FINGERPRINTING A NUMBER OF *PRUNUS PERSICA* VARIETIES CULTIVATED IN DUHOK PROVINCE USING SRAP MARKERS

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Abstract:

In order to evaluate the genetic relationship among the germplasms of *Prunus persica*, sequence related amplified polymorphism (SRAP) marker was used to analyze genetic diversity of nine genotypes of peach cultivated in Duhok Governorate/ Kurdistan Region- Iraq. Twelve primer pairs generated 658 bands，379 bands were polymorphism; level of polymorphism observed in the present study with 12 primers pairs was 60.89 %. Revealed by NTSYS software the SM coefficient of genetic similarity ranged from 0.056 to 0.35. A dendrogram was constructed based on SRAP data using UPGMA cluster method. The nine genotypes of peach were classified into three groups and five sub-groups which were basically corresponded with the genetic relationships based on SRAP marker data.

Key words: *Prunus persica*, Fingerprinting, SRAP Marker**,** Duhok Province

INTRODUCTION

Peach (*Prunus persica* L. Batsch) is considered as one of the important and favorable stone fruits farmers worldwide (Nagaty *et al*., 2011). Peach is a deciduous tree in nature belonging to the subfamily *Prunoideae* of the family *Rosaceae* and classified in the subgenus *Amygdales* within the genus *Prunus* (Watkins, 1976). It is provides vitamins, minerals, and fiber also contains antioxidant compounds for healthy diets (Verde *et al*.**,** 2013).

Peach had been considered a traditional and important fruit crop of Kurdistan region and ranks first in genus *Prunus*. The role of germplasm diversity is important in the establishment of peach fruit crop (Bakht *et al*., 2012).

For the study and characterization the genetic variations among different species and population, molecular markers are the marker of choice (Graham *et al.,* 2004). For this reason an attempt was made in the present study to evaluate the level of genetic diversity in some varieties of Peach in Duhok province using SRAP markers (Sequence-related amplified polymorphism), this technique was developed by Li *et al*., (2001). The evaluation of genetic relationships among these varieties provides the basic information for breeding programs, and to identify SRAP markers that could be used to follow up important flowering and fruiting traits in peach breeding programs.

Materials and Methods

DNA Extraction: Experiments were carried out in Plant molecular biology lab, Scientific Research Center, College of Science, University of Duhok., peach cultivars were collected from Duhok Agriculture station \ Duhok governorate Kurdistan/ Iraq. Genomic DNA of all the samples were extracted from young leaves according to the modified CTAB method described in Weigand *et al*., (1993).

For the present study of genetic diversity among different peach varieties, twelve SRAP combinations were used and produced amplified product. The primers combination used during the present study were: (EM 16; Me 11, E16;Me1, E16;Me6, EM16; Me4, EM15;Me1, EM16;Me9, EM15;Me13, EM17;Me1, EM16;Me10, EM17;Me2, EM1;Me4, EM15 and Me12).Table (1) represents the forward and reverse sequences of these primers.

Table (1): Sequences of forward and reverse SRAP Primers:

Reverse	5' 3'	Forward	5' 3'
EM1	GACTGCGTACGAATTAAT	ME4	TGAGTCCAAACCGGACC
EM15	GACTGCGTACGAATTCTG	ME1	TGAGTCCAAACCGGATA
EM15	GACTGCGTACGAATTCTG	ME13	TGAGTCCAAACCGGCAT
EM15	GACTGCGTACGAATTCTG	ME12	GGTGAACGCTCCGGAAG
EM16	GACTGCGTACGAATTCGG	ME9	TGAGTCCAAACCGGTCA
EM16	GACTGCGTACGAATTCGG	ME10	TGAGTCCAAACCGGAAA
EM16	GACTGCGTACGAATTCGG	ME11	TGAGTCCAAACCGGAAC
EM16	GACTGCGTACGAATTCGG	ME1	TGAGTCCAAACCGGATA
EM16	GACTGCGTACGAATTCGG	ME2	TGAGTCCAAACCGGAGC
EM16	GACTGCGTACGAATTCGG	ME4	TGAGTCCAAACCGGACC
EM17	GACTGCGTACGAATTCCA	ME1	TGAGTCCAAACCGGATA
EM17	GACTGCGTACGAATTCCA	ME2	TGAGTCCAAACCGGAGC

SRAP reaction mixture and amplification protocol:

The reaction system was 20μL, including: 1×PCR buffer, MgCl2 2.0 mmol·L-1, dNTPs 0.1 mmol·L-1, primer 0.5 μmol·L-1, template DNA15 ŋg, Taq DNA polymerase 1.5U. The protocol for PCR amplification was: initial denaturation (5 min at 94°C); denaturation (60s at 94°C), annealing (60s at 35°C), extension (90s at 72°C), for 5 cycles; denaturation (60s at 94°C), annealing (60s at 50°C), extension (90s at 72°C), for 35 cycles; final extension (10 min at 72°C). The amplification products were separated by electrophoresis on agarose gels.

Data analysis: According to the results of electrophoresis, if there was an amplified band (band present) it was scored as 1, otherwise (band absent) scored as 0. Using NTSYS software Version 2.1 (Applied Biostatistics) program (Rohlf**,** 2004) using the program editor. The data were analyzed using SIMQUAL (Similarity for Qualitative Data) routine to generate genetic similarity index. (Nei and Li.,1979).

Results and Discussion:

The twelve SRAP primer pairs produced a total of 658 bands, with a mean of 54.8 per primer combination, of which the highest polymorphic rate was obtained from the primer pair (EM1/ME4) and the lowest polymorphism rate was obtained from the primer pair (EM15/ME12). The level of polymorphism observed in the present study with 12 primers pairs was 60.89 % (Table 2). In a study of Ahmed *et al*., (2004) For SRAP marker they obtained an average of 21.8 per primer combination.

Table (2): The present total numbers of bands, number of polymorphic bands and polymorphic rate of the twelve combinations of SRAP primers.

Primer combination	Total number of bands	Number of Polymorphic Bands	Polymorphism rate (%)
EM1/ME4	49	40	81.63
EM15/ME13	40	22	55.00
EM15/ME1	31	13	41.94
EM15/ME12	55	28	50.90
EM16/ME9	47	29	61.70
EM16/ME10	33	24	72.72
EM16/ME11	65	47	72.30
EM16/ME1	44	35	79.54
EM16/ME2	66	30	45.45
EM16/ME4	79	43	54.43
EM17/ME1	107	62	57.94
ME17/ME2	42	24	57.14
TOTAL	658	379	60.89

The genetic similarity among the nine peach varieties based on the data of the twelve combinations SRAP primers, were showed in Table (3). The highest genetic distance was between Elberta and Florida sun, and lowest genetic distance was between July Elberta and Cornet and between Cornet and Santa Rosa.

Table (3): The present genetic similarity coefficient matrix of the nine peach varieties based on the data of the twelve combinations SRAP primers.

	Cornet	Dixi Red	Double Delight	Earilirich	Elberta	Florida Sun	Goldenmine	July Elberta	Santa Rosa
Cornet	0.0000								
Dixi Red	0.2052	0.0000							
Double Delight	0.1965	0.1280	0.0000						
Earlirich	0.40504	0.28551	0.2751	0.0000					
Elberta	0.28057	0.17321	0.1973	0.1653	0.0000				
Florida sun	0.26913	0.20744	0.1481	0.2167	0.0885	0.0000			
Golden mine	0.41165	0.29142	0.2231	0.1937	0.1292	0.0962	0.0000		
July Elberta	0.42791	0.26393	0.2202	0.2337	0.2052	0.24320	0.1722	0.0000	
Santa Rosa	0.42791	0.28163	0.3421	0.3290	0.3410	0.36509	0.2880	0.2492	0.0000

Cluster Analysis:

A dendrogram was obtained by the UPGMA method using the total number of SRAP bands (Fig. 1). There were three main groups in the dendrogram: Group 1 consisted of one genotype Santa rosa. The second group consist four sub-groups which genotype Erlirich appear alone, Elberta, florida sun grouped together while Golden mine and July elberta clustered separately. The third group consists of two sub-groups, the first one consist of genotype Cornet. The second consist of genotypes Dixi red and Double delight. The cluster analysis showed the similarity among peach genotypes were high, and that might be refer to possible frequent gene flow from one genotype to another and the chance of crossing thus gene exchange was few. (Wang *et al.,* 2002; Dirlewanger *et al.,* 2002).

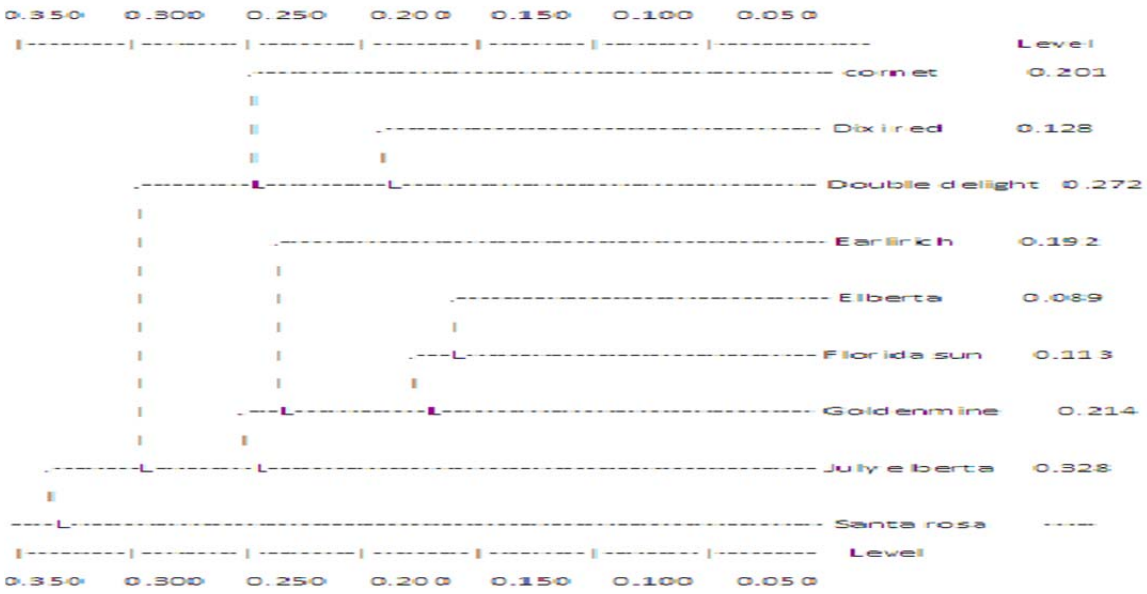

Figure (1): A dendrogram Neighbor–joining tree representing the genetic relationships among Peach genotypes

In the present study the SRAP markers were used for the first time in *Prunus persica* and distinguished cultivars efficiently with high level of polymorphism. The results obtained in this study showed that there were high levels of polymorphism in peach cultivars and were distinguished with the SRAP data.

The SRAP marker system had been used for characterization and finger printing studies in a wide range of plants (Uzun *et al*., 2010). The study described in this paper shows that SRAP analysis is a powerful tool for the characterization of peach cultivars. In our study the SRAP markers had been used to distinguish Peach cultivars for the first time.

The results of the present study will allow for future studies on the appropriate use of these cultivars in breeding programs, proper biodiversity assessment and better conservation of germplasm resources In order to breed new cultivars or new germplasm of peach it is nesscery to cross cultivar that genetically are distance to increase the chance of crossing genes (Wang *et al.,* 2002; Dirlewanger *et al.,* 2002).

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استخدام مؤشرات SRAP في دراسة التنوع الوراثي لجنس الخوخ *Prunus persica* في محافظة دهوك – اقليم كوردستان \ العراق

الخلاصة :

من أجل تحليل العلاقة الوراثية للمادة الوراثية لجنس الخوخ (*Prunus persica*) ، استخدمت تقنية (SRAP) لتحليل التنوع الوراثي لتسعة المورثات من الخوخ المزروعة في محافظة دهوك /اقليم كردستان العراق. تم استخدام 12 توليفة من البرايمرات وانتجت 658 حزمة منها 379 حزمة كانت متابينة، حيث كان مستوى التباين بين الحزم 60.89 % . اظهر برنامج ال NTSYS لاظهار البعد الوراثي بين الاصناف المدروسة حيث تراوح بين 0.056 الى 0.035 . كما أظهرت نتائج التحليل التجميعي والتي استندت على نتائج تقنية ال SRAP الى ثلاثة مجاميع وراثية رئيسية و خمسة مجاميع فرعية.

بكارئينانا نيشاندەرێن SRAP بۆ دیارکرنا جوراوجوریا ژنتیکی یا خوخێ (*Prunus persica*) ل پارێزگەها دهوکێ یا هەرێما کوردستانا عێراقێ

پوختە

ڤەکولینا پەیوەندیا بوماوەیی ونەوەکهەڤیا بوماوەیی بو چەشناندا خوخێ ل پارێزگەها دهوکێ هاتەکرن ل. وژبەر وێ نیشانێن SRAP هاتنە بکارئینان. ژئەنجامێ بکارئینانا (12) نیشاندەرێن SRAP د ڤی ڤەکولینێ دا وەکهەڤیا ژنتیکی هاتیە هەژمارتن بۆ نەھ چەشناندا یێت خوخێ سەرجەمێ وان شەریتێن ل 12 نیشاندەراندا 658 شەریتێن و 379 ژێ شەریتێن جوراوجورن . وەکهەڤیا ژنتیکی دەست پێ دکەت ژ 0.056 بۆ 0.035. پولین کرنا ڤان چەشناندا لسەر بنەمایێن نیشاندەرێن SRAP سێ گروپێن سەرەکی دیار کرن و پێنج گروپێت دووەمی .