Identification of Three Egyptian Annona Cultivars Morhologically and Biochimically using Rapd Analysis

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ABSTRACT

To detect remarkable fingerprinting and evaluate genetic variation and similarity among three Egyptian cultivars *Annona* sp. (which cultured in Sabahia Horticulture Research Station, during 2016 and 2017 seasons atSabahia Horticulture Research Station (HRI), namely: Abd Elrazek, Baladi, and Senigalinsis) first morphologically using Leaf diameter, then seed number and fruit dimension, it was found that fruit and seeds could be used to evaluate variation and genetic similarity among cultivars. Where Abdelrazik was the lowest in seed number with 37 seeds comparing with Baladi and Sengalinsis with 73 and 127 respectively.

Moreover, fruit could be used to evaluate genetic similarity among cultivars. Abdelrzak was the lowest in seed number with 37 seeds comparing with Baladi and Senigalinses with 73 and 127 respectively.

Leaf parameters were used as a species and varieties diversity and found useful with longest leaf length for Abdelrzak and Sengalinsis 13.5 cm and 12.43 cm respectively. While Baladi was the shortest with 0.30 cm.

`Finally molecular marker techniques (Random Amplified polymorphic DNA (RAPD) fingerprinting technique,) were used Different polymorphism percentages were recorded for four random primers (OPG1, POG2, OPG3 and OPG4) through this investigation. Generally, all genotypes could be distinguished via four random primers under study. First primer reflected high polymorphic percentage with 82 % of polymorphism. On the other hand, high similarity percentage and lowest polymorphism percentage were recorded for fourth primer with 50 % of polymorphism.,,, A dendrogram was done to depict the pattern of relationships between the studied cultivars and their genetic diversity Not only it was fairly good But Also it reveal the need of further studies with new suitable techniques.

keywords: RAPD, *Annona* sp, genetic similarity, morphological.

INTRODUCTION

Annona squamosa L.(Sugar Apple) could be considered an important fruit crop grown commercially worldwide. Although, few breeding programs for sugar apple around the world were founded. Little propagated vegetative cultivars were lunched in India, China and Taiwan as described by (Nakasone & Paul, 1998).

Considering this family Annonaceae is one of the most uniform, morphological characters like, morphology of the ruminated seed and tiny embryo, which was considered, applied for taxonomic purposes. In the past few years, morphological traits have been used as tools to characterize germplasm resources. Unfortunately, morphological characters tend to be influenced by environmental factors and problems with ambiguity are frequent. Molecular markers have provided a powerful tool for proper characterization of germplasm diversity (Williams et al., 1990)

RAPD (random amplified polymorphic DNA) is a deep-rooted methodology to provide information regarding variable of DNA-level for many applications in genetic analyses (Ferreira and Grattapaglia, 1995). Commonly of these markers in conservation programs can be for any kind of organism and to their accelerated results (Lopes et al., 2002). Nevertheless, molecular markers gave effective help for detecting characterization of germplasm diversity due to influence morphological characters by environmental factors (Williams *et al*., 1990). Also, RAPD markers have been used for diversity analysis in different plant etc, Cacao (Leal *et al*., 2008). Ronning *et al*., (1995) indicated efficiency of RAPD markers as fingerprinting method genotypes within and between *Annona* species via performed RAPD analysis of *Annona. cherimola.* `Campa' and `Jete,' *A. squamosa* `Lessard,' and the atemoyas `Ubranitzki,' `Malali,' and `Kaspi' resulted in very distinctive patterns. Also, Fifty-two polymorphic loci were identified, which segregated in an expected Mendelian fashion.

It well known that, *Annona* contains more than 50 species and interspecific hybrids, many of which are cultivated in tropical and subtropical America for their edible fruit. Five groups and 14 sections composed genus *Annona* (based on morphological characters) (Safford, 1914).

Different variations in *Annona* fruits shapes and sizes were detected. Fruit flesh is sweet, white to light yellow, and resembles and tastes like custard. It can maintain the charming appearance, strengthen immune system of the body, prevent scurvy, and act as an anticancer; it is known as the upper tonic, high nutritional value in the ancient times of China (Xie et al., 2009). Also Keny *et. al*. (2010) was found that The most important indirect effects were obtained for number of seeds and pericarp weight, obtained via pulp weight, on fruit

weight, and for fruit length and width, obtained via mean fruit weight, on fruit yield.

Medicinal purposes of *Annona muricata* were anciently detected. Bark, leaves, root and fruit-seeds of *A. muricata* tree are used in natural medicine in the tropics. Bark, leaves and roots are considered sedative, antispasmodic, smooth muscle relaxant and nervine and a tea is made for various disorders, fruit and fruit juice is taken for worms and parasites, to cool fevers, to increase mother's milk after childbirth (lactagogue), and as an astringent for diarrhea and dysentery. Finally, seeds crushing are used as a vermifuge and anthelmintic against internal and external parasites and worms (Adewole and Caxton-Martins 2006).

MATERIALS AND METHODS

A-Plant material

The present work carried out during 2016 and 2017 seasons to identify and distinguish the three Annonas varieties namely Abdelrazik of (*A.squamosa Sugar* apple), Senigalensis *(A. Senigalinses*) African Sugar Apple and Balady (*A. squamosa*)-Sugar Apple thats' comertially cultivated in Egypt.

The Three *Anonna* cultivars were grown in the Sabahia experimental Station Farm in heavy clay with snail soil, at (Sabahia) Alexandria Governorate, where each cultivar was represented by three trees selected as uniform as possible were labeled.

For leaf parameters measurement ten mature leaves were used from each of the three replicate tree replicates at end of July and all measurement were taken including (Medial width of the leaf blade(W_1), in centimeter, width for the leaf at the basal quarter of the leaf as well as Leaf length from petiole conjunction to blade tip(L_1) in centimeter,) were mesured.

For fruits features of the three studied cultivars ten fruits were randomly taken from every selected trees and all the measurement were taken including (fruit Wight in grams(W_0) width of the leaf blade(W_0), in centimeter, width for the leaf at the basal quarter of the leaf as well as Leaf length from petiole conjunction to blade tip(L_1) in centimeter,) were measured.

Statistical analysis were performed using the Compete Randomized Design (CRD.) according to Snedecore and Cochran(1982)

DNA extraction Procedure for total DNA and Preparation of the PCR master mixture:

Genomic of The three *Annona*s extracted according to manufacturer protocol of Omega Co. (USA.LMt.)Kit.

Under the PCR cabinet, amplification reaction was prepared in a separate room rather than that in which the extraction were done. In Eppindorf tube, the components of the PCR were prepared as a master mix containing the reagents needed to amplify the required number of samples as well as positive and negative control (Table, 1) then 4µl (25 ng) of the DNA were added in the PCR tubes and 1.0Pmol of random primer was added random primer listed in table1) 12.5µl of the master mix were, to reach 25µl as a final reaction volume.

Table 1: Random Amplified Polymorphic DNA primers under study.

Primer	Sequences
OPG1	ATTTATCGTC
OPG12	GCTGAGCGTC
OPG3	GACCTAGCGA
OPG4	ATGCACAGTG

D. RAPD -PCR amplification:

Total genomic DNA was amplified through GeneAmp Polymerase Chain Reaction (PCR) system cycler. PCR for amplified genomic DNA was carried out. RAPD reactions were performed as described by Williams *et al*. (1990).

E. Agarose gel electrophoresis and detection of the amplification products:

1.5% agarose solution was prepared by adding 0.75g a arose to 50ml of 1x TBE electrophoresis buffer. Run was performed via adjusted current at 80 Volts for 100 min.

F. Data analysis*:*

Gel documentation system (Geldoc-it, UVP, England) and data was interpretated using software analysis,ww.totallab.com, (Ver.1.0.1).

RESULTS AND DISCUSSIONS

Morphological character variations could be noticed visually for the three *Annona*s under study the data presented in (Table 2) Morphological traits could be illustrated as follow:

Abdelrazik was the lowest in seed number with 37 seeds comparing with Baladi and Sengalinsis with 73 and 127 respectively. for the three studied Annona varieties.

Concerning the fruit weight Balady *CV.* Had the lowest fruit weight W_0 with 151.9 grams, While Elrazek had the highest fruit weight with 398.17 grams. while uneatable Senigalinsis *CV*., followed by Senigalinses with 7.67 centimeters while Balady *CV*. Was the lowest Length with 6.67 centimeters.

Fruit Peel weight in grams also employed to describe the three studied varieties variation where, Elrazek found to have the highest Fruit Peel with 98.067 grams followed by Balady with 44.23 grams and finally Senigalinses with 26.96 grams.

Fruit Peal to the total fruit weight P/W Ratio were also studied and fount to had a different trend than that for every single parameter alone that Balady found to have highest ratio (0.291) followed by Abdelrazik (0.246) and finally Senigalinses for (0.152)

Table 2: Fruit, Leaf and seed parameters

variety	Fruits parameters					Leaf parameters		seed parameters	
Abbreviation/ Units	weight grams	Length cm(L_0)	diameter cm	Peel weight grams (P)	P/W Ratio	Length cm (L_1)	Width cm W	No.#	weight grams
Abdelrazik	398.17a	9.40a	9.07 a	98.067a	0.246a	13.50a	6.20a	37 c	23.73 a
Balady	151.9. c	6.67 c	6.43 c	44.23 b	0.291a	10.3b	5.77 a	73 b	14.07 b
Senigalinses	177.33b	7.67b	7.43 b	26.96 b	0.152b	12.43a	6.0 a	127a	21.76 a
LSD(.05)	18.36	0.99	0.151	18.19	0.93	1.24	1.44	6.05	3.27

This Ratio found to express the importunacy of the fruit value better than the fruit w weight only. Andthat found to agree with the findings of Keny *et. al*. (2010) whenthey found Positive correlations were obtained between number of seeds and seed weight, and between number of fruits and yield. The greatest direct effects were those obtained for pulp weight on fruit weight and for mean number and weight of fruits on fruit yield. The most important indirect effects were obtained for number of seeds and pericarp weight, obtained via pulp weight, on fruit weight, and for fruit length and width, obtained via mean fruit weight, on fruit yield.

`Moreover, Leaf parameters including ((Leaf Length L_1) and (Leaf Width cm W) were utilized to characterize the three studied varieties variation where, Abdelrazik found to have the Longest Leaf Length with 13.5 cm. followed by Sengalinsis at 12.43 cm. While Baladi was the shortest with 10.30 cm.

Figure (1) with Photograph. A, B C, E,F,, K,L, H and G). charactarize, fruit and seeds. as morphological characters to distinguish morphological and genetic relationships for the three *Annona*s. namely, Abd Elrazek(A, E and H), Baladi(B,F and K) and Sengalinsis(C, G and L).

Random amplified polymorphic DNA (RAPD) technique:

To detect the differences among the three *Annona*s. namely, Abd Elrazek, Baladi and Sengalinsis through four arbitrary primers (OPG1, POG2, OPG3 and OPG4), Random amplified polymorphic DNA(RAPD) technique were performed. The numbers and sizes of genomic bands and Polymorphic bands resulted from applying those four primers with three *Anonna*s. are shown in (Figure 2) and (Table 3). Based on Random amplified polymorphic DNA (RAPD) data were founded 82, 53, 69 and 50 polymorphism percentages for first, second, third and fourth random primers.

Using primer 1 with the genomic DNA from the three *Annona*s. reflected eleven genomic bands with various sizes range. Nineteen bands with various sizes range were recorded for genomic from the three *Annonas*Ten bands were recorded as polymorphic bands with 53 % of polymorphism. After genomic amplifications of for genomic from three *Anonna sp.* cultivars with third primer, thirty one bands were obtained. In addition, nine polymorphic bands were recorded with 69 % of polymorphism. Sixteen bands with various sizes rang were recorded for genomic for genomic from three *Annona sp*. cultivars. Eight bands were recorded as polymorphic bands with 50 % of polymorphism. Numbers of Polymorphic bands were nine bands with 82 % of polymorphism Fourth primer reflected high similarity relation among cultivars under study as results of polymorphism percentage decreasing. Highest variation among the three *Annona*s. could be detected via first primer with 82 % of polymorphism. Thus, highly is similarity percentage among cultivars was founded through employed first random primer.

Genetic similarity for the three *Annona*s. Namely, Abd Elrazek, Baladi and Sengalinsis was evaluated distinctly based on RAPD fingerprinting technique. Almost identical fingerprinting for Abd Elrazek and Baladi indicate high genetic similarity between Abd Elrazek and Baladi. In accordance of morphological distinguished traits (fruit and seeds number) Sengalinsis was separated in an independent cluster.

DISCUSSION

Noticeable morphological characters for Annona sp. was previously remarked by Pimenta et al, (2012). They rely on morphology of *Annona crassiflora Mart* seedling. They indicate that, all morphological data of araticum fruits, seeds and seedlings can be used to recognize the botanical family and, when associated to other features, to recognize the species in the field. More evidences were added to our study for depending on *Annona sp.* morphological characters by Hayat (1963) who studied morphology of seed germination and seedling in *Annona squamosa*. Furthermore Sandra *et tal* (2016) working on collection composed of 167 accessions of the species *Annona cherimola, A. glabra, A.reticulata,* and *A. squamosa, Rollinia sp.,* and the interspecific hybrid *A. squamosa* x *A. cherimola* found the morpho-agronomic evaluation was carried out on 98 accessions using 25 qualitative and quantitative descriptors, which identified the phenotypic traits in *A. squamosa* and *A. reticulate* that are related to fruit quality, such as size, weight, symmetry, degrees Brix, pH, acidity, seed weight and seed number, which are the more discriminating descriptors, differentiating six clusters in *A. squamosa* and four clusters in *A. reticulata.*

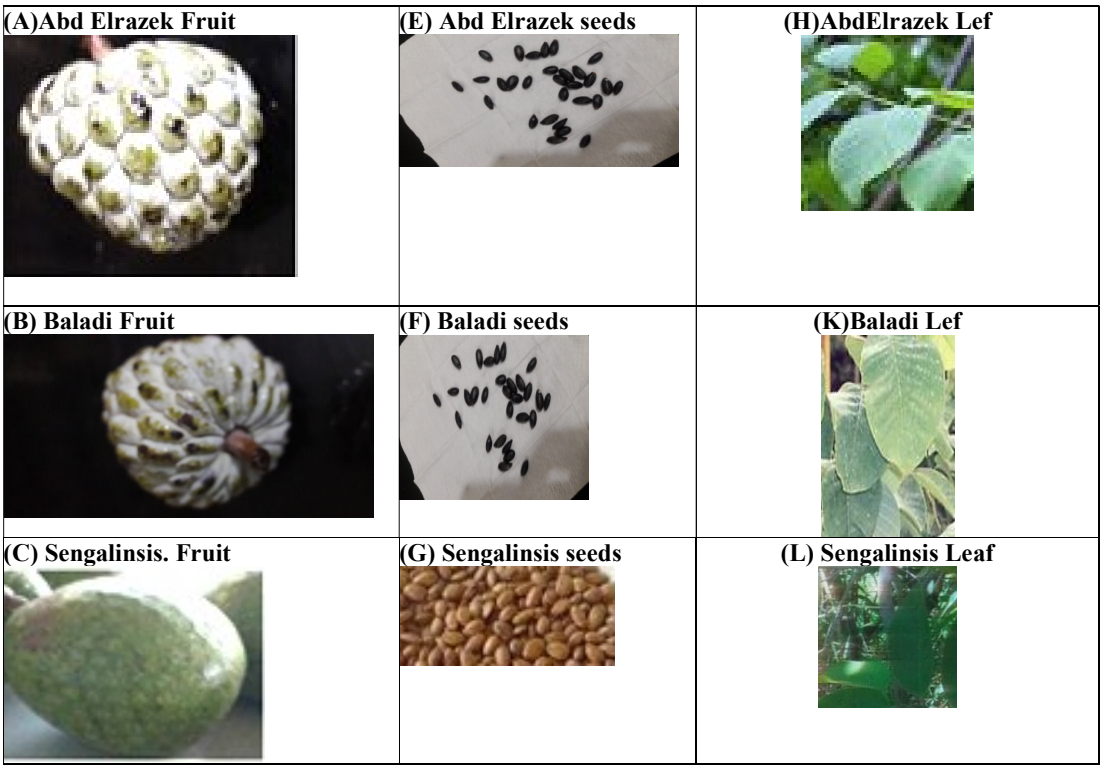

Fig1: Fruit, Leaf and Seeds for the three studied Annona CV.s

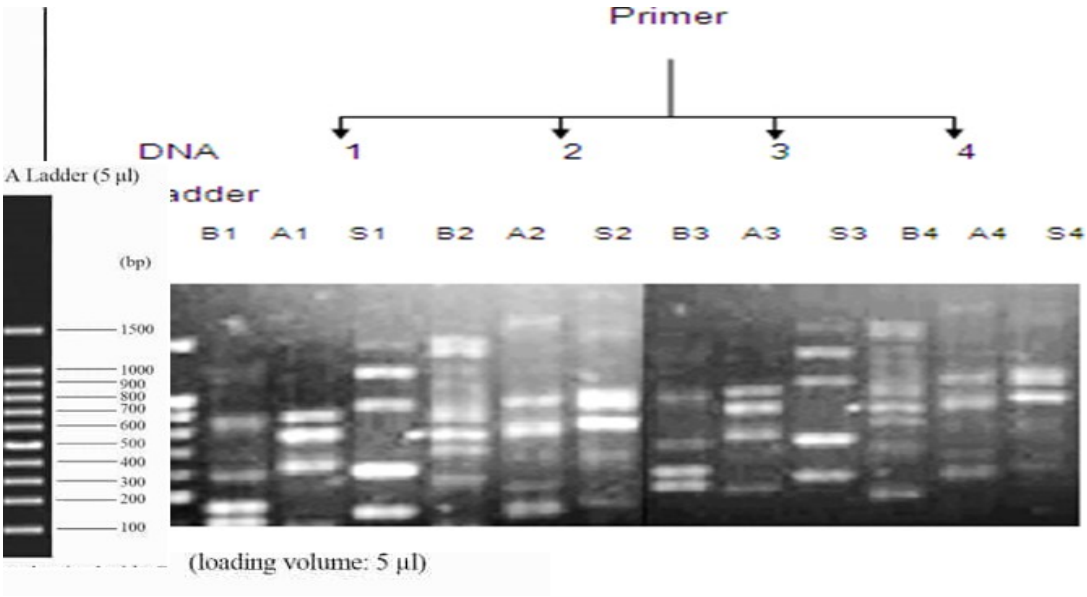

Figure 2: Photograph showing the Random Amplified Polymorphic DNA results.

Where:

DNA Ladder
A1: Abd Elrazek cultivar screened with first primer.
B2: Baladi cultivar screened with second primer.
S2: Sengalinsis cultivar screened with second primer.
A3: Abd Elrazek cultivar screened with third primer.
B4: Baladi cultivar screened with fourth primer.
S4: Baladi cultivar screened with first fourth primer.
B1: Baladi cultivar screened with first primer.
S1: Sengalinsis cultivar screened with first primer.
A2: Abd Elrazek cultivar screened with second primer.
B3: Baladi cultivar screened with third primer.
S3: Sengalinsis cultivar screened with third primer.
A4: Abd Elrazek cultivar screened with fourth primer.

Table 3: Summarized Random amplified Polymorphic DNA (RAPD) fingerprinting results for three *Annona* sp.

	Primer	Total amplified bands	Polymorphic bands	Monomorphic bands	polymorphism %
1	ATTTATCGTC	11	9	2	82
2	GCTGAGCGTC	19	10	9	53
3	GACCTAGCGA	13	9	4	69
4	ATGCACAGTG	16	8	8	50

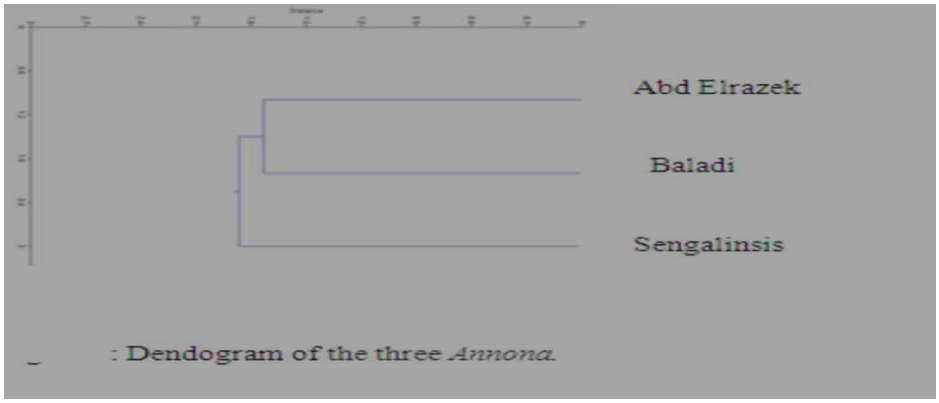

Figure 3: Dendogram of the three *Annona*s.

Our obtained results which included using RAPD marker were in agreement with Telles *et al*. (2003). They evaluated the genetic diversity of *Annona crassiflora* through 20 selected RAPD primers. Generally, RAPD showed a low percentage of polymorphism in the germplasm collection. Small er genetic distance among individuals of the same location was found. Thus, greater genetic variability is necessary and can be achieved by collection of few individuals from a number of different locations rather than a large number of individuals from the same location. Low percentage of polymorphism (< 29%) was observed by using the set of primers indicating low level of genetic variation among the 64 accessions evaluated. Dendogram revealed five clusters.

Nevertheless, more support was added to our findings by Cota et al., (2011). They studied seventy-two individuals of *Annona crassiflora* from four natural populations were genotyped using RAPD markers. moderate genetic diversity among populations were found, with Shannon's I index varying between 0.31 and 0.44, and Nei's genetic diversity (HE) for the population set equal to 0.31.

More light added to our findings by using RAPD fingerprinting technique for surveying variation by Brown et al., (2003). Via applied fourten polymorphic primers for Venezuela accession of *Annona* sp. and identified two groups which indicate great genetic variability for Venezuela accession of *Annona* sp. which need more efforts in breeding programs.

CONCLUSION

Morphological and molecular marker techniques (Random Amplified polymorphic DNA (RAPD) fingerprinting technique) were employed to remark unique fingerprinting among three Egyptian cultivars *Annona* sp. Fruit seeds and number were employed as morphological characters to evaluate genetic relationships for three Anonna sp. Noticeable morphological characters which enable us to depending on morphological futures as a fingerprinting. More distinctly molecular marker techniques (Random Amplified polymorphic DNA (RAPD) fingerprinting technique) was applied and add more evidence for economical and taxonomical position of Egyptian Annona sp. further studies needed to carried out for huge importance of this tropical fruits, specially for its medical applications combined with genetic engineering techniques.

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تعريف وتقييم الصفات لثلاثه اصناف من القشطه الناميه فى مصر مورفولوجيا وبيوكيميائيا بأستخدام تقنيه الرابد للبصمه الوراثيه

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الملخص العربي

أجريت هذه الدراسه لتقدير وايجاد الاختلافات الوراثيه بين ثلاث اصناف من القشطه المصريه الناميه فى محطه البحوث الزراعيه بالصبحيه أسكندريه والمسماه، عبدالرازق، بلدى وسنجالنسيس خلال الفتره من٢٠١٦ الى٢٠١٧ حيث ادت دراستنا لأثبات أهميه المقاييس المظهريه الخاصه بالأوراق فضلا عن مقاييس الثمار والبذورالمظهريه حيث أظهرت النتائج أختلافات معنويه فى مقاييس الأوراق والبذور حيث تم دراسه الأختلافات المرورفولوجيه الخاصه بالنباتات وأجزائها أظهر الصنف عبدالرازق أطول قياس لنصل الورقه بمتوسط ١٣,٥ سنتيمتر ويليه السنجالنسيس بمتوسط ١٢,٤٣سنتيمتر بينما كان البلدى أقلهم فى طول نصل الورقه بمتوسط ١٠,٣٠ سنتيمتر.

حيث وجد أن مقاييس طول وعرض الثمار وعدد البذور بها ستكون مفيد عند أستخدامها للمقارنه وراثيا بين الأصناف حيث أعطى الصنف عبد الرازق أقل متوسط لعدد البذور بالثمره بمتوسط ٣٧ بذره فى حين كان العدد فى البلدى وعبد الرازق هو ٧٣ و١٢٧ على التوالى.

كما تم أستخدام تقنيه البصمه الوراثيه العشوائيه(الرابد) أو تقنيه التضاعف للدى ان أيه عشوائيا بأستخدام أربعه بادئات عشوائيه مختلفه وهى (OPG1, POG2, OPG3 and OPG4) وتم حساب وتقديرالأختلافات والتشابهات بين الأصناف والناتجه عن كل برايمرمنفردة لقد أظهرت الاصناف المختلفه أختلافات فيما بينها للاستجابه لكل بادئ من الأربعه المستخدمه، فبالنسبه للبرايمراالأول أظهر بوليمورفيزم عالى بنسبه ٨٢% على العكس من البرايمرالرابع والذى أظهر بوليمورفيزم أو تشابهات بنسبه ٥٠%،، كما تم عمل شجره وراثيه لتوضيح علاقه القرابه والبعد وراثيا مابين الأصناف الثلاثه تحت الدراسه حيث ادت النتائج لاهميه والتوصيه بأجراء المزيد من الدراسات بأستخدام تقنيات جديده أكثر ملائمه وفائده.