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EVALUATION OF GENETIC DIVERSITY OF SOME GENOTYPES IN ALMOND (*Prunusdulcis* L.) USING SRAP

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ABSTRACT

Almond (*Prunusdulcis*) is one of the most important nut fruits worldwide and its seed has been recognized as a health nutrient supply for human consumption. In this study, as well as their genetic diversity and natural variations using SRAP marker, aiming at exploiting valuable traits for breeding new cultivars. Our study of identified Molecular evaluation of genetic diversity at the DNA level for relationships and population structure among 12 almond genotypes, Identifying alleles (s-alleles) of the genre of self-incompatibility and self-compatibility gene, and preservation of these important resources, the wild distribution of almond and closely related species. A total of 48 bands ranging from 100 to 2000 bp were detected using the 3SRAP primer combinations in the twelve populations (Table 4). The numbers of bands per primer combination ranged from 6 to 21, with an average of 11.57.161 (99.35%) were polymorphic, and the percentage of polymorphic bands produced by each primer ranged from 100%.

Key words: Almond, genetic diversity SRAP, *Prunusdulcis*.

INTRODUCTION

The genus almond (Prunusdulcis Miller (D.A Webb) syn *P. amygdalus* Batsch) is one of the oldest tree nut crops and one of the most important nut fruits worldwide (**Kester and Gradziel, 1996; Hala´sz *et al.,* 2008; Sorkheh *et al.,* 2009**). Almonds are the largest source of commercial tree nut products, with an annual world production of nearly 1.9 million tons and 75 thousand tons from Turkey (**FAO, 2012**).

Almond was reported to originate in West and Central Asia, but almond species grow in any region with a subtropical Mediterranean climate of mild wet winters and warm, dry summers (**Kester and Gradziel, 1996**). Since ancient times, almond cultivation rapidly spread throughout the Mediterranean regions from central Asia (**Sorkheh *et al.,* 2007**).

Almond, scientifically known as *Prunusdulcis*, under the family Rosaceae, almond tree is the number one tree nut produced on a global basis, it is especially spread through and well adapted to the whole Mediterranean region, from which about 28% of the world production is obtained. *Prunusdulcis* (Mill.) D.A. Webb. is grown as an economically valuable crop in a number of countries worldwide, but large-scale cultivation has been primarily restricted to semiarid and arid regions with mild, temperate climates. Considering the species' wide native range and inherent genetic, morphologic, and phenologic diversity, almond remains quite underused in areas outside those currently in cultivation. Globally, almond is one of the most important nut crops, yet the full horticultural potential of this species has yet to be realized. In recent years, various

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molecular markers have been used to evaluate the genetic diversity and relationships among almonds, such as RAPD, AFLP, SSR, ISSR, and SNP (**Bartolozzi *et al.*, 1998; Martins *et al.*, 2003; Xu *et al.*, 2004; Xie *et al.*, 2006; Shiran *et al.*, 2007; Sorkheh *et al.*, 2007; Gouta *et al.*, 2010**). Sequence-related amplified polymorphism (SRAP) is a frequently used molecular marker, since it is simple, reliable and effective (**Guo et al., 2012**). Many studies evaluated the level of genetic variability in almond using various types of markers (**Xu *et al.,* 2004; Xie *et al*., 2006; Sorkheh *et al.,* 2007; Pe´rez-Campos *et al.,* 2011; Rahemi *et al.,* 2012; Distefano *et al.,* 2013**).

These studies identified high level of variation and heterozygosity resulting from its self-incompatibility. Numerous studies have been conducted to assess the genetic diversity held in different almond collections around the world. These analyses have used a variety of molecular techniques including the analysis of allozymes and amplified fragment length polymorphism, random amplified polymorphic DNA, simple sequence repeat, and inter simple sequence repeat markers (**Sorkhe*et al*., 2007, 2009**).

MATERIALS AND METHODS

Plant Materials

We have been using a total of 12 varieties in this study (Table 1) samples were obtained sheet young people of the three source genetic, gene Bank almonds Sheikh Zuid research station belonging to the Desert Research Centre and the Directorate for Agriculture and South Sinai.

DNA Extraction

The DNA from the young leaves was extracted using a modified CTAB method (**Huang *et al.*, 2000**). DNA quality was detected by electrophoresis on 0.8% (w/v) agarose gel. DNA concentration was measured by a UV–VIS spectrophotometer, and adjusted to 40 ng ml^{-1} and then stored at 20 C for SRAP-PCR analysis.

SRAP-PCR Amplification

SRAP primer sequences were synthesized by Beijing Aoke Biological Technology and Service Co. Ltd (Beijing). 357 primer combinations were screened among five plants from different population. From these, 14 primer combinations were selected for the present study based on reproducibility, clarity of bands, and their polymorphism (Table 2).

PCR amplification reactions were carried out according to previously established protocols by **Jing *et al.* (2013)** in 20 mL volume, which contained 40 ng of template DNA, 10 PCR buffer (100 Mm Tris–HCl, pH 8.3; 500 mMKCl), 0.18 mM of each dNTP, 0.75 mM of each primer, 1.87 mM of $MgCl_2$, and 1 unit of Taq DNA polymerase (TaKaRa Biotechnology Dalian Co., Ltd., China). PCR reactions were performed using the following program: an initial step of 5 min at 94 C, followed by 5 cycles of 1 min at 94 C, 1 min at 36 C, and 1 min at 72 C. In the following 30 cycles, the annealing temperature was increased to 51 C, and a final 7 min extension at 72 C. PCR products were separated on 6% denatured polyacrylamide gels and detected by silver staining. Then clear and reproducible distinguished bands were recorded and used in the following analysis. DL2000 DNA ladder (TaKaRa Biotechnology Dalian Co., Ltd., China) was used as DNA markers.

Data Analysis

In SRAP analysis, each clear, reproducible, amplified DNA polymorphic bands between 200 bp and 2000 bp were scored as 1 for presence or 0 for absence to form a binary matrix for further analysis. POPGENE version 1.32 (**Yeh *et al.,* 2000**) was used to compute the number of effective loci, the percentage of polymorphic loci, observed

Table (1): Almond cultivars and wild species assayed including the origin, parentage and main agronomic characteristics

No.	Varieties	Collection sites	Pedigree	Shell	Flowering	GPS	
						Latitude	Longitude
S_1	*PrunusSaint Catherine Monastery1*	Saint Catherine Monastery South Sinai		Farkk		28.557149	33.974250
S_2	*Prunus Saint Catherine Monastery2*	Saint Catherine Monastery - South Sinai		Sami hard		28.556816	33.974785
S_3	*Prunus Saint Catherine wadishrezz*	Saint Catherine - *wadishrezz* -South Sinai		Farkk		28.560364	33.957002
S_4	*Prunus Wadi40*	WadiElarabeen Saint Catherine South Sinai		Hard		28.551731	33.949981
S_5	PrunusZwyed1	El Sheikh Zuweid North Sinai El-Gora		Hard		30.952595	34.307769
S_6	PrunusZwyed 2	El Sheikh Zuweid North Sinai		Soft		31.222419	34.117095
S_7	Prunus Nonpareil	مرسى مطروح	Unknown	paper	Middle	31.349200	27.150591
S_8	Prunusferdal	مرسى مطروح				31.350141	27.138680
S_9	Prunustexas	USA	Unknown	Semi-hard	Late		مزرعة جروبى التابعة للهيئة العامة للتشجير
S_{10}	Prunusperles (mal)						
S_{11}	Prunus IXL	USA	Unknown	Soft	Middle		مزرعة جروبى التابعة للهيئة العامة للتشجير
S_{12}	Turkey	El Sheikh Zuweid Research Station, North Sinai				31.236689°	34.117520°

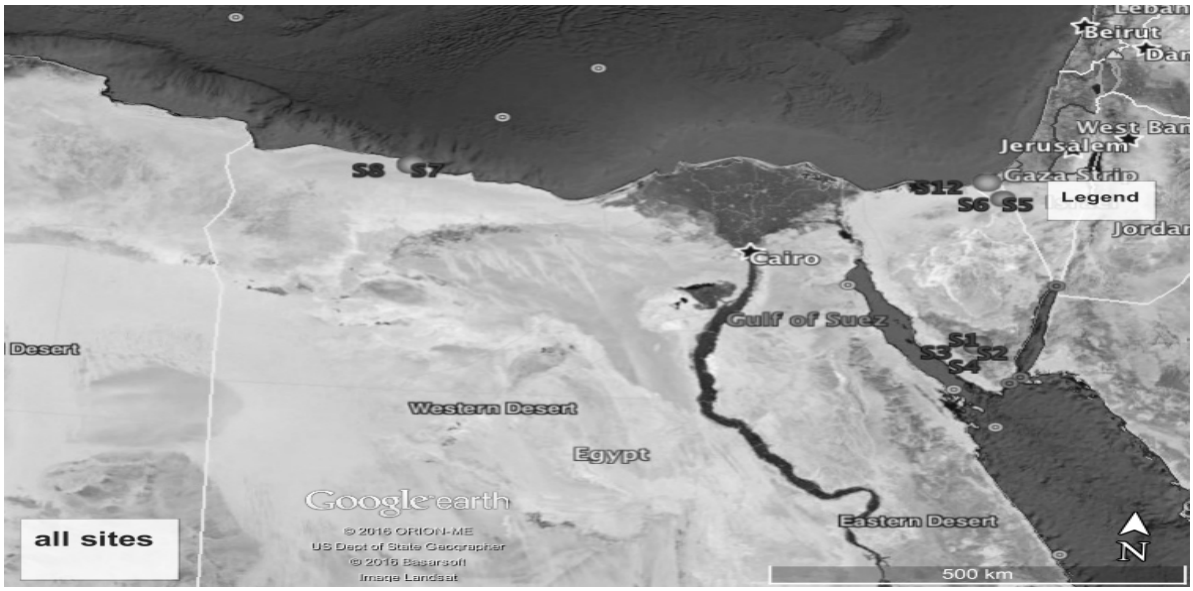


Fig. (1)

Fig. (2)

Fig. (3): Sweet almond in saint catherine

Fig. (4): Sweet almond in wadies of saint catherine south sinai

Fig. (5): Almond in wadies of saint catherine south sinai

Fig. (6): Almond leaves and immature fruit

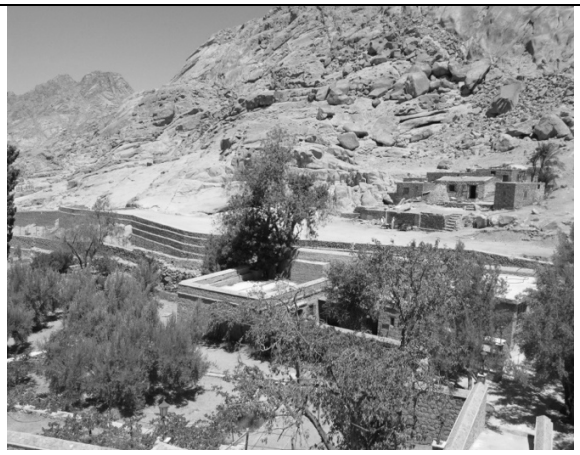


Fig. (7): Sweet almond in saint catherine monastery

Fig. (8): Mature sweet almond in saint catherine

Fig. (9): لوز فرك غير ناضج

Fig. (10): Almond fruit showing the immature fruit

Fig. (11): Hard almond

Fig. (12): Hard almond

number of alleles (na), effective number of alleles (ne), Nei's genetic diversity (h) (**Nei, 1973**), Shannon's information index (I), Nei's coefficient of genetic differenti- ation (Gst), Nei's genetic distance (GD) and gene flow (Nm). An analysis of molecular variance (AMOVA) was carried out using AMOVA program version 1.5 (**Excoffier *et al.*, 1992**). The UPGMA dendrogram were constructed using MEGA 4.0 (**Tamura *et al.*, 2007**). All data were produced using the program DCFA 1.1 (**Zhang and Ge, 2002**). In addition, relationships between the genetic distance matrix and the geographic distance matrix were estimated with the Mantel test (**Mantel, 1967**) in the NTSYS- pc program (**Rohlf, 2000**) by 1000 permutations of bootstrapping.

RESULTS

SRAP Polymorphism Analysis

A total of 30 SRAP primer combinations were used to screen polymorphism among 12 genotypes from different almond. Among them 3 SRAP primer combinations amplifying clear and abundant fragments were used to evaluate the genetic diversity of twelve populations. A total of 48 bands ranging from 100 to 2000 bp were detected using the 3SRAP primer combinations in the twelve populations (Table 2). The number of bands per primer combination ranged from 6 to 21, with an average of 11.57. 161 (99.35%) were polymorphic, and the percentage of polymorphic bands produced by each primer ranged from 100%.

SRAP amplification

In the present study, 3 appropriate primer combinations (PCs) were screened out of a total 30 PCs. Among them 3 PCs yielded stable, reproducible amplification patterns in two repetitive experiments. From the 11 PCs and 36 accessions used in this study, a total of 48 fragments ranging from 38 to 1348 bp were scored, of which 48 (100%) were polymorphic, with an average of 100 polymorphic bands per PC (Table 2). This results in agreement with the observations of **Budak *et al.* (2004a)** in buffalograss (Buchloedactyloides) Englem, **Ferriol *et al.* (2004)** in Cucurbitamoschata and **Li and Quiros (2001)** in *Brassica oleracea* L., which has reported the presence of 10–20 polymorphic bands per PC. The percentage of polymorphic bands produced by each PC 100 All primers used in the present study. Effective number of alleles (Ne), expected heterozygosity

Primer Em 8 nd DN8

The results of Primer Em8&DN8 are illustrated in Fig. 1 and Table 1. It give 12 polymorphic bands with different fragment sizes ranging from 1172 to 218 bp for almond.

Primer Em12&DN6

The results of Primer Em12&DN6 are illustrated in Fig. 2 and Table 2. It gives 17 polymorphic bands with different fragment sizes ranging from 767 to 42 bp for almond.

Primer Em2R&DN9

The results of Primer Em2R&DN9 are illustrated in fig.3 and table3. it give19 polymorphic bands with different fragment sizes ranging from 767 to 42 bp for almond.

Genetic similarity and cluster analysis

Pair-wise differences among almond genotypes calculated by statistical package for social science (SPSS ver.13) computer program. The analysis was based on the number of markers that can differentiate between any given pair of genotypes or species to calculate the similarity matrix.

SPSS computer program was applied. The degree of similarity was represented by similar values, which ranged between 0-1 for the most dissimilarity and most similar between any given pair of species. To perform and draw the phylogenetic Dendrogram SPSS program was applied. All the Almond species and accessions were distinguished with identification of 36

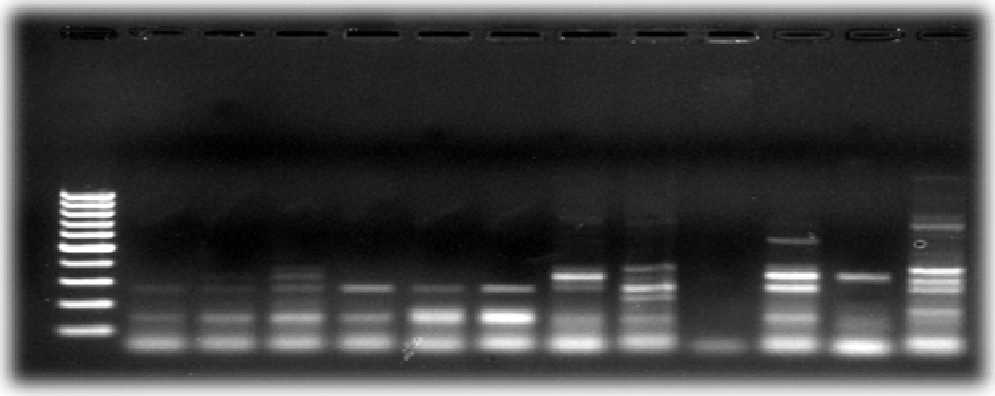


Photo (1): DNA polymorphism of the twelve species in almond genotype using SRAP-PCR with Primer Em8&DN8

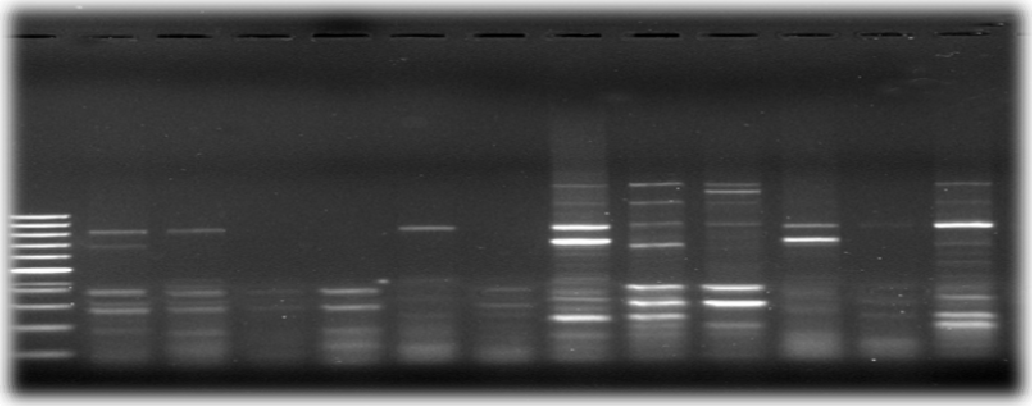


Photo (2): DNA polymorphism of the twelve species in almond genotype using SRAP-PCR with Primer Em12&DN6

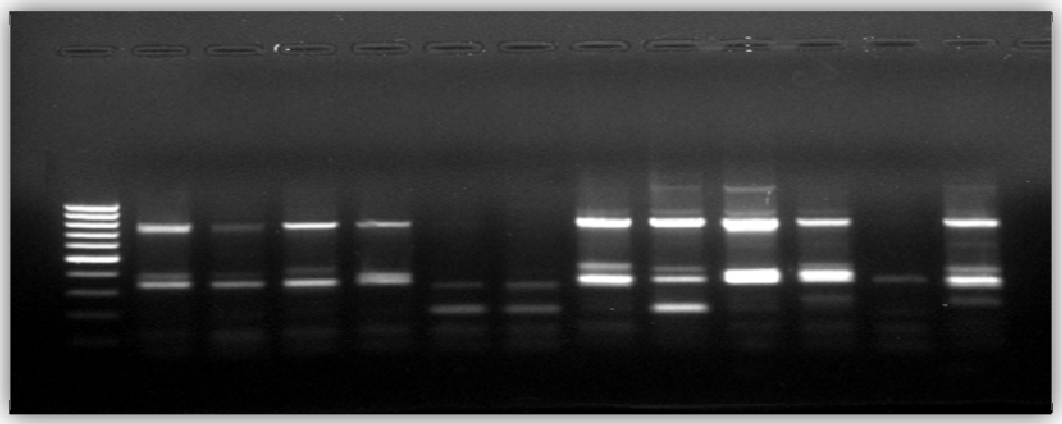
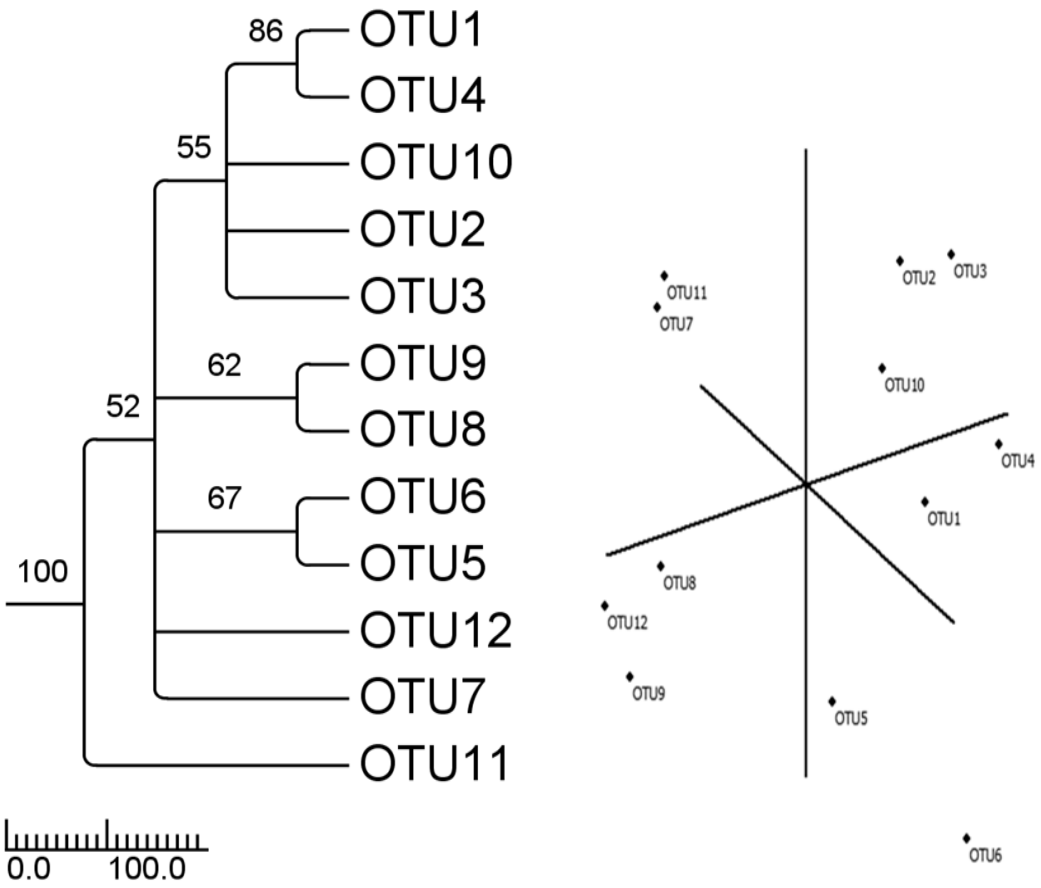


Photo (3): DNA polymorphism of the twelve species in almond genotype using SRAP-PCR with Primer Em2R&DN9

Table (2): The 11 SRAP primer pairs used in this study and their total, polymorphic fragments

Statistics / Primer	No. Bands	Monomorphic	Polymorphic	% Polymorphism
SRAP-Em2-DN9	**12**	**0**	**12**	**100**
SRAP-Em8-DN8	**17**	**0**	**17**	**100**
SRAP-Em12-DN6	**19**	**0**	**19**	**100**
Total	**48**	**0**	**48**	**100**

A total of 48 bands were scored from all primer pairs for all the 12 genotyps 48 bands were polymorphic.



different fingerprints, revealing the high power of discrimination of the used SRAP PCs and attesting the great diversity of Almond accessions. The results showed that the highest genetic distance (85) was recorded between "OTU1", an accession of Prunus Saint Catherine Monastery 1 from Saint Catherine Mon-astery South Sinai, and "OTU4", an accession of Prunus Wadi40, and the lowest one (52) was counted between "OTU7" and "OTU1, 2, 3, 4,10".

The data obtained from SRAP analysis of 12 Almond accessions was subjected to cluster analysis. The dendrogram of genetic relationships among accessions constructed by Neighbor-Joining method with genetic distance are presented in Fig.1. The cluster analysis revealed species-based grouping, with all the Almond genotypes separated from each other. The almond accessions were analyzed, though separated in several different sub-clusters and displayed a high level of genetic variability probably due to the geographical distribution of the materials. Different *prunes dulicus* accessions grouped together in a same cluster. It can be assumed that the domesticated genotypes of Almond evolved from "Sarakhs" and then this wild accession likely developed to other local Almond. Therefore, it seems that the wild Almond accession plays an important role in evolutionary of the prunus genus, consistent with the results of **Arab-Nejhad *et al.* (2009)** and **Salimi *et al.* (2009)**. Our SRAP-based cluster results also indicated that P. dulcus clustering was related to the geographical locations and climatic conditions of its growing regions, and that the relationship of varaites. Almond accessions in the same place were close (Fig. 1). This result suggests the possible influence of climatic conditions and geographic distances on diversity of different pistachio accessions confirming the results of **Barazani *et al.* (2003) and Hormaza *et al.* (1994,1998)** who have shown significant differences among accessions coming from different area using a variety of morphological and molecular markers, such as RAPDs. The principal coordinate analysis (PCoA) based on genetic similarity matrices were used to visualize the genetic relationships among accessions. The first three eigenvectors together accounted for 37.6% of the total variation at the molecular level, indicating the suitability of the SRAP approaches for genetic clustering. Since the original data are not highly correlated in PCoA, the first few principal coordinates do not explain much of the original variation. Therefore, assessment of genetic relationships on the basis of the first three principal coordinates could lead to misleading interpretations and analysis of genetic relationships among accessions should be based on cluster analysis and also optimal number of principal coordinates that explain maximum amount of original data.

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الملخص العربى

تقييم التنوع الوراثى لبعض التراكيب الوراثية فى اللوز

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أجريت هذه الدراسة بمعمل الهندسة الوراثية بكلية زراعة عين شمس ومركز بحوث الصحراء ويهدف البحث إلى دراسة التنوع الوراثي لعدد من التراكيب الوراثية لبعض انواع اللوز والتي تم تجميعها من شمال وجنوب سيناء ومطروح تم اختيار بعض انواع من اللوز في شمال وجنوب سيناء وتحتوى هذه الأنواع على مواصفات مورفولوجية (حجم الثمر ومقاومتها للنيماتودا وتحملها الظروف البيئية الصعبة)، كخطوة أساسية لعمل خريطة وراثية لهذه العائلة، وذلك بدراسة الخصائص الوراثية لكل تركيب وراثي على المستوى الوراثي. للوصول لهذا الهدف أستخدم البحث أنواع غير معروفة من اللوز منطقة شمال ووسط سيناء وأنواع معروفة من اللوز عالمياً من منطقة مطروح لأثنى عشر نباتات، يتم تحديد العلامات الوراثية الجزيئية التي تميز كل تركيب وراثي للاستفادة منها في وضع أطلس للنباتات الطبية الموجودة في مصر وكانت النتائج التى توصل إليها البحث هي كما يلي: دراسة الخصائص الوراثية الجزيئية باستخدام - SRAP PCR لأثنى عشر نوع من اللوز: تم استخدام عدد ٣ بادئ وتم التوصل إلى أن العدد الكلى للحزم المتضخمة هو ٤٨ حزمة تراوح طولها ما بين ٢٦٦- ١٥٠٠ زوج من القواعد، أظهر منها ٥٧ حزمة متعددة المظهر بنسبة قدرها (٦٤,٢%). وخلاصة القول: يمكننا الاستنتاج أن الانعزال الجغرافي لأي نوع نباتي يؤدى إلى اختلاف في الخلفية الوراثية للتراكيب الوراثية لكل نوع تحت الدراسة و التي تم جمعها من مناطق شتى، وهذه النتائج من الأهمية بمكان حيث تبرز أهميتها في مجال حفظ المصادر الوراثية التي تتطلب معرفة مدى التنوع الوراثي داخل كل نوع وكذلك وضع استراتيجيات لحفظ هذه الأنواع وادخالها في برامج التربية.

الكلمات الإسترشادية: التنوع الوراثي، التراكيب الوراثية، اللوز، تقنية SRAP.

المحكمـــــون:

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