculated for dominant markers that the allelic relationship between their bands was unclear with the formula:

PIC=Σ [2fi (1-fi)].

Dice similarity matrix was obtained using the software NTSYS-pc 2/02 **(Rohlf, 1998)** and similarity dendrogram was constructed using the UPGMA (The unweight Pair Group Method with Arithmetic) cluster analysis was performed.

The cophenetic correlation test was applied for estimating the correlation between each of the similarity matrices, and the corresponding phenogram the estimated correlation coefficient values showed the goodness of fit of cluster analysis performed on the basis of each of SM (simple maching coefficient), J (Jaccards Coefficient) and D (Dices, coefficient of similarity). In order to evaluate the tree generated from clustering by genetic similarity, coefficients, Consensus fork indices (CIC) were calculated using the strict consensus method of the NTSYS program clustering. CIC measures how resolved the tree is. The Best-Mitted similarity matrix coefficient was then employed for assessment of the genetic diversity. Accordingly Dice similarity coefficient and UPGMA were chosen as the most compatible clustering and Similarity Coefficient.

Results and Discussions

Molecular genetic analysis:

Some techniques of molecular markers application have been demonstrated by several investigators for extracting their patterns through using special reproducible primers for each technique.

In the present study the techniques namely:

IRAP-PCR = Inter Retro transposon Amplified Polymorphism –Polymerase chain Reaction technique was employed for the molecular genetic analysis study of five sweet orange Citrus sinensis cultivars namely: a- White Khalil, b-Red Khalili, c- Succary, d-Navel orange and e- Mazizi Cvs. As lane 1,2,3,4 and 5, respectively.

.IRAP –PCR

The Inter Retrotransposon Amplified Polymorphism –Polymerase chain Reaction, technique allow for detecting insertional polymorphism via amplification of the DNA fragments between two Retrotransposons in plant genomes.

In this study five IRAP primers composed of short tandom repeat sequences were used to analysis the DNA of five samples for five Citrus sinensis cultivars (white Khalili, Red Khalili, Succary, Navel orange and Mazizi Cvs.)

Five arbitrary oligonucleotide primers were used for establish IRAP-PCR finger prints of the five Citrus sinensis cultivars under study. Data obtained as shown in **Tables** (**2**),(**3**),(**4**), (**5**) and (**6**) revealed that all were successful in generating reproducible and polymorphic products (bands / fragments). Each of the five used primers displayed its own potentiality ranged from an acceptable to strong amplification rate with distinct fragments. The fingerprint pattern generated by the five IRAP-primers revealed characteristic profiles for each of the five sweet orange cultivars in terms of number and position of IRAP bands.

Tables (**2), (3), (4), (5), (6), and (7**) as well as **Figures (2),(3), (4),(5) and (6**) display that the total number of the reproducible fragments amplified by the five IRAP primers reached 59 from which 37 were polymorphic fragments with a polymorphism percentage ranging from 38 to 86 % with an average 63% as shown in **Table** (**7**) .

The primer F1 &B8 revealed clear variation in the IRAP products between the studied sweet orange cultivars. **Figure (2**) and **Table** (**2**) illustrate the amplified fragments obtained by this primer. Six polymorphic fragments out of eight amplified ones were scored in the studied cultivars as shown from **Tables** (**2) and (7**). Two only of the total amplified fragments were monomorphic with molecular size at 613.661 and 97.634 bp. However six unique polymorphic DNA fragments amplified by the F1 &B8 primer three of them were positive (3^+), and three others were negative(3^-). The three positive unique DNA bands were amplified at molecular sizes about (415.927& 154.432 bp) and (156.34bp) in red Khalili and white Khalili Cvs. respectively. Meanwhile, the three negative unique polymorphic bands were detected at 894.346bp which absent in white Khalili cv. Only and present in four other cultivars, besides both amplified fragments at 502.118 &305.961bp absent in red Khalili cv. only and presented in four other cultivars. In addition no unique fragments were recorded by the F1&B8 primer. The positive unique polymorphic fragments discriminate between two white and Red Khalili cultivars from one hand the three other ones from the other. Since, Red Khalili cv. Characterized by the presence of both amplified bands at about 415.927 and 154.432 bp which completely absent in four other Cvs. Under study. The white Khalili CV, Identified than four other cultivars under study by Presence of the amplified fragment at 156.341 bp.

As for the analysis of the IRAP_PCR banding pattern of investigated five sweet orange Cultivars generated by the F4&B8 Primer **Figure** (**3**) and **Tables** (**3&7**) display that a total number of amplified fragments reached 6. Three of them were monomorphic at 643.247, 403-933 and 292,620 bp. Meanwhile, three other amplified bands all were unique polymorphic with a polymorphism 50% . These three unique polymorphic bands were Positive and generated at 1409, 317 and 209.879 bp (detected in Succari cv.) as well as at 207.797 in Red Khalili cv. So, the F4&B8 primer discriminate between Succari cv. which characterized by the presence of two unique positive bands with a molecular size of about 1409.317 and 209.797 bp that consequently it could be Identified than the remainder sweet orange cultivars. Moreover, Red Khalili cv. was also distinguished by the presence of the positive unique fragment generated by the F4& B8 Primer at the 207 .797 bp molecular size which was completely absent in four remainder sweet orange cultivars under study.

Concerning the IRAP F5&B10 primer, data obtained are illustrated in **Figure** (**4)**and **Tables** (**4)** and (**7**) .The total number of the generated amplified DNA bands among the five sweet orange cultivars under study by such primer reached 11from which five fragments were polymorphic with 45% polymorphism. The six monomorphic amplified fragments across the studied cultivars were scored at about 820.441, 720.125, 478.562, 351.473 ,308.498 and 165.680bp.

However, the five polymorphic DNA bands were comprised of one no unique fragment amplified at 2053.103 bp which presented in Red Khalil and Succari Cvs only but absent in remainder cultivars i.e, White Khalili, Navel orange and Mazizi. Other Generated polymorphic fragments among the5 sweet orange cultivars under study Via F5 &B10 primer were positive unique (4+) Amplified at (1501.331 & 1033.023 bp) occurred in Red Khalili cv. and (1527.665& 997.715 bp) in Succary cv. According to the presence or absence of positive unique fragments amplified by the F5&B10 primer two sweet orange cultivars were obviously discriminated form the remainder ones under study. Whereas, the Red Khalili cv. was characterized by the presence of two unique fragments with a molecular size at about (1501.331 &1033 bp), while Succary cv. was identified by the presence of the pair unique bands at about 1527.665 &997.715 bp. Other cultivars were characterized by the absence of such a foresaid four bands.

Regarding the characteristic profiles of the five sweet orange cultivars in terms of number and position of the IRAP bands generated by the F9 &B3 primer, data obtained are illustrated in **Figure** (**5**) and **Tables** (**5**) and (**7**). Analysis of the IRAP banding pattern of the studied orange cultivars generated by the F9&B3 primer revealed that the obtained patterns exhibited a molecular weight ranged from about 974.754 to 79.691 bp. Thirteen amplified fragments were produced by the F9&B3 primer from which five only were polymorphic with relative lower polymorphism percentages (38%) . However , eight monomorphic fragments were amplified at about 974.754 ,448.074, 310.283, 258.623, 214.865, 169.459, 99.737, and 79.69 bp. On the other hand , the five polymorphic bands regenerated by F9&B3 primer were identified as two no unique and three unique fragments. Two no unique fragments with a molecular

weight amplified at about 839.321 and 661.955bp both were recorded in the three Red Khalili , Succary and Navel orange but absent in two other cultivars under study (White Khalili & Mazizi). On the other hand ,two of the three unique polymorphic DNA fragments generated via F9&B3 primer were positive and amplified with a molecular weight at 510.320 and 500.459bp, whereas each was solely presented in a single cultivar i.e, Succary and Red Khalili Cvs , respectively and in parallel both were absent in three other cultivars under study .Meanwhile , the third unique fragment was negative with a molecular size at 373.474 bp which was presented in the four White Khalili ,Red Khalili , Succary and Navel orange Cvs, while absent in Mazizi cv. only. It is too interesting to be considered that the presence or absence of a given one or more of the amplified DNA fragments in or out of the characteristic profile for each of the 5 sweet orange cultivars under study are representative of a real scientific tool for discrimination between them. Herein , the no unique polymorphic fragment generated by F9&B3 primer at molecular size of about 839.321 and 661.955 bp recorded in three Red Khalili, Succary and Navel orange Cvs. distinguished such cultivars than two other ones. Moreover, the presence of the positive unique DNA fragment at 510.320bp in Succary cv. only discriminate between such cv. and other ones. Nevertheless, the same was true pertaining the presence of the positive unique fragment at 500.459bp in Red Khalili cv. distinguished it clearly than other genotypes.

In addition, the presence of two amplified DNA fragments at 839.321 and 661.955bp molecular size associated with absence of both 510.320 and 500.459 bp amplified fragments discriminate obviously Navel orange cultivars than other ones. As for the negative unique band representative of presence of DNA fragment at 373.474 bp in all cultivars under study except Mazizi only discriminate it than remainder cultivars.

With regard to analysis of IRAP banding pattern (amplified bands) of the five studied sweet orange cultivars generated by the F10&B6 primer, data obtained are illustrated in **Figure** (**6**) and **Tables** (**6&7**).It is quite clear that 21 DNA fragments were generated by the F10&B6 primer from which 18 were polymorphic with 86.0% polymorphism, while three other ones were monomorphic as shown from tabulated data in **Table** (**7**). From the eighteen polymorphic fragments seventeen were positive unique and only one was no unique. The no unique polymorphic fragment generated by the F10&B6 primer was dealing with the presence of the amplified fragments at 25.603bp which recorded in two White Khalili and Red Khalili orange Cvs. from one hand and its complete absence in Succari ,Navel orange and Mazizi Cvs. from the other. This no unique fragment discriminate partially between the investigated five genotypes, especially as the distribution of the amplified unique primers among all sweet orange cultivars was taken into consideration.

Herein, the positive unique polymorphic fragments generated by the F10&B6 primer at 26.92 bp , 35.987 bp and 96.590 molecular size in Succary , Navel orange and Mazizi Cvs. respectively associated with the absence of the fragment at 25.603 bp clearly distinguished between such three cultivars.

The F10&B6 primer showed the strongest amplification potentiality among the five studied sweet orange cultivars with comparison to the other four IRAP primers used. Anyhow, this primer revealed a clear variation in IRAP products between studied sweet orange genotypes.

As for the number of total producible amplified fragments from the 5 Citrus sinensis cultivars by each of the five IRAP primers and polymorphism % , **Table** (**7**) shows that a considerable variations were clearly observed .Since , the F10&B6 was the superior (21 total amplified bands) , descendly followed by F9&B3 primer (13 fragments) , F5&B10 (11 fragments) , F1&B8 (8 fragments) and F4&B8 which ranked last (6 total amplified bands) .However, ranking was slightly modified with the polymorphism % , whereas F10&B6 come also ,1st (86%), followed in a descending order by F1&B8 primer (75%) , F4&B8 primer (50%) ,F5&B10 primer (45%) and F9&B3 (38%).

Nevertheless, **Table** (**8**) display obviously that the number of positive unique polymorphic DNA fragments (specific markers) exhibited / generated across the five Citrus sinensis cultivars by the F10&B6 primer varied greatly from one cultivar to another .Since, Navel orange cv. occurred the highest number (5 positive unique fragments) ,followed by Red Khalili cv.(4 specific markers), both White Khalil and Succary Cvs (3specific markers per each) and finally Mazizi cv. exhibited the least value (2 specific markers).

A similarity matrix was calculated using IRAP data according to Dice Coefficient. Similarity, dendrogram was constructed using the UPGMA cluster analyses **Figure** (**1**). The 5 studied had similarity values ranging from 0716 to 0.877.Results of similarity matrix showed that the highest genetic similarity (0.877) was existed between two White Khalil and Mazizi orange Cvs, While the lowest genetic similarity value (0.716) was observed between both Red Khalili and White Khalili Cvs.An UPGMA dendrogram was generated by IRAP data and the similarity (0.77) for all cultivars pairs was used as the clusters cut off value (**Fig.1)**

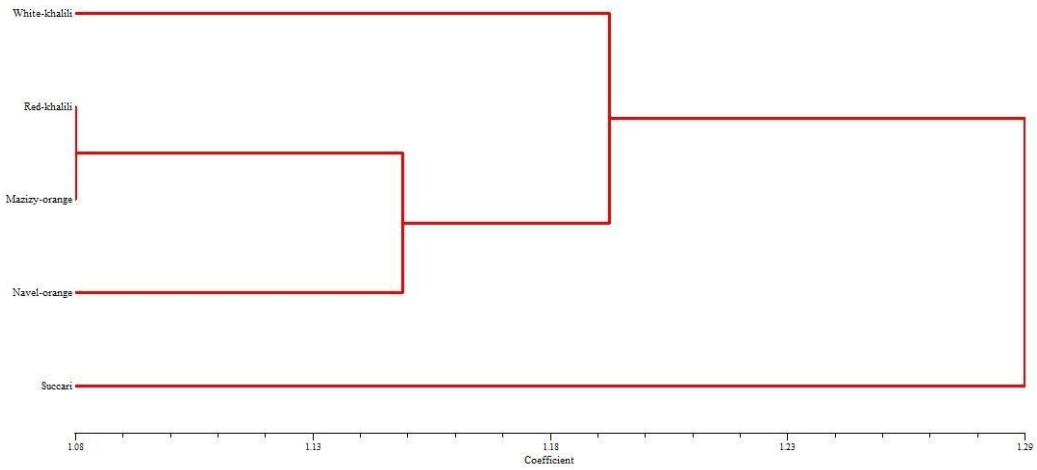

Figure (**1**): Dendrogram generated using UPGMA, revealing relationships between 5 Citrus sinensis cultivars using IRAP data.

From this dendrogram, the five genotypes could be classified into two main classes (A and B). Considering the dendrogram (**Fig**. **1**), cluster A include Succari orange only. The cluster B, the largest group, consisting of two sub-clusters, C and D; C consisted from White Khalili only while D is consisted from E and F sub sub-clusters; E include only Navel orange. Meanwhile F include two genotypes; Mazizi orange and red Khalili. These two genotypes revealed 0.877 genetic similarity.

The rise of a possible mutation related to white or red Khalili orange can be suggested. Usually Bud mutations happen in citrus trees and are generally detected by growers in branches of trees displaying altered horticultural traits, such as maturity and flowering time, or fruit characteristics. Contrasting with this diversity for agronomic traits, very low genetic variability has been found in cultivated citrus using molecular markers. This study revealed that IRAP markers can distinguish mutation- derived species such as white and red Khalili oranges. The use of IRAP markers allowed efficient differentiation of tightly linked genotypes. Generally our results regarding the suitability and efficiency of using IRAP-PCR technique for discriminating and distinguishing between some citrus sinensis cultivars.

Our results are in agreement with the finding of **Hajar *et al.*, (2014**) on using IRAP markers to determine genetic diversity among 29 Citrus genotypes. They suggested that retrotransposon based fingerprinting methods are useful tool for rapid characterization of Citrus and its related genera .This approach could be efficiently employed also for conservation and management as Citrus germplasm genetic resource.

Moreover, findings of **Wei (2007**) on estimating Phylogenetic relationships among 24 Citrus cultivars and **Saleh, (2013)** on *Ficus sycomores* genotypes gave support to the present results regarding the suitability of using IRAP-PCR technique for identifying and discriminating of Citrus cultivars and other species .

However, studies of **Huang *et al,* 2012**) on some mandarin cultivars (*Citrus reticulata*) are in disagreement with our data. They reported that no difference were observed between two mandarin cultivars under study by using 100 retrotransposon primers.

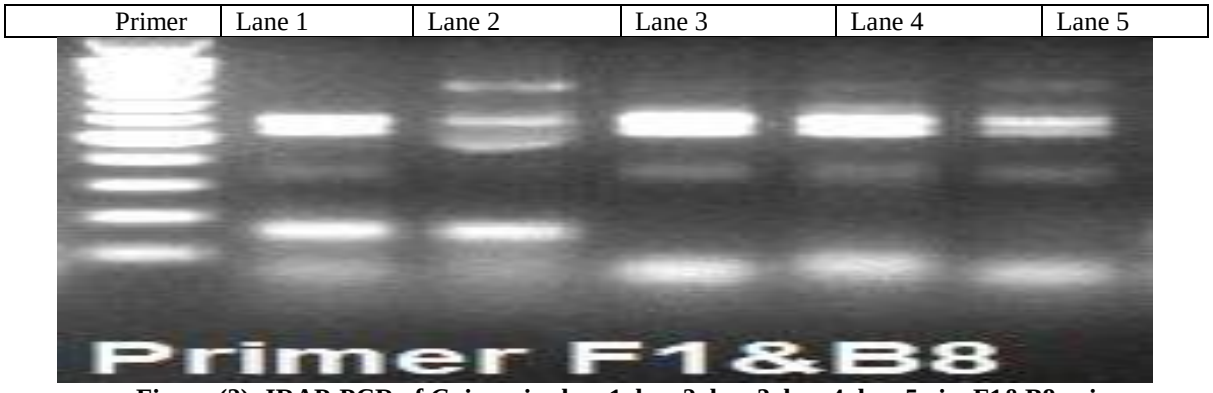

Figure (2): IRAP-PCR of *C.*sinensis , lane1, lane2, lane3, lane4, lane5 via F1&B8 primer.

Table, 2. Number and size n bp of the amplified DNA fragments generated via an oligonucleotide (F1&B8) primer used to establish IRAP- PCR finger prints for five citrus sinensis cultivars.

MW	White Khalili	Red Khalili	Succary	Navel orange	Mazizy
894.346	**0.000**	**1.000**	**1.000**	**1.000**	**1.000**
613.661	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
502.118	**1.000**	**0.000**	**1.000**	**1.000**	**1.000**
415.927	**0.000**	**1.000**	**0.000**	**0.000**	**0.000**
305.961	**1.000**	**0.000**	**1.000**	**1.000**	**1.000**
156.341	**1.000**	**0.000**	**0.000**	**0.000**	**0.000**
154.432	**0.000**	**1.000**	**0.000**	**0.000**	**0.000**
97.634	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
8	**5**	**5**	**5**	**5**	**5**

Primer	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5

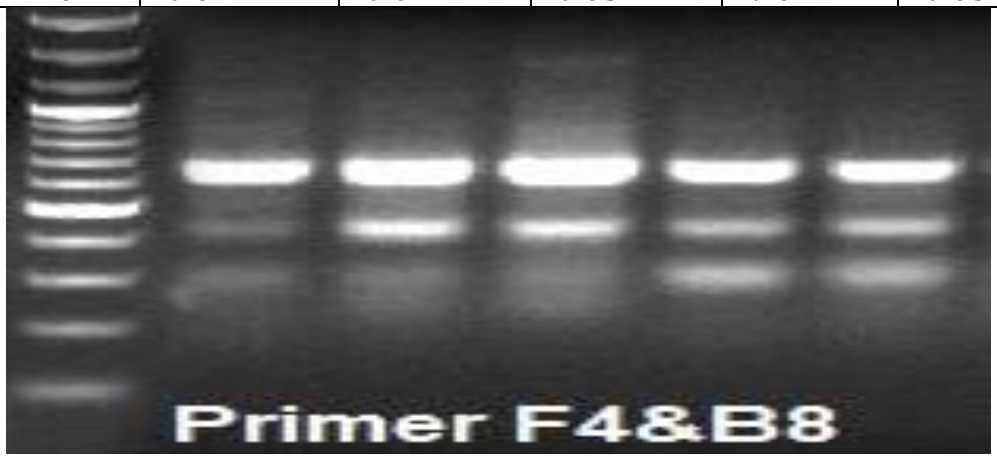

Figure (3): IRAP-PCR of *C. sinensis* , lane1,lane2,lane3,lane4,lane5 via F4&B8 primer .

Table, 3. Number and size n bp of the amplified DNA fragments generated via an oligonucleotide (F4&B8) primer used to establish IRAP- PCR finger prints for five citrus sinensis cultivars**.**

MW	White Khalili	Red Khalili	Succary	Navel orange	Mazizy
1409.317	**0.000**	**0.000**	**1.000**	**0.000**	**0.000**
643.247	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
403.933	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
292.620	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
209.879	**0.000**	**0.000**	**1.000**	**0.000**	**0.000**
207.797	**0.000**	**1.000**	**0.000**	**0.000**	**0.000**
6	**3**	**4**	**5**	**3**	**3**

Primer	**Lane 1**	**Lane 2**	**Lane 3**	**Lane 4**	**Lane 5**

Figure(4):IRAP-PCR of *C.*sinensis , lane1, lane2, lane3, lane4, lane5 via F5&B10 primer .

Table 4. Number and size n bp of the amplified DNA fragments generated via an oligonucleotide (F5&B10) primer used to establish IRAP- PCR finger prints for five citrus sinensis cultivars**.**

MW	White Khalili	Red Khalili	Succari	Navel orange	Mazizy
2053.103	0.000	1.000	1.000	0.000	0.000
1527.665	0.000	0.000	1.000	0.000	0.000
1501.331	0.000	1.000	0.000	0.000	0.000
1033.023	0.000	1.000	0.000	0.000	0.000
997.715	0.000	0.000	1.000	0.000	0.000
820.441	1.000	1.000	1.000	1.000	1.000
720.125	1.000	1.000	1.000	1.000	1.000
478.562	1.000	1.000	1.000	1.000	1.000
351.473	1.000	1.000	1.000	1.000	1.000
308.498	1.000	1.000	1.000	1.000	1.000
165.680	1.000	1.000	1.000	1.000	1.000
11	6	9	9	6	6

Primer	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5

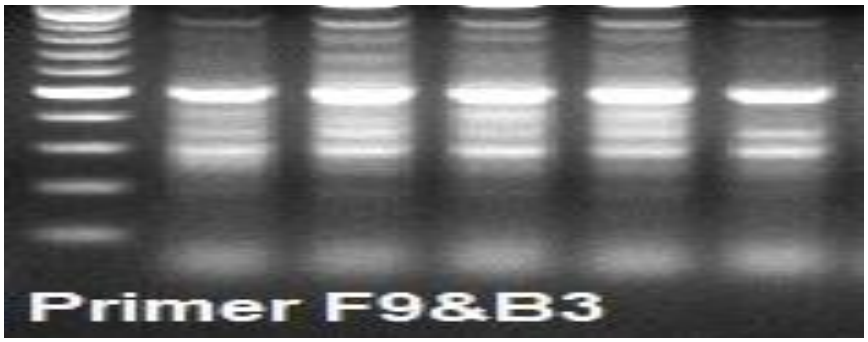

Figure (5): IRAP-PCR of *C. sinensis*, lane1,lane2, lane3, lane4, lane5 via F9&B3 primer .

Table, 5. Number and size n bp of the amplified DNA fragments generated via an oligonucleotide (F9&B3) primer used to establish IRAP- PCR finger prints for five citrus sinensis cultivars.

MW	White Khalili	Red Khalili	Succary	Navel orange	Mazizy
974.754	1.000	1.000	1.000	1.000	1.000
839.321	0.000	1.000	1.000	1.000	0.000
661.955	0.000	1.000	1.000	1.000	0.000
510.320	0.000	0.000	1.000	0.000	0.000
500.459	0.000	1.000	0.000	0.000	0.000
448.074	1.000	1.000	1.000	1.000	1.000
373.474	1.000	1.000	1.000	1.000	0.000
310.283	1.000	1.000	1.000	1.000	1.000
258.623	1.000	1.000	1.000	1.000	1.000
214.865	1.000	1.000	1.000	1.000	1.000
169.459	1.000	1.000	1.000	1.000	1.000
99.737	1.000	1.000	1.000	1.000	1.000
79.691	1.000	1.000	1.000	1.000	1.000
13	9	12	12	11	8

Primer	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5

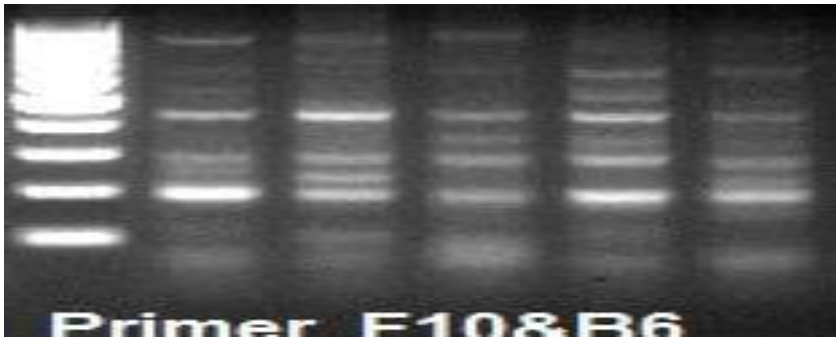

Figure (6): IRAP-PCR of *C. sinensis*, lane1, lane2, lane3, lane4, lane5 via F10&B6 primer .

Table 6. Number and size n bp of the amplified DNA fragments generated via an oligonucleotide (F10&B6) primer used to establish IRAP- PCR finger prints for five citrus sinensis cultivars.

MW	White Khalili	Red Khalili	Succary	Navel orange	Mazizi
1414.265	0.000	0.000	1.000	0.000	0.000
1291.524	0.000	1.000	0.000	0.000	0.000
1262.541	1.000	0.000	0.000	0.000	0.000
878.076	0.000	1.000	0.000	0.000	0.000
687.968	0.000	0.000	0.000	1.000	0.000
467.733	1.000	0.000	0.000	0.000	0.000
446.975	0.000	0.000	0.000	1.000	0.000
327.151	1.000	1.000	1.000	1.000	1.000
207.782	0.000	0.000	1.000	0.000	0.000
198.560	0.000	0.000	0.000	1.000	0.000
142.069	1.000	1.000	1.000	1.000	1.000
103.395	0.000	1.000	0.000	0.000	0.000
96.590	0.000	0.000	0.000	0.000	1.000
75.677	1.000	1.000	1.000	1.000	1.000
46.986	1.000	0.000	0.000	0.000	0.000
39.407	0.000	1.000	0.000	0.000	0.000
35.987	0.000	0.000	0.000	1.000	0.000
26.792	0.000	0.000	1.000	0.000	0.000
25.603	1.000	1.000	0.000	0.000	0.000
24.467	0.000	0.000	0.000	0.000	1.000
23.381	0.000	0.000	0.000	1.000	0.000
21	7	8	6	8	5

Table, 7. Number and type of the amplified DNA fragments generated via (F1&B8), (F4&B8), (F5&B10), (F9&B3) and (F10&B6) IRAP primers from five citrus sinensis cultivars under study.

Primer code	Total amplified fragments	Length range (bp)	monomorphic	Polymorphic fragments			of polymorphism %
				Nonunique	unique	total	
F1&B8	8	894,346-97,634	2	--	3(+) 3(-)	6	75
F4&B8	6	1409,317-207,797	3	--	3(+)	3	50
F5&B10	11	2053,103-165,680	6	1	4(+)	5	45
F9&B3	13	974,754-79,691	8	2	2(+) 1(-)	5	38
F10&B6	21	1414,265-23,381	3	1	17(+)	18	86
Total	59	----	22	4	33	37	63

Table, 8. Numbers and molecular weight (bp) of specific markers (positive unique polymorphic DNA fragments) generated via 5 IRAP primers from different sweet orange, *Citrus sinensis* under study.

	White Khalili		Red Khalili		Succari		Navel Orange		Mazizi Orange		
primers	Numbers	M.w. (bp)	Numbers	M.w (bp)	Numbers	M.w. (bp)	Numbers	M.w. (bp)	Numbers	M.. (bp)	total
F1&B8	1	156.341	2	154+415.927 .432	-	-	-	-	-	-	3
F4&B8	-	-	1	207.797	2	1409.317 209.879+	-	-	-	-	3
F5&B10	-	-	2	10+1501.331 33.023	2	1527.665 997.715+	-	-	-	-	4
F9&B3	-	-	1	500.459	1	510.320	-	-	-	-	2
F10&B6	3	1262.541 467.733+ 46.986+	4	87+1291.524 103.3+8.076 39.407+95	3	1414.265 207.782+ 26.792+	5	446.97+687.968 35.+198.560+5 23.381+987	2	96.59 24+0 .467	17
TOTAL	4	---	10	---	8	---	5	---	2	---	29

Table, 9. Pairwise similarity matrix of five Citrus generated from IRAP data analysis.

Similarity Coefficient					
Case	**Matrix File Input**				
	White Khalili	**Red Khalili**	**Succary**	**Navel orange**	**Mazizi**
White Khalili	**1**	**-**	**-**	**-**	**-**
Red Khalili	**0.716**	**1**	**-**	**.-**	**-**
Succary	**0.757**	**0.72**	**1**	**-**	**-**
Navel orange	**0.806**	**0.732**	**0.8**	**1**	**-**
Mazizi	**0.877**	**0.697**	**0.769**	**0.819**	**1**

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دراسه علي تعريف خمسة اصناف برتقال علي اساس التشابهة و التباين في بعض القياسات الوراثيه لتهيئه جزيئات احماضها النوويه (دنا) باستخدام تقنيه IRAP –PCR

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أجريت هذه الدراسة علي خمسة اصناف برتقال (خليلي أبيض , خليلي احمر , سكري , ابو سره, مزيزي) لإلقاء بعض الضوء علي تباينات التراكيب الوراثية و البصمة الوراثية بينهما من خلال تضخيم خمسة مواقع وراثية لها بإستخدام خمسة معلمات لتكنيك IRAP حيث تميزت هذه المعلمات بقدراتها العالية و المتوسطة للتمييز بين هذه الاصناف تحت الدراسة . وقد أظهرت نتائج تحاليل البصمة الوراثية أن العدد الكلي للحزم المضخمة 59 حزمة من بينهم 37 حزمة متباينة (بنسبه تباين 63%) و أن 29 حزمه من بين هذه الحزم المتباينة كانت منفردة موجبة اي تعتبر نوعية Specific markers قد تكون مرتبطة بجينات وظيفيه جديده مميزة ويمكن استخدامها في برامج تحسين البرتقال لاحقا .

وعليه بأن هذه الاصناف لم تعطي بصمة وراثية واحدة و محددة فلم يوجد تطابق كامل ولا حتي تباين كامل فيما بينهم فلم تتشابه في كل الحزم المضخمة بل ايضا التباينات في عدد الحزم المضخمة وكذلك المتباينة من صنف لأخر . كما هذه المعلمات الخمسة المستخدمة إختلفت فى قدرتها على التضخيم فكان المبادئ F10&B6 هو الأكثر تفوقا (21 حزمة منها 18 حزمة متباينة بنسبة تباين عالية 86 %) وإختلفت الأصناف المختبرة فى عدد الحزم المنفردة الموجبة (النوعية) من صنف لأخر حتى للبرايمر الواحد فقد أظهر البرتقال أبوسره أكبر عدد من هذه الحزم المنفردة الموجبة 5 حزم يليها فى ترتيب تنازلى الخليلى الأحمر 4 حزم والخليلى الأبيض والسكرى (كل 3 حزم) والمزيزى 2 حزمة .

وعن التمييز بين الأصناف على أساس قيم التشابه الوراثى وإن كانت عاليه نسبيا فكانت أعلى قيمة هى 0.877 وعند مقارنة المزيزى بالخليلى الأبيض والعكس كان صحيحا بمقارنة الخليلى الأحمر بالخليلى الأبيض (0.716) . أما بالنسبة لشجرة القرابة الوراثية فقد أظهرت النتائج إمكانية تمييز هذه المجموعة عن بعضها البعض مما يثبت فعالية وكفاءة تكنيك IRAP فى هذا الصدد حيث قسمت هذه الأصناف إلى مجموعتين (أ, ب) تضمنت المجموعة (أ) صنف السكرى منفردا و أما المجموعة (ب) فقسمت إلى تحت مجموعتين (ج ,د) شملت تحت المجموعة (ج) أبوسره منفردا أما تحت المجموعة (د) قسمت إلى تحت تحت مجموعتين (هـ ,و) جاء الخليلى الأحمر تحت تحت المجموعة (هـ) والمزيزى فى تحت تحت المجموعة (و) .