Molecular genetic variability of some deciduous fruit rootstocks in Egypt

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ABSTRACT

The study was executed during two successive years (2016 and 2017) in the Orchard of Deciduous fruit department and Biotechnology Research lab of Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The aim of the work was using one year old plants of five and four seedy strains of Pear (Betulaefolia) and Peach (Mit-Ghamr) deciduous fruit rootstocks, where the study carried out to evaluate the molecular genetic variability between the two seedy strains rootstock respectively by using two molecular genetic markers, ISSR and SCoT based on PCR techniques to support the use of marker-assisted selection (MAS) for detection of a biotic and biotic stress in seedy strains.

Six and Ten primers were used with ISSR and SCoT molecular genetic markers had successfully generated reproducible polymorphic products to study the genetic variability between Betulaefolia and Mit-Ghamr rootstocks. Data cleared that high levels of polymorphism among the strains studied of each rootstock, where a total bands of five seedy strains of Betulaefolia rootstock with ISSR- SCoT primers were recorded 105 band (43-62 respectively), and the total monomorphic bands were 29 (11-18) added to total polymorphic bands was recorded 76 (32-44) with polymorphic percentage (74.44% - 71%) where the specific marker bands were 30 (13 -17) respectively. On the other hand, four seedy strains of Mit-Ghamr rootstock with ISSR- SCoT primers data recorded 97 total bands as (31-66 respectively).

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These bands identified as 43 (14-29) monomorphic ones and 54 (17-37) polymorphic bands with polymorphic percentage were (54.8% - 56%) and the specific markers bands scored 40 (13-27).

Keywords: Seedy strains– Betulaefolia and Mit-Ghamr deciduous fruit

Rootstocks – ISSR and SCoT Molecular Markers.

INTRODUCTION

Peach and Pear from (Rosaceae family), their binomial name were (*Prunus persica - Pyrus comunus*) and they considered a highly demanded by Egyptian consumers. There are many peach varieties growing more widely now throughout the world، and Pear is one of the most important deciduous fruit trees all over the world, where it takes the second rank after apple in production. Rootstocks play an important role in Peach and Pear production, since, the proper choice of rootstock is as important as the choice of variety and site. This is true because the rootstock is involved in determining two key factors: the variety susceptibility to several serious diseases - added to the tree's performance in the climate and the orchard site.

Four and five strains of shoot tips and stem segments of young branches of peach var. Meet Ghamr rootstock and pear var. Betulaefolia with a chromosome number 2n=2x=16 and Genome size of 265 Mb., and chromosome number 2n=34 and Genome size of 577 Mb. respectively. https://www.rosaceae.org/organism/Prunus/persica

These strains were selected after detection of salt stress genes using marking assistant selection (MAS) by BADH and Rubisco genes. Molecular markers are of interest to plant geneticists and breeders as a source of new genetic information on plant genomes and for use in trait selection.

Lisek and Rozpara (2010), Mohamed and El-Sharabasy (2009), Gihan *et al*., (2009), Abo Rekab *et al*., (2010), Gad and Mohamed (2012), Fathi *et al*., (2013), Ozyurt *et.al*.,(2013),Mohamed *et al*., (2015) and Khorshidi *et al*.,(2017) recorded that ISSR primers had successfully generated

reproducible polymorphic products between pear, peach strains and Okinawa Rootstock, grape cultivars, date palm cultivars, some pinus species and apricot strains with a high levels of polymorphism among the studied cultivars and strains.

Start Codon Targeted (SCoT) polymorphic markers were used to assess genetic relationships among grape varieties, Cicer, Pear and Sweet potato, revealed start codon targeted polymorphism technique can be utilized to identify DNA polymorphisms and fingerprint cultivars in domesticated peanut reported that SCoT markers were more informative and the efficiency of SCoT for fingerprinting of varieties was more than other markers based on the average percentage polymorphism conducted to different references, Gorji *et al*., (2011), Xiong *et al*., (2011), Amirmoradi *et al*.,(2012),(Guo *et al*.,2012), Shahlaei *et al*.,(2014), Etminan *et al.,* (2016) and Nair *et al*.,(2016). Mohamed *et al*.,(2017).

The objective of the study was to obtain further additional information about the genetic variability between five seedy strains of Betulaefolia rootstock also between four seedy strains of Mit-Ghamr rootstock by using ISSR and SCoT molecular genetic markers.

MATERIALS AND METHODS

Plant Material

This study were conducted for two years (2016 and 2017) in the orchard of Deciduous fruit Department and Biotechnology Research Lab of Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. One year old plants of five seedy strains of Betulaefolia rootstock and four seedy strains of Mit-Ghamr rootstock were used obtained in spring-summer and autumn. After using a marker assisted selection (MAS) for detection of a biotic and biotic stress in seedy strains, the five pear strains were (1, 2, 9, 10 and 11), and four peach strains selected with number (2, 3, 8 and 14) takes serial numbers (stI, II, III, IV and V) and (stI, II, III, and IV) respectively according to their bands intensity and abundance

DNA Extraction

Freshly excised leaves for each seedy strains of Betulaefolia and Mit-Ghamr rootstocks, frozen in liquid nitrogen and subsequently stored at -20 ºC until processed, were collected from the orchard of Deciduous fruit department, Horticulture Research Institute, Agriculture Research Center. Genomic DNA was extracted by DNeasy plant mini kit (Qiagen) with the enclosed manual. DNA quality was examined by electrophoresis 1% agarose and DNA concentration was quantified by means of calibration dependence was measured by photo Capt Mw v 3.0 software. For ISSR and SCoT analysis, aliquots of 10 ng/ul were prepared and stored at -20ºC.

Molecular Genetic Marker Analysis

ISSR assays were performed as described by Lisek and Rozpara (2010), Fathi, *et al*., (2013), Ozyurt *et.al*.,(2013) and Khorshidi *et.al,(*2017).Six ISSR primers (Table 1) were selected from different published papers to be employed in ISSR analysis. SCoT amplification was performed as described by Xiong *et al*. (2011) and Guo *et al*.(,2012), using ten primers with 18-mer Sequence 5'- 3' (Table 1). These primers were selected from published papers Nair *et.al.,(*2016), Mohamed *et.al*.,(2017) and Dora *et.al*.,(2017).

Table (1): ISSR and SCoT primers used and their sequences.

ISSR Primers	**Sequence 5'- 3'**	**SCoT Primers**	**Sequence 5'- 3'**
14A	CAGGCCCTTC	**SCoT 1**	ACG ACA TGG CGA CCA CGC
89A	GAAAGGGGTG	**SCoT 2**	ACC ATG GCT ACC ACC GGC
44B	GAAAGGGGTG	**SCoT 3**	ACG ACA TGG CGA CCC ACA
HB-12	GTTGGTGGCT	**SCoT 4**	ACC ATG GCT ACC ACC GCA
HB-14	GTA GACCCGT	**SCoT 6**	CAA TGG CTA CCA CTA CAG
HB-15	GTGGTGGTGGC	**SCoT 8**	ACA ATG GCT ACC ACT GAG
		SCoT 9	ACA ATG GCT ACC ACT GCC
		SCoT 10	ACA ATG GCT ACC ACC AGC
		SCoT 11	ACA ATG GCT ACC ACT ACC
		SCoT 12	CAA CAA TGG CTA CCA CCG

Data Analysis

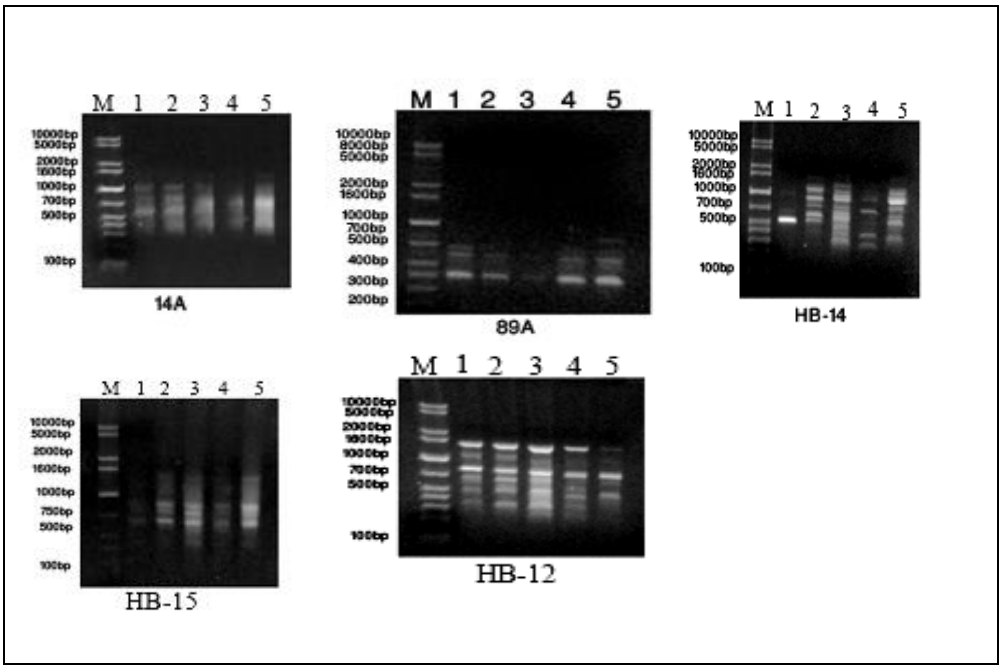
The banding patterns generated by ISSR and SCoT were examined to determine the level of polymorphism and the genetic relatedness among the five seedy strains of Betulaefolia rootstock and four seedy strains of Mit-Ghamr rootstock treatments. The amplified fragments were scored as present (1) or absent (0), and were typed into computer file as a binary matrix. The matrix was then analyzed by PAST (free programs on web) software (Nei and Li, 1979).

RESULTS AND DISCUSSION

I- Molecular genetic variability in Betulaefolia rootstock strains

1- ISSR-PCR molecular genetic markers:

All the Betulaefolia rootstock strains recorded with ISSR primers (Plate1 and Table2), where they were visualized across 43 bands as a total number with molecular size from 255 to 1544 bp, the results obtained 32 total polymorphic bands at polymorphic percentage was (74.44%) and the highest amplified polymorphic bands was (13 bands) its percentage was (92.8%) produced with primer HB-14, while the lowest polymorphic percentage was (42.8%) present with primer 14A, primer 89A has lowest number of monomorphic bands (4bands). On the other hand, the total results of monomorphic and specific marker bands were 11 and 13 in five primers respectively. There were some specific markers discriminated each cultivar from the others as indicated in table (2) where Primer 14A showed two specific markers for strains (stIV and stV)., Primer 89A showed one specific markers for (stV) strain, Primer HB-12 showed five specific markers, one of them for stV strain and the other four specific marker present with stIV strain. While, Primer HB-14 showed three specific marker one of them for (stII) and the other two specific markers were for (stIII). Also, Primer HB-15 exhibited two markers for (stI).



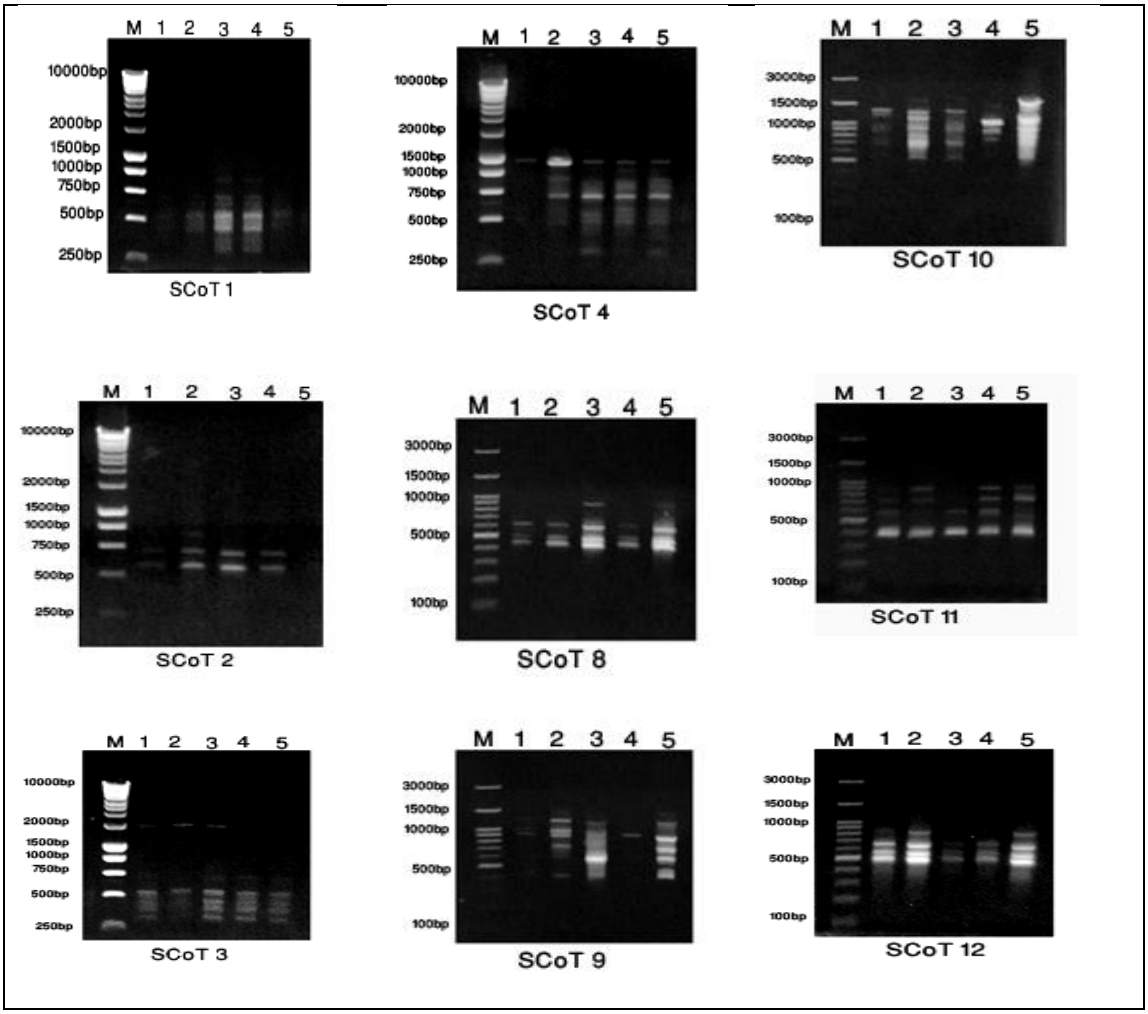
Plate(1): DNA polymorphism using ISSR-PCR for the five seedy strains(stI,stII,stII,stIVand stV)of Betulaefolia rootstock amplified with five ISSR primers .

Table(2): List of ISSR primers of five seedy strains (stI,stII,stII,stIVand stV) of Betulaefolia rootstock. Percentage of polymorphism and Specific Marker bands (SM).

Primer Name	Total Bands	M .size range (bp)	Monomorphic Bands	Polymorphic Bands	Polymorphism %	Specific marker SM
14A	7	345-1470	4	3	42.8%	2(*stIV,stV*)
89A	4	315-730	2	2	50%	1(*stIII*)
HB-12	8	330-1460	2	6	75%	5(*stIV,stV*)
HB-14	14	255-1510	1	13	92.8%	3(*stII,stIII*)
HB-15	10	310-1544	2	8	80%	2(*stI)*
Total	43	255-1544	11	32	74.44%	13

2- SCoT-PCR molecular genetic markers

Total number of bands was 62 with molecular size range (320 to 2000 bp) as represented in Plate (2) and Table (3). Five investigated rootstock strains were resulted from nine primers SCoT molecular genetic markers. The results obtained were 44 of total polymorphic bands with total polymorphic percentage (71%), the highest polymorphic percentage was (88.9%) produced with primer SCoT-9 and the lowest polymorphic percentage was (25%) produced in case of primer SCoT-8. On the other hand, 18 monomorphic bands and seventeen specific marker bands in six primers were appeared. Finally, the highest total amplified bands (11bands) were recorded in case of primer SCoT-4 and SCoT-10 whereas, primer SCoT-2 had the lowest amplified bands (3bands). The specific markers discriminated each strain from the others. Primer SCoT 2 showed one specific marker for strain (stII) and SCoT 3 showed three specific markers for the same strain. Primer SCoT 4 exhibited four specific markers for strain (stI). While, Primer SCoT 9 illustrated two specific markers one of them for (stIII) strain and the other specific marker for (stIV). Primer SCoT 10 exhibited four markers two for strains (stII) (stV) and the other two specific markers were for strain (stIV). Thus, it is clear that the highest specific markers was present with SCoT 4.



Plate(2): DNA polymorphism using SCoT-PCR for the five seedy strains of Betulaefolia rootstock amplified with nine SCoT primers.

Table(3):List of SCoT primers of five seedy strains of Betulaefolia rootstock. Percentage of polymorphism and Specific Marker bands (SM).

Primer Name	Total Bands	M. size range (bp)	Monomorphic Bands	Polymorphic Bands	Polymorphism %	Specific marker SM
SCoT- 1	7	340-865	2	5	71.4%	-
SCoT-2	3	665-965	2	1	33.3%	1(*stII*)
SCoT-3	6	320-2000	2	4	66.7%	3(*stII*)
SCoT-4	11	345-1500	2	9	81.8%	4(*stI*)

SCoT-8	4	455-915	3	1	25%	-
SCoT-9	9	425-1470	1	8	88.9%	2(*stIII,stIV*)
SCoT-10	11	5301680	2	9	81.8%	4(*stII,stIV,stV)*
SCoT-11	5	385-840	2	3	60%	3(*stII , stIII)*
SCoT-12	6	430-825	2	4	66.7%	-
Total	62	320-2000	18	44	71%	17

ISSR and SCoT Molecular genetic markers combination analysis

Resulted data of five seedy strains of Betulaefolia rootstock in table (4) clear that ISSR and SCoT primers recorded a sum (105 bands), these bands were identified as 29 monomorphic ones and 76 polymorphic bands with polymorphic percentage (72.38%) and the polymorphic bands were scored as 30 specific markers.

Table (4): Polymorphic, Monomorphic, Specific Markers and Polymorphic percentage generated by the (ISSR and SCoT) analysis of five seedy strains of Betulaefolia rootstock.

Primers Names	Total Band	Monomorphic Band	Polymorphic band	Specific Markers	Polymorphic %
ISSR	43	11	32	13	74.44%
SCoT	62	18	44	17	71%
Total	105	29	76	30	72.38%

II- Molecular genetic variability in Mit-Ghmar rootstock strains

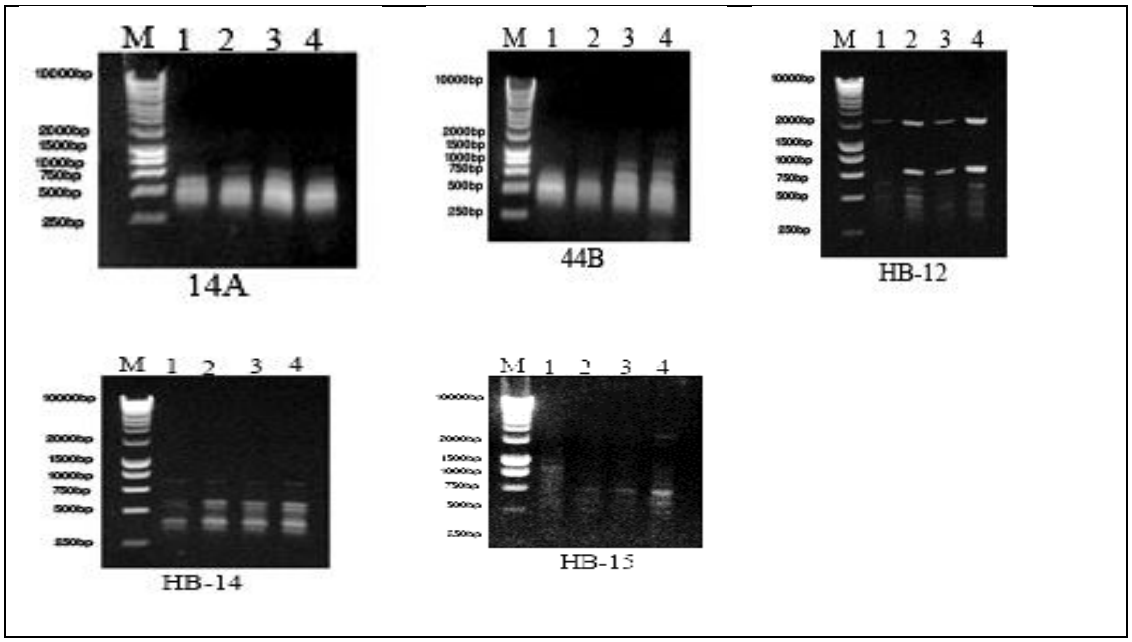
1- ISSR-PCR molecular genetic markers

All the examined five primers (Table 5 and plate 3) produced different ISSR-PCR banding patterns. The number of bands generated per primer varied between 5 was lowest amplified bands at 14A and primer HB-14 to 8 bands was the highest amplified bands at Primer 44B. Total number of 31 bands were visualized across the four investigated rootstock strains with molecular size ranging from 330 to 3460 bp, 17 of total polymorphic bands were scored with total polymorphic percentage (54.8%), and the

highest polymorphic percentage was 85.7% produced by primer HB-12, while the lowest polymorphic percentage was 20% illustrated with primers 14A and HB-14. On the other hand, total of 14 monomorphic bands were appeared and 13 specific marker bands in all five primers.

There were some specific markers discriminated each cultivar from the others as follows:-

Primer 14A showed one specific marker for strain (stI), Primer 44B showed three specific markers for (stIV) and four specific markers were produced from Primer HB-12 where three of them for(stI) strain and other specific marker was for (stIII) strain. While, Primer HB-14 exhibited one specific marker for (stIV) strain, and Primer HB-15 exhibited four specific markers, three of them were for (*stI)* strain and the other one for (*stIV*) strain. So, it is noted that specific marker in each 14A and HB-14 were the lowest number while the highest number of monomorphic Band was present at the same primers.



Plate(3):DNA polymorphism using ISSR-PCR for the four seedy strains of Mit- Ghamr peach rootstock amplified with five ISSR primers.

Table(5):List of ISSR primers of four seedy strains of Mit- Ghamr peach rootstock. Percentage of polymorphic bands (PB) and Specific Marker bands (SM).

Primer Name	Total Bands	M. size range (bp)	Monomorphic Bands	Polymorphic Bands	Polymorphism %	Specific marker SM
14A	5	425-735	4	1	20%	1(*stI*)
44B	8	330-1875	3	5	62.5%	3 *(stIV*)
HB-12	7	365-2630	1	6	85.7%	4(*stI*,*stIII*)
HB-14	5	430-940	4	1	20%	1(*stIV*)
HB-15	6	480-3460	2	4	66.7%	4(*stI*,*stIV*)
Total	31	330-3460	14	17	54.8%	13

2- SCoT-PCR molecular genetic markers

A high level of polymorphism was generated utilizing the nine SCoT-PCR primers (Plate 4 and Table 6) represent a total number of 66 bands were visualized across the four investigated rootstock strains with molecular weights ranging from 185 to 1480 bp. Total amplified bands were 37 with polymorphic percent (56.1%) and the highest polymorphic percentage recorded at (77.8%) with primer SCoT-9. No polymorphic percentage was produced with primer SCoT-12. On the other hand, total monomorphic bands appeared 29, where twenty-seven specific marker bands present in eight primers. Finally, Primer SCoT-3 showed the highest amplified bands (twelve bands) whereas, primer SCoT-8 and primer SCoT-12 were the lowest amplified bands (four bands).

Primer SCoT1 exhibited two specific markers for strain (st.I) and (st. IV). On the other hand, Primer SCoT 3 showed six specific markers, one for each (stI and stIV) and two specific markers for two strains (stII and stIII).

Four specific markers were resulted from primer SCoT 4 and all of them for (stI) strain. In case of Primer SCoT 6 two specific markers one of them for (stII) strain and the other was for (stIV) strain. In the same way, primer SCot 8 produced one specific marker for (stIV) strain and four specific marker for *(*stI) strain exhibited with primer SCoT 9. While, primer SCoT 10 produced four specific markers, three of them found in (stI) strain and the other one for (stIII) strain and four specific markers were illustrated from primer SCoT 11, where three of them for (stIII) strain and the other one for ***(***stII).

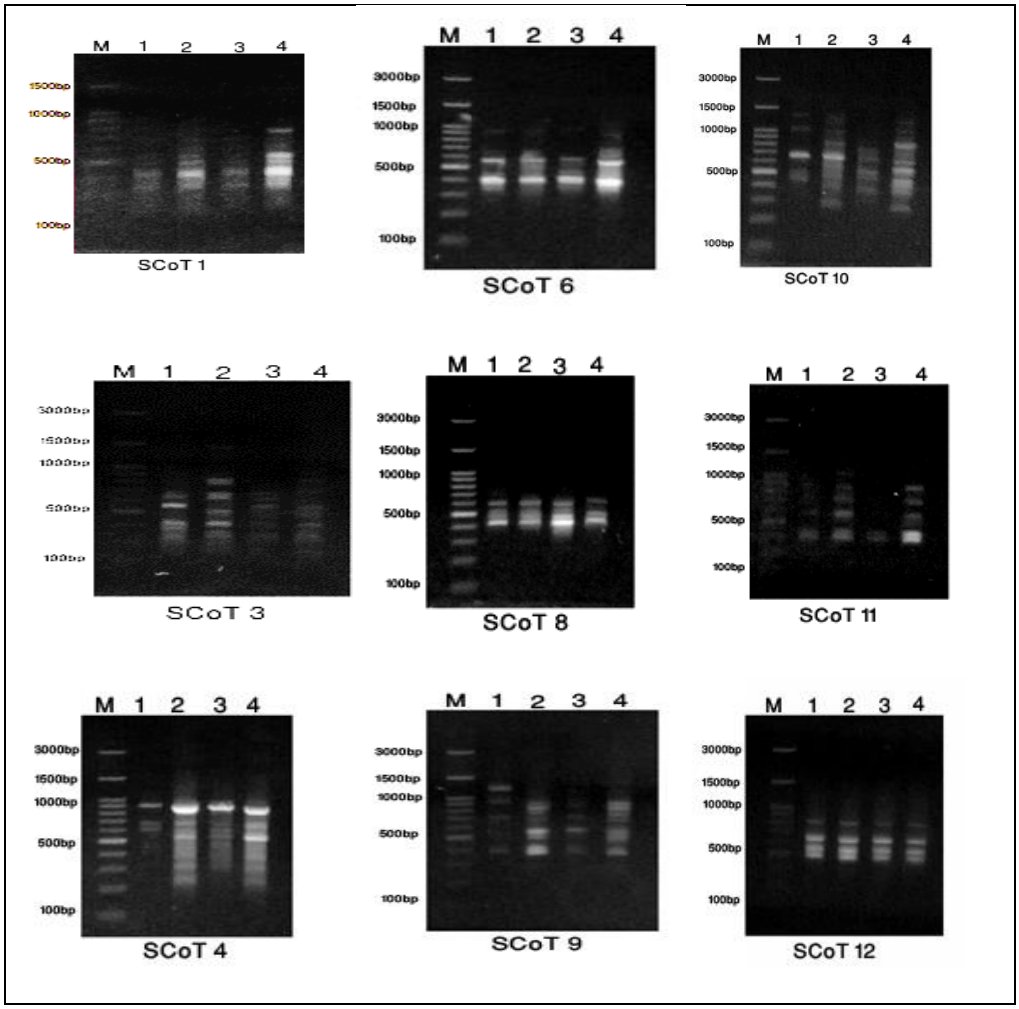


Plate (4): DNA polymorphism using SCoT-PCR for the four seedy strains of Mit-Ghamr peach rootstock amplified with nine SCoT primers.

Table (6): List of SCoT primers of four seedy strains of Mit- Ghamr peach rootstock. Percentage of polymorphic bands (PB) and Specific Marker bands (SM).

Primer Name	Total Bands	M.size range (bp)	Monomorphic Bands	Polymorphic Bands	polymorphism %	Specific marker SM
SCoT-1	7	335-770	5	2	28.6%	2(*stI,stIV*)
SCoT-3	12	185-1480	5	7	58.3%	6(*stI*, *stII, stIII* , *stIV*)
SCoT-4	7	245-935	2	5	71.4%	4(*stI*)
SCoT-6	6	350-925	3	3	50%	2(*stII*, *stIV*)
SCoT-8	4	435-610	3	1	25%	1 (*stIV*)
SCoT-9	9	400-1270	2	7	77.8%	4(*stI*)
SCoT-10	11	285-1380	3	8	72.7%	4(*stI,stIII)*
SCoT-11	6	385-1070	2	4	66.7%	4(*stII,stIII)*
SCoT-12	4	500-815	4	-	-	-
Total	66	185-1480	29	37	56.1%	27

ISSR and SCoT Molecular genetic markers combination analysis

Table (7) illustrate combination data of ISSR and SCoT primers for four seedy strains of peach Mit-Ghamr rootstock, where produced 97 bands as a total bands, these bands were identified as 43 monomorphic ones and 54 polymorphic bands with polymorphic percentage (55.6%) and 40 specific markers.

Table (7): Polymorphic, Monomorphic, Specific Markers and Polymorphic % generated by the (ISSR and SCoT) analysis four seedy strains of Mit-Ghamr rootstock.

Primers Names	Total Band	Monomorphic Band	Polymorphic band	Specific Markers	Polymorphic %
ISSR	31	14	17	13	54.8%
SCoT	66	29	37	27	56%
Total	97	43	54	40	55.67%

General the results indicated that ISSR and SCoT analysis possible use to detect molecular genetic variability in seedy strains of Betulaefolia and Mit-Ghamr peach Rootstocks and characteristic Marker Assisted Selection (MAS) for detect biotic and a biotic stress for these strains and we can obtain a new fruit rootstocks tolerance to salinity and drought and other different a biotic stress.

Conclusion, all ISSR and SCoT primers used in the present study allowed for enough distinction among the five seedy strains of Betulaefolia and four seedy strains of Mit-Ghamr Rootstocks. Overall comparison among seedy rootstocks across the used primers revealed the power of studied molecular genetic markers in distinguishing genetic variability between strains of seedy deciduous fruit rootstocks grown in the same location and these results were in line with Mohamed *et al*., (2015), Khorshidi *et.al,(*2017), Shahlaei *et.al*.,(2014), Etminan *et. al*, (2016) and Nair *et.al.,(*2016).

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التباين الوراثى الجزيئى لبعض أصول الفاكهة المتساقطة فى مصر

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أجريت هذه الدراسة بمزرعة قسم بحوث الفاكهة المتساقطة وبمعمل البيوتكنولوجي – معهد بحوث البساتين – مركز البحوث الزراعية بالجيزة خلال الفترة من ٢٠١٦-٢٠١٧، وذلك على خمس سلالات بذرية من الكمثرى أصل البيتشوفوليا وأربعة سلالات بذرية من الخوخ أصل ميت غمرعمر سنة.
تم إستخدام التضاعف لأجزاء من الدنا والبوادئ المتخصصة داخل التتابعات المتكررة بإستخدام جهاز تفاعل سلسلة البلمرة (ISSR-PCR) و (SCoT-PCR) لتقدير التباين الوراثي لهم .
نجحت ستة بوادئ ISSR وعشرة بوادئ SCoT مع السلالات البذرية الخمسة من الكمثرى والسلالات البذرية الاربعة من الخوخ في اظهار مستويات عالية من التباين الوراثي.
وصل العدد الكلي الناتج إلى ١٠٥ حزمة (٤٣-٦٢ عل التوالى) بين السلالات البذرية الخمسة من الكمثرى محل الدراسة منها ٢٩ (١١-١٨) حزمة متماثلة و٧٦ (٤٤-٣٢) حزمة متغايرة بنسبة تباين ٧٤،٤٤ %-٧١ % والحزم المميزة كانت ٣٠ جزمة (١٣-١٧) ووصل العدد الكلي الناتج بين السلالات البذرية الاربعة من الخوخ محل الدراسة إلي٩٧حزمة (٣١-٦٦ عل التوالى) منها ٤٣ (١٤-٢٩) حزمة متماثلة و٥٤ (٣٧-١٧) حزمة متغايرة بنسبة تباين ٥٤،٨ % - ٥٦ % والحزم المميزة كانت ٤٠ جزمة (١٣-٢٧)