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Analysis of Genetic Diversity and Genetic Fingerprint of Some Egyptian Strains of 'Meet-Ghamr' Peach Using SSR Markers

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ABSTRACT

The present study was carried out to elucidate the molecular characterization of six peaches (*Prunus persica* L.) strains derived from Egyptian Meet-Ghumr cultivar. In addition, further understanding of the structure of genetic diversity using sixteen previously described SSR primer pairs to determine if the primers could discriminate between the strains. The loci were low polymorphic, the different alleles found at each locus only ranged from 1- 4 including null allele. The observed heterozygosity ranged from 0.00 - 0.66, the heterozygosity level within the genotype varied from 0.00 - 0.30, gene diversity or the polymorphic information content (PIC) values were between 0.00 to 0.51, and the power of discrimination (DP) for each locus differed from 0.00 – 0.61. Regarding the probability of matching fingerprints, two of the strains 'Soltany and Shamiea' were identical in the DNA profile with all the sixteen loci and thus considered to be genetically identical. The similarity degree ranged from 0.83 to 1.00 with an average of 0.91, the lowest similarity degree was between Mawwy and Farkk Meet-Ghumr, indicating the low level of genetic variation that exists in the edible peach gene pool. This is confirmed the importance of benefiting from other genetically close species of peach such as almond in the genetic improvement of peach.

Keywords: Peach, *prunus persica* L., SSR markers, Molecular characterization, DNA fingerprints, genetic diversity.

INTRODUCTION

Peach [*Prunus persica* (L.)] is belonging to the Rosaceae family, which classified as the third most important plant family agronomically in temperate zones located between southern and northern latitude from 30° to 45° out of distribution of peaches in the world (Sosinski *et al*. 2000). This family comprises of economically important species as apple, apricot, plum, sour cherry, almond and strawberry. In contrast to numerous of the other family members, peach is a self-pollinated, diploid (2n =2x =16) and has a tiny genome size of ~ 5.9 $\times 10^8$ base pairs per diploid nucleus (Baird *et al.* 1994), approximately twice the size of the genome of *Arabidopsis thaliana* L.. On the other hand, little is known about how divergences in mating system affect changes in diversity during domestication of species (Dianne *et al*. 2016). The explicit use of related *Prunus* species and interspecific crosses as rootstocks for peach and almond has been reported by [Kester and Hansen (1966) and Denisov. (1988)] suggesting the importance of closely related *prunus* species in peach breeding.

Moreover, the peach has a relatively short juvenile stage. Therefore, molecular markers linked to fruit-specific characters are importance for identifying and selecting peach genotypes before the traits are expressed. This makes peach the exemplary genetic system for identifying and characterizing the important genes in Rosaceae species. Cultivar identification is depending on genotype morphological and agronomic characteristics. It is difficult to distinguish genotypes on the basis of their outer morphology alone because of the actual intraspecific variation in leaf and fruit characters. However, some phenotypic traits are difficult to describe. Rootstocks are difficult to observe their morphological traits after grafting. Moreover, these phenotypic traits are influenced by the growth period of the plant and environmental factors. This means that the evaluation is expensive and needs a longer time during the entire vegetative growth periods to obtain morphological data for genetic diversity evaluations. In research and breeding programmes it is very important that the genotypes used are identified accurately and fairly (Pedersen 2006) because the results are being used and compared both nationally and internationally in other scientific experiments. Therefore, molecular markers such as isozymes (Chaparoo *et al*. 1994), RFLP [Eldredge *et al*. (1992) and Quarta *et al*. (1996)], RAPD [Rajapakse *et al.* (1995) and Warburton and Bliss (1996)], SSR [Dirlewanger *et al.* (2002) and Aru´s *et al*. (2003)], ISSR (Seba Sarhan *et al*. 2015) and AFLP [Dirlewanger *et al*. (1998) and Manubens *et al*. (1999)] have been performed to genetic diversity studies and genetic fingerprints in the situation of peach gene pools. The preference of the marker to use for a particular application depends on its easiness and simplicity of use and the particular targets under investigation.Peach with a narrow genetic base (Scorza *et al*. 1985) leads to low variability within the genotypes. Microsatellites or simple sequence repeat (SSR) currently becoming the selected marker due to their high level of polymorphism, single locus detection, transportable between peach genotypes and across closely related species and abundance with co-dominant inheritance [Sosinski *et al.* (2000) and Gupta *et al*. (1996)]. So this molecular marker is ideal for the molecular characterization, assessing genetic variability and understanding the genetic relationships of different plant species, in addition to DNA fingerprints where it showed high reproducibility.Until now many isolated SSRs from peach and other species of genus *Prunus* were identified. A huge amount of expressed sequence tags (EST) developed in the last few decades in genus of Prunus has given a precise method for detecting SSRs in transcribed regions (Jung *et al.* 2005). EST derived SSRs are more easily transferred across taxa mostly for polymorphic than those from intergenic regions. As a result, these transcribed sequences become more conserved. Moreorver, they are more linked to genes of agronomic importance (Jung *et al*. 2005). This study is intended to analyze the molecular characterization, genetic diversity and genetic fingerprints for six Peach strains derived from Egyptian Meet-Ghumr cultivar by SSRs fingerprinting, in order to contribute of conserve and employ genotypes of Peach to be more effectively in accordance with molecular results for cultivar improvement.

MATERIALS AND METHODS

Plant material:

Young leaf samples of six Peach strains (Soltany, kelaby, Hegazy, Mawwy, Shamiea and Farkk Meet Ghumr) derived from Meet Ghumr cultivar were collected from a private orchard of Ismailia Governorate, Egypt. The samples were stored at – 20 °C until utilization and then ground to powder in liquid nitrogen.

DNA extraction:

The extraction of total genomic DNA was conducted following the modified method of the basic DNA extraction protocol (Dellaporta *et al*. 1983) by (Porebski *et al*. 1997): 500 mg. of a fine powder of young leaves (grinding was done in liquid nitrogen) was extracted by 10 ml preheated (65ºC) from cetylhexadecyl-trimethyl ammonium bromide (CTAB) extraction buffer [3% CTAB (w/v), 100 mM Tris- HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) PVP (Polyvinyl pyrrolidone)], and then added 1% (v/v) of β-mercaptoethanol (15 mM). The obtained mixture was incubated at 65ºC for 60 min, followed by twice extractions with chloroform/isoamyl alcohol (24:1). Next, the nucleic acids precipitated with Cold isopropanol, and the pellet was dissolved in 1 mL TE 0.1X (Tris-EDTA) buffer. RNA was separated with RNaseA 4 µl (10 mg/mL). The DNA was purified by 300 µl phenol: chloroform: isoamyl alcohol (25:24:1), then was kept overnight at (-20 ºC) with 1/10 vol. of 2 M Na acetate (pH 8.0) and one vol. of cold isopropanol alcohol. The obtained precipitated was washed twice, and the pellet was dissolved in 0.1X TE buffer. The total DNA was quantified by gel electrophoresis, and its quality confirmed by spectrophotometry.

PCR (SSR) amplification and product electrophoresis:

The used sixteen SSR primer pairs in PCR amplification were previously described by [Sosinski *et al*. (2000); Vendramin *et al*. (2007)]. These primers were manufactured by VBC-Biotech, Vienna, Austria (www.vbc-bioch.com). PCR reactions were conducted following the modified procedures of (Viruel *et al*. 2005). Microsatellites amplification was done in 10 µl volume contained: 0.02 µM forward primer, 0.2 µM reverse primer, 0.2 mM of each dNTP, 1.8 mM $MgCl_2$, 0.05 U Taq polymerase, 1X PCR buffer, and 10 ng of template DNA. The amplification was done using a `Primus` 384 well thermocycler (MWG Biotech, Germany). A first denaturation step was done at 94 ºC for 2 min, the reaction had 30 cycles with (94 ºC for 1 min, 0.5 ºC/sec to 51 ºC, 51 ºC for 30 sec, 0.5 ºC/sec to 72 ºC, 72 ºC for 1 min) followed by a final extension step of 5 min at 72 ºC. The PCR reactions were repeated at least twice to guarantee the reproducibility of the findings. PCR products were separated on 2 % agarose gel and labeled with ethidium bromide to prove the PCR amplification and determine the size of the amplified fragments. After that, The PCR products of the Microsatellite were detected by electrophoresis on Polyacrylamide non-denaturing gels, because Microsatellite alleles may vary in length by only few base pairs. Therefore, 7 % Polyacrylamide gels were used to exact allele sizing of the SSR loci, and then stained with ethidium bromide solution and documented by gel documentation model. Quantity-one software was used to estimate the sizes of the products by comparison to size marker.

Data analysis:

Twenty two distinct bands produced from selected primers were scored as presence (1) or absence (0) for the six strains tested. This information was utilized to compile a binary data matrix. The total number of alleles and composition of allelic of each strain was measured for each SSR locus. Putative alleles were indicated by the estimated size in bp. The genetic similarity, using Dice similarity index, was estimated among all possible pairs of strains (Sneath and Sokal, 1973). Dice formula: GS (ij) = 2a/ (2a+b+c)

Where GS (ij) is the measure of the genetic similarity between individuals i and j, (a) is the number of bands shared by i and j, (b) is the number of bands present in (i) and (c) is the number of bands absent in i and present in j.Phylogenetic tree was produced by clustering the data with the average Linkage (Between Groups) using the SPSS software ver. 16. The genetic information was evaluated for single locus SSRs by number of alleles per locus (A), effective number of alleles (NE), observed heterozygosity (Ho, direct count), Wright's fixation index (F) and polymorphic information content values for each locus (PIC) or the expected heterozygosity were calculated as follows:

He or PIC = 1- $\sum$ pi2 where pi is the frequency of the ith allele, and summation extends over n alleles (Nei 1973); the discrimination power (DP) for each locus was calculated as above formula, whereas the allele frequency was replaced by the genotype frequency, according to (kloosterman *et al*. 1993). The probability of matching fingerprints was estimated according to (Jones 1972). In addition, the heterozygosity level within the assayed six genotypes. Every observed DNA fragment was considered as a co-dominant SSR marker alleles.The computations were conducted by the programs GENEPOP version 1.31 (Raymond and Rouset 1995), Quantity one, Irfanview and Microsoft Excel.

RESULTS AND DISCUSSION

Polymorphism of Microsatellite markers and DNA fingerprints:

Utilizing the extraction and amplification method of DNA with selected primers, clear patterns for various strains was obtained. The polyvinyl pyrrolidone (PVP) was used in extraction solution to get better PCR for the extracted DNA samples from tissues, which are affluent in the polyphenolics and polysaccharides (Khanuja *et al*.1999). Amplification was successful with the assayed 16 primer pairs, which generated SSR fragments for all the six peach strains. This reveals a high level of homogeneity between SSR flanking regions in these peach samples and other peach genomes. These amplified primers did not produce more than two alleles for each genotype or complex band. Based on that, all 16 microsatellite loci were described as a single locus. Four loci only were polymorphic (25 %) and all loci

showed a total of 22 alleles ranged between one to four putative alleles per locus (1.4 alleles on average), implying the presence of zero alleles (Table 1). However, these values are lower than the number of alleles that [Cantini *et al.* (2002) and Pedersen (2006)] found in their work on sweet and sour Prunus that developed in such tetraploid species. It may be a result of using another group of microsatellite loci and using different genotypes described more diverse in their study. In this study, the expected size range and the low numbers of the amplified bands are consistent and similar to those obtained in peach by [Vendramin *et al*. (2007) and Sosinski *et al*. (2000)], The obtained amplification pattern is shown in (Fig. 1) with the primers Pchgms1 and Pchcms5 . The effective number of alleles (NE) is the measure of allelic evenness. In our study, the effective number of alleles varied from 1.00 to 2.057 with the mean of 1.177. Consequently, the 16 SSR loci used in this study were less useful for the evaluation of genetic diversity in the six peach genotypes studied. Most of genotypes studied appear to be the same but with different names using the combination of SSRs. In addition, all the primers pairs produced a maximum of two bands per genotype, similar to the diploid construction of this cultivated species. The heterozygosity levels of genotypes ranged from 0.00 for Hegazy strain to 0.30 for Farkk Meet-Ghumr strain with an average of 0.133 (Table 2). Peach is a normally self-pollinated thus, a low level of genetic heterozygosity can be explained within the genotypes. Additionally, the genotypes studied may be not received free external gene flow, which is likely the cause of the excess heterozygosity as previously reported (Bodian *et al* 2012). Moreover, peach has a narrow genetic base (Scorza *et al.* 1985) causing low variability within the genotypes. In contrast, high level of heterozygosity in almond as reported by (Shiran *et al*. 2007). The observed heterozygosity (Ho), estimated from the direct count of the identified loci and ranged from 0 to 0.667 with an average of 0.083 for all loci used (Table 1). The polymorphic information content (PIC or He) ranged from 0 to 0.514 for the locus Pchcms1, included the presence of zero allele. While based upon discriminating power, the most informational locus was Pchcms1with a DP = 0.612, whilst the average of DP parameter for all loci was low (0.113) as shown in (Table 1). Expected/observed heterozygosity values were compared by the fixation index (F), and the lowest of F values (Table 2) revealed the general behavior of the strains under study. Which means that they are very closely–related, but not identical. These values as a whole are responsible for the low level of polymorphism in peach, featured lower than the values reported by (Sanchez-Perez *et al*. 2005), using SSRs originated from genomic libraries but are in agreement with those obtained from other cDNA libraries (Vendramin *et al*. 2007). In this study, Pchgms and Pchcms groups of SSRs loci usually revealed high level of polymorphism than EPPISF loci. It may be a result of differences in the SSR origins. For instance, the used EST-EPPISF group of markers was developed in peach from cDNA libraries by (Vendramin *et al*. 2007). Therefore, it was expected that these group of markers have less polymorphism. Whereas, the transcriptome are more conserved and less variable (Jung *et al*. 2005).

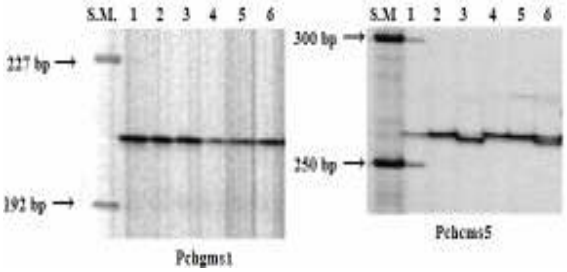

Figure 1. An example of SSR polymorphism obtained with primers Pchgms1 and Pchcms5 for six peach strains, S.M. size marker and the numbers from left to right refer to Soltany , Kelaby, Hegazy, Mawwy, Shamiea and Farkk Meet Ghumr strains respectively.

Moreover, they located foremost near or in genes. While, the group of Pchgms markers also designed in peach by (Sosinski *et al.* 2000) from genomic DNA sequences. These loci one more likely to include non-coding regions with high mutation rate and more variable. Generally, the results in this study showed a high grade of symmetry for the SSR loci between the used peach strains in this study and other peach cultivars. Even, with different species belonging to genus of *Prunus*, such almond, apricot, plum, sweet and sour cherry. Where, these loci were originally developed by [Sosinski *et al*. (2000) and Vendramin *et al*. (2007)]. These results confirmed the narrow evolutionary distance between species which were confirmed by (Watkins 1976). On the other hand, Shiran *et al*. (2007) reported the existence of significant differences in the range of allelic diversity between peach and almond. These agree with what found [Martinez-Gomez *et al*. (2003); Sanchez-perez *et al*. (2005) and Seba Sarhan (2015)] with the use of those SSR markers in different *Prunus* species. Aranzana *et al.* (2002) found a high degree of similarity between species of this genus. This homology for the SSR loci between different peach genotypes in the present study or, even among *Prunus* species would partly explains reducing of breeding obstructions to transfer the genes between species successfully. Whereas, peach strains in this study have excellent fruit and agronomic properties hence, closely related species or genotypes from Egypt or any other country should be demoralized to get the chance for transferring gene successfully between them for cultivar improvement purposes. Especially that, microsatellite loci originated from c DNA sequences, which, are more likely located near or in genes linked to the important agronomic and economic traits as reported [Hamilton *et at*. (1999) and Jung *et al*. (2005)].

Consequently, the performance of a given primer pairs to measure the polymorphism, is not dependent only on diverse of germplasm but more dependent on the primers itself, where it designed.

The SSR profiles were combined with all the sixteen loci and compared to illustrate whether any strains were genetically identical. Regarding this, two of the strains (0.33) Soltany and Shamiea did not differ at any locus and described that they are identical, 100 % of the peach strains had 12 identical loci, none of the strains were different at

all loci with the rest strains, and 83 % of the strains had 13 identical loci. In this regard, the probability of matching fingerprints which indicate that, the probability of two genotypes or more has similar SSR profiles. It was high (100 %) with 12 SSR loci (Table 1). This high value reflects that the six strains were genetically identical at the level of those 12 loci. In a related context, the high average of the probability of matching fingerprints (0.887) between the six strains at the level of all loci, confirms the high degree of similarity between them. A possible explanation is that these strains derived from the same cultivar (Meet-Ghumr cultivar).

While, matching the genetic fingerprint between Soltany and Shamiea strains at the level of all 16 loci, it is possible due to that these strains are the same genotype but with different names. This is consistent with the non-registration of any unique bands (alleles) with those strains (Table 2). On the other hand, the other four strains (0.67%) kelaby, Hegazy, Mawwy and Farkk Meet Ghumr did not gave identical DNA-fingerprints.

Where three of these strains had produced five unique bands (Table 2), four of them considered as positive unique alleles; Pchcms 1_204 bp, Pchcms 1_192 bp, Pchcms 1_162 bp and Eppisp 011_ 246 bp; and were amplified from two loci. While the fifth unique band was considered as a negative and amplified from locus Pchcms 5_262 bp;. These three loci originated from c DNA regions. Therefore, Hegazy, Mawwy and Farkk Meet Ghumr strains in the present work not considered as identical genotypes. 3.2. Genetic similarities and clustering of strains.

Table 1. Characterization of the PCR products of simple sequence repeats (SSR) markers in peach strains studied.

SSR locus and Pol*	Reference	Repeat type	Numbers of alleles (A)	Effective number of alleles (NE)	Heterozygosity (Ho)[a]	Probability of matching fingerprints[b]	Discrimination power (DP)[c]	PIC or He Values[d]	Fixation index (F)
EPPISF001(N)	Vendramin *et al*. (2007)	$(GATG)_5$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF002 (N)	Vendramin *et al*. (2007)	$(AG)_2/(GTGGT)_3$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF004(N)	Vendramin *et al*. (2007)	$(CCA)_5$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF010 (N)	Vendramin *et al*. (2007)	$(AG)_{10}$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF011 (Y)	Vendramin *et al*. (2007)	$(TC)_7$	2	1.18	0.167	0.755	0.245	0.153	-0.091
EPPISF014 (N)	Vendramin *et al*. (2007)	$(CAG)CCA(CAG)_6$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF018 (N)	Vendramin *et al*. (2007)	$(TCT)5(TCC)_3$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF020 (N)	Vendramin *et al*. (2007)	$(CT)9(TC)_3$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF025 (N)	Vendramin *et al*. (2007)	$(TC)_7$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF027 (N)	Vendramin *et al*. (2007)	$(CAG)_5$	1	1	0.00	1.00	0.00	0.00	0.00
EPPISF032 (N)	Vendramin *et al*. (2007)	$(ACCC)3/(TC)_5$	1	1	0.00	1.00	0.00	0.00	0.00
Pchgms 1(N)	Sosinski *et al*. (2000)	$(AC)_{12}(AT)_6$	1	1	0.00	1.00	0.00	0.00	0.00
Pchcms 1 (Y)	Sosinski*et al*. (2000)	$(CA)_9(TC)_{14}/(GA)_{13}$	4	2.057	0.167	0.388	0.612	0.514	0.686
Pchcms 2 (N)	Sosinski *et al*. (2000)	$(CA)_8$	1	1	0.00	1.00	0.00	0.00	0.00
Pchgms 4 (Y)	Sosinski *et al*. (2000)	$(CT)_{21}$	2	1.8	0.667	0.52	0.48	0.444	-0.50
Pchcms 5 (Y)	Sosinski *et al*. (2000)	$(CA)_9(TA)_8$	2	1.8	0.333	0.531	0.469	0.444	0.25
Mean			1.375	1.1773	0.0832	0.887	0.113	0.097	0.023

***Polymorphism within the genotypes: Y if locus is polymorphic and N if the locus is monomorphic.**

[a] Direct count heterozygosity or observed heterozygosity is calculated according to Nei (1973). 0.00 indicates that the locus is homozygous over all the studied genotypes.

[b] refers to, the probability of two genotypes or more having similar SSR profiles.

[c] refers to, the probability of discriminating between two genotypes or more with every locus. It is calculated as 1-P (P = probability of matching fingerprints).

[d] refers to the polymorphism information content or expected heterozygosity, and it is calculated according to Nei (1973) and reflect the ability of a marker for detecting polymorphism between the genotypes, depending on the numbers of detectable alleles and their frequency.

Table 2. Heterozygosity grade and specific/Unique alleles within peach genotypes examined.

No.	Genotypes	Heterozygosity	specific/Unique alleles
1	Soltany	0.10	-
2	kelaby	0.20	-
3	Hegazy	0.00	Pchcms 1_204 bp$^{(+)}$,Pchcms _5_262 bp$^{(-)}$
4	Mawwy	0.10	Pchcms 1_192 bp$^{(+)}$, Pchcms 1_162bp$^{(+)}$
5	Shamiea	0.10	-
6	Farkk Meet Ghumr	0.30	Eppisf 011_ 246 bp$^{(+)}$
	Mean	0.133	

$^{(+)}$ indicates that the unique alleles are positive or present; $^{(-)}$ negative unique alleles.

Our results found that, the similarity coefficient between the strains ranged from 1.00 to 0.833 (Table 3). The low level of genetic variation was evident by the average of 0.91 similarity degree in the edible peach gene pool. These were in agreement with previous results [Zhongping Cheng (2007); Verde *et al*. (2013) and Cao *et al*. (2014)]. The maximum similarity degree was observed between Soltany and Shamiea strains (100 %) Followed by (0, 97) between Soltany and kelaby, kelaby and Shamiea also between kelaby and Farkk Meet-Ghumr as shown in (Table 4). The four strains had 15 identical loci and differed in one allele only. Therefore they considered to be very closely-related or almost identical. It is possible over many years that the

genotypes of peach have been transplanted to areas other than the area of their origin and they may have adapted with different names. Also, probably due to that these strains derived from the same cultivar (Meet-Ghumr cultivar). Similarly, the previous genome scans of peach and almond and despite its domesticated status; almond possess more genetic diversity than any of the studied peach species as reported (Dianne *et al*. 2016), suggesting that mating system more influential in diversity among species than domestication. It was noticed that the genetic matching between Soltany and Shamiea strains in this study at the level of the sixteen loci are consistent with the similarity in some characteristics of the fruits such as, the fruit length, width of the fruit and specific gravity, in addition to sugar percentage (the data not shown). The same situation was observed in terms of the high degree of genetic similarity by 97% between Soltany and kelaby, kelaby and Shamiea and between kelaby and Farkk Meet-Ghumr. Whereas, these strains is showing match or high degree of similarity in some characteristics of fruits, like acidity, TSS, sugar percentage, specific gravity, dimensions of length and width of the fruit. Which indicates the possibility that these loci may be responsible or in partnership with other loci for inheriting of these traits. While the lowest similarity degree was 0.83 between Mawwy and Farkk Meet-Ghumr, also the similarity degree was low comparatively between Mawwy and Hegazy strains by 0.84 (Table 3). Means that, the three strains have the highest genetic distance and therefore considered being genetically different from each other, through registration of unique bands (alleles). There may be some reasons to explain this reduction. It is likely that genetic diversity developed among clonal offspring of the same cultivar, due to the accumulation of somatic mutations over many years and through selections by the propagators for improving the properties like climatic adaptation or fruiting characteristics. All of that would have caused the release of several genetically different lines of the same cultivar. These genetic differences were generally consistent with the observations that indicating the significant differences in fruit characteristics such as sugar %, acidity %, total phenol, vitamin C, TSS % and the color index in addition to the dimensions of the length and width of the fruit between the three strains (the data not shown). This confirms that these loci may be responsible for the inheriting of these traits, especially that most of the loci used in this study, own sequences located near or within genes responsible for important economic traits in peach.

In the present study, peach strains were clustered (Fig.2) with data of amplified loci. The dendrogram could be divided into three sections or clusters. Hegazy and Mawwy strains formed a separated two clusters. Where, both strains showed the highest genetic distance with the rest of peach strains.Whereas, cluster 3 consisted of four strains (Soltany, kelaby, Shamiea and Farkk Meet-Ghumr). In this cluster, it was observed that kelaby and Farkk Meet-Ghumr were very closely related to each other, the same case with Soltany and Shamiea strains but they were fully genetically identical on level of the 16 SSR loci. Because of, the limited genetic variability of the peach become the basis for preservation or conservation and use of peach germplasm. So, modern molecular techniques could provide more information for discovering genetic diversity of peach at the level of DNA. The combination between morphology and molecular characterization can give orientation for conservation and use for breeding purposes.

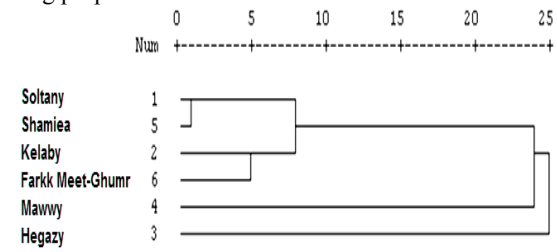

Figure 2. Dendrogram for six peach strains derived from Meet-Ghumr cultivar constructed from SSRs data based on Average Linkage (Between Groups), using Similarity computed according to Dice coefficient, with 22 fragments.

Actually, the dendrogram usually indicates the genetic relationship among group of genotypes in a population and may be expresses the evolutionary and parentage history of the species, this is the case whether the samples of population under study were numerous and diverse enough (Tran 2005). In our study the population was six strains derived from the same cultivar (Meet-Ghumr cultivar) rather than a wider gene pool. So, the dendrogram was just utilized to assessment the structure of genetic variation between the samples, not to understand any evolutionary relationship. In conclusion, the cluster results showed the presence of high similarity between the peach strains under study. Which could be interpreted as these strains did not received free external gene flow which, might be occurred because of the hybridization for new cultivar breeding in the history. Also may be, due to limited number of samples in this study. However, this study using microsatellite markers has elucidated that SSR fingerprinting able to distinguish between most of peach strains, in spite of the high similarity degree. As well as, molecular characterization analysis for the SSR loci used according to their origin could provide sufficient power of resolution to discriminate between peach strains and it is possible to help and serve in the construction of the molecular genetic data base for peach germplasm.

Moreover, the clustering of genotypes and its relation with important agronomic traits is highly useful for selection of genotypes in breeding programs for purposes of improvement. Thus would ensure conservation, in addition an optimal use and management of Egyptian peach germplasm collection.

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Table 3. Similarity indices (%) calculated by Dice computer package among six peach strains derived from Meet-Ghumr cultivar.

	6 Strains drived from mitt-Ghamer cultivar					
Strains/Genotypes	**Soltany**	**kelaby**	**Hegazy**	**Mawwy**	**Shamiea**	**Farkk Meet-Ghumr**
Soltany	1.000					
kelaby	.971	1.000				
Hegazy	.848	.882	1.000			
Mawwy	.882	.857	.848	1.000		
Shamiea	1.000	.971	.848	.882	1.000	
Farkk Meet-Ghumr	.944	.973	.857	.833	.944	1.000

REFERENCES

Aranzana, M. J.; J. Garcia-Mas; j. Carbo and P. Arus (2002). Development and variability analysis of microsatellite markers in peach. Plant breed 121, 87-92.

Aru´ s, P.; M. J. Aranzana and J. Carbo´ (2003) SSR and AFLP markers for germplasm evaluation and cultivar identification in peach. Acta Hort. 606, 35–40.

Baird, W. V.; A. S. Estager and J. K. Wells (1994). Estimating nuclear DNA content in peach and related diploid species using laser flow cytometry and DNA hybridization. Journal of the American Society for Horticultural Science. 119: 1312 – 1316.

Bodian, A.; A. Mohamed and N. Khadidiatou (2012). Genetic diversity analysis of date palm *(Phoenix datylifera* L.) cultivars from Figuig oasis (Morocco) using SSR markers. International Journal of Science and Adanced Technology. Volume 2 No 3: 96-104.

Cantini, C.; A. F. Iezzoni; W.F. Lamboy; M. Boritzki and D. Struss (2002). DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. Journal of the American Society for Horticultural Science.126, 205-209.

Cao, K.; Z. Zheng; L.Wang; X. Liu and G. Zhu (2014). Comparative population genomics reveals the domestication history of the peach, Prunus persica, and human influences on perennial fruit crops. Genome biology 15: 415.

Chaparoo, J. X.; D.J. Werner; D. Malley and R. R. Sederoff (1994). Targeted mapping and linkage analysis of morphological, isozyme and RAPD markers in peach. Theor. Appl.Genet.87, 805–815.

Denisov, V. P. (1988). Almond genetic resources in the USSR and their use in production and breeding, Acta. Hort., 1988, 224: 299-306.

Dianne, V.; H. josh; A. Mallikarjuna and R. Jeffrey (2016). Evolutionary genomics of peach and almond domestication. G3; genes /Genomes / Genetics volume x: 1-8

Dirlewanger, E.; P. Cosson; M. Tavaud; M. J. Aranzana. C. Poizat; A. Zanetto and P.Aru´s(2002). Development of microsatellite markers in peach and their use in genetic analysis in peach and sweet cherry. Theor. Appl. Genet. 105, 127–138.

Dirlewanger, E.; V. Pronier; R. Parvery; C. Rothan; A. Guye and R. monet (1998). Genetic Linkage map of Peach [*Prunus Persica* (L.) Batsch] using morphological and molecular markers. Theor. Appl. Genet. 97, 888 – 895.

Eldredge, L.; R. Ballard; W.V. Baird; A. Abbott; P. Morgens; A. Callahan; R. Scorza and R. Monet (1992). Application of RFLP analysis to genetic mapping in peaches. Hortic. Sci. 27, 160–163.

Gupta, P.; H.S., Balyan; P.C Sharma and B. Ramesh (1996). Microsatellites in plants: A new class of molecular markers, Curr. Sci, 70: 45-54.

Hamilton, M. E.; A. Pincus; A. Di-Fiore and R. Fleischer (1999). Universal Linker and Ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques, 27: 500-507.

Jones, D.A. (1972). Blood samples: probability of discrimination. Journal of the Forensic Science Society, 12: 355-359.

Jung, S.; A. Abbott; and C. Jusudurai (2005). Frequency, type, distribution of simple sequence repeats in Rosaceae ESTs. Functional integrated Genomics, 5: 136-143.

Kester, D. E. and C.J. Hansen (1966). Rootstock potentialities of F1 hybrids between peach (*Prunus persica* L.) and almond (Prunus amygdalus Batsch.), Journal of the American Society for Horticultural Science, 89: 100-109.

Khanuja, S.P.; A.K. Shasany; M.P. Darokar and S. Kumar (1999). Rapid isolation of DNA from dry and fresh samples of plant producing large amounts of secondary metabolites and essential oils. Plant Mol. Biol. Rep., 17: 1-7.

Kloosterman, A.D.; B. Budowlw and P. Daselaar (1993). PCR-amplification and detection of human DIS80 VNTR locus-Amplification condition, population genetics and application in forensic analysis. International Journal of legal Medicine,105:257-264.

Manubens, A.; S. Lobos Y. Jadue; M. Toro; R. Messina; M. Liadser and D. seelenfreund (1999). DNA isolation and AFLP fingerprinting of nectarine and peach varieties (*prunus persica*). Plant Mol. Biol. Rep. 17, 255 – 267.

Martinz-Gomez, P.; S. Arulsekar; D. Potter and T. M. Gradziel (2003). An extended interspecific gene pool available to peach and almond breeding as characterized using simple sequence repeat (SSR) markers. Euphytica, 131: 313-322.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321-3323.

Pedersen, B. H. (2006). DNA fingerprinting of 51 sweet and sour prunus accessions using simple sequence repeats. Journal of Horticultural Science& Biotechnology, 81: 118-124.

Porebski, S.; L. Grant Bailey and B.R. Baum (1997). Modification of a CTAP DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter, 15 (1): 8-15.

Quarta, R.; M. T. Dettori; I. Verde; P. Laino; A. Sabatini; A. Vantaggi and R. Sciarroni (1996). Characterization and evaluation of genetic diversity in peach germplasm using RAPD and RFLP markers. Acta. Hort. 546, 489–496.

Rajapakse, S.; L. E Belthoff; G. He; A.E. Estager; R. Scorza; I. Verde; R. E. Ballard; W. V. Baird; A. Callahan; R. Monet and A. G. Abbott (1995). Genetic linkage mapping in peach using morphological, RFLP, and RAPD markers. Theor. Appl. Genet. 90, 503–510.

Raymond, M. and Rouset F. (1995). GENEPOP (version 1.31): population genetics software for exact test and ecumenicism. J. Hered 86:248-249. http:// whbiomed.curtin.edu.au/gene-pop/.

Sanchez-Perez, R.; D. ruiz; F. Dicenta; J. Egea and P. Marinez-Gomez (2005). Application of simple sequence repeat (SSR) markers in apricot breeding: molecular characterization, protection and genetic relationships. Sci. Hort. 103: 305-315.

Scorza, R; S. Mehlenbacher and W. Lighner (1985). Inbreeding and coancestry of freestone peach cultivars of eastern United States and implications for peach germplasm improvement. Journal of the American Society for Horticultural Science. 110, 547 – 552.

Seba, S.; F. Hamed; S. lawnd and W. Al- youssef (2015). Relationships among peach, almond and related species as detected by SSRL ISSR markers. International Journal of ChemTech Research, vol. 8, No. 1, 82-88.

Shiran, B.; N. Amirbakhtiar; S. Kiani and S. Mohammadi (2007). Molecular characterization and genetic relationships among almond cultivars assessed by RAPD and SSR markers. Scientia Horticulturae, 111: 280-292.

Sneath, P.H. and R. R. Sokal (1973). Numerical taxonomy. Freeman. San Francisco. 573 pp.

Sosinski, B.; M. Gannavarapu; L. D. Hager; L. E. Beck; G. J. King; C. D. Ryder S. Rajapakse; W. V. Baird; R. E. Ballard and A. G. Abbott (2000). Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theor. Appl. Genet.101: 421- 428.

Tran, Thi. M. (2005). Genetic variation in cultivated coffee (*Coffea arabica* L.) accessions in Northern New South Wales, Australia. MSc thesis. Southern Cross University.

Vendramin, E; M. Dettori; S. Giovinazzi; S. Micali; R. Quarta and I. Verde(2007). A Set of EST-SSRs isolated from Peach fruit transcriptome and their transportability across Prunus species. Molecular Ecology Notes. 7, 307 – 310.

Verde, I.; A. G. Abbott; S. Scalabrin; S. Jung and S. Shu (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genetics 45: 487–494.

Viruel, M. A.; P. Escribano; M. Barbieri; M. Ferri and J.I. Hormaza (2005). Fingerprinting, embryo type and geographic differentiation in mango (Mangifera indica L., Anacardiaceae) with microsatellites. Molecular breeding, 14: 383-383.

Warburton, M. L. and F. A. Bliss (1996). Genetic diversity in peach (Prunus persica L./ Batsch) revealed by randomly amplified polymorphic DNA (RAPD) markers and compared to inbreeding coefficients. Journal of the American Society for Horticultural Science. 121: 1012–1019.

Watkins, R. (1976). Cherry, Plum, Peach, apricot and almond. In: Simmond, N.W. (Ed.) evolution of Crop plants. Longman, London, pp.342-347.

Zhongping Cheng (2007). Genetic characterization of different demes in *Prunus persica* revealed by RAPD markers. Scientia Horticulturae. 111, 242 – 247.

تحليل الأختلافات الوراثية والبصمة الوراثية لبعض سلالات خوخ ميت غمر المصري بواسطة معلمات التتابعات المكررة البسيطة (الميكروستالليت)

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أجريت هذه الدراسة للوقوف على الخصائص الجزيئية لستة سلالات خوخ نشأت من صنف خوخ ميت غمر المصري. بالأضافة لفهم مكونات الأختلافات الوراثية بين هذه السلالات من خلال تكبير ستة عشر موقع وراثي بمساعدة ستة عشر زوج من بوادىء معلمات التتابعات المكررة البسيطة أو المعروفة بالميكروستالليت و المتخصصة لجينوم الخوخ والني سبق تصميمها وتوصيفها، أيضا لمعرفة قدرة هذه البوادىء على التمييز والتفريق بين السلالات الستة تحت الدراسة بما يعرف بالبصمة الوراثية. أوضحت النتائج أن المواقع الوراثية المكبرة كانت منخفضة التباين وتراوح عدد الأليلات الناتجة من ١ الى ٤ أليلات فقط بمتوسط ١,٣٧ أليل لكل موقع ، في حين أن نسبة الخلط على مستوى المواقع الوراثية كانت منخفضة حيث تراوحت من صفر الى ٦٦% كذلك أظهرت نسبة الخلط داخل التراكيب الوراثية على مستوى الستة عشر موقع وراثي إنخفاضا ملحوظا أيضا ما بين صفر الى ٣٠%. في حين كانت قيمة الأختلافات في الجين أو مقدار ما يوفرة الموقع الوراثي من معلومات عن الإختلافات الوراثية الموجودة بين التراكيب الوراثية كانت منخفضة أيضا حيث تراوحت ما بين صفر الى ٥١%، في حين أن طاقة أو قدرة التمييز لكل موقع وراثي بين السلالات تحت الدراسة إختلفت وتراوحت من الصفر الى ٦١% بمتوسط ١١%. بينما أحتمال تطابق زوج أو أكثر من التراكيب الوراثية في بصمة الحامض النووي "دنا" والتي تعتمد على شكل وعدد الحزم المكبرة, أظهرت النتائج أن سلالتي السلطاني والشامية متطابقتين في البصمة الوراثية في كل المواقع الوراثية التي تم تكبيرها ومن ثَم وصفوا بأنهم متطابقين وراثيا. وفي سياق متصل كانت درجة التشابه الوراثي بين السلالات الستة مرتفعة حيث تراوحت ما بين ٨٣ % الى ١٠٠% . النتائج أكدت على ما توصلت اليه الدراسات السابقة من إنخفاض مستوى الأختلافات الوراثية في المستودع الجيني لخوخ الأكل. مما يشير أيضا لأهمية الأستفادة من الأنواع الأخرى القريبة وراثيا من الخوخ مثل اللوز كمصدر للجينات الهامه و للإ ختلافات الوراثية المطلوبة للتحسين الوراثي للخوخ.