Abstract: Employing DNA markers allowed determining genetic diversity and relationships amongst five apricot genotypes. In this study, two relative gene expressions pertaining to ParARF3 gene, which could be distinguished from the genotypes that were exposed to various concentrations of marine alga treatments. As per our results, screening of seven primers with the DNA of 5 apricot genotypes was done, and six primers were generated while the primer OPN–16 gave negative results. The total quantity of fragments generated by 6 primers was 80 at an average of 13.33 fragments/primer. The highest unique percentage band depicted in U–17 touched 23%, and the total number of polymorphic bands touched 17 fragments with the average reaching 2.83 fragments/primer. The number of monomorphic lied in the range of 5 to 10, with a total of 47 monomorphic. Primer M 32 yielded the highest number of monomorphic bands reaching 10. Between Zaghenia and Zinni, a maximum genetic distance value of 0.8 was reached with less similarity value of 20%. A minimum genetic distance value of 0.44721 was noted between Kaisy and Baia while the high similarity value touched 55.3%. According to the cluster tree analysis, the genotypes were generally split into two key groups: A and B. The Zinni group, which included one apricot genotype, showed genetic similarity of 20% with the other genotypes present in B group. The B group was split into two sub-clusters B1 and B2 and the genetic similarity reached 44%. The ParARF3 relative gene expression pertaining to Zinni genotypes was second as well as convergent with that of gene expression for Zaghenia genotype results. Baia and Kaisy genotypes lied in between the lowest and highest ParARF3 value gene expression exposed to Marine Alga. These outcomes showed that RAPD markers offer an effectual alternative for the plant species genetic characterisation.

Keywords: Plant, DNA, RAPD markers, Genetic diversity, Gene expression.

Introduction

The family Rosaceae comprises few species carrying a lot of economic significance, and one of them is the genus Prunus that comprises apricot (Prunus armeniaca L.), a native to Asia (Layne et al., 1996). Prunus
Armeniaca L. is a major medicinal edible plant species generally called as “apricot”. Apricot is a very delectable and globally traded fruit (Erdogan-Orhan & Kartal, 2011). The fruit is loaded with minerals and vitamins and is frequently utilised in folk medicine for curing infertility, spasms, eye inflammation, and haemorrhages (Liu et al., 2020). The plant is enriched with mono- and polysaccharides, fatty acids, olyphenols, sterol derivatives, cyanogenic glucosides, carotenoids, and volatile constituents. It is characterised by an alluring smell. The efficacy of *P. armeniaca* has been studied for different biological activities like antimitagentic, antimicrobial, inhibitory against different enzymes, anti-inflammatory, cardioprotective, antinociceptive and antioxidant (Erdogan-Orhan & Kartal, 2011).

Algae extract as a bio fertilizer, comprises S, Mg, Ca, K, P, and N; some growth regulators; Co, Mo, Cu, Mn, Fe, and Zn; polyamines as well as vitamins for enhancement of vegetative growth, yield, nutritional status and fruit quality; and can be used in various vineyards and orchards (Abd El-Moniem & Abd-Allah, 2008; Spinelli et al., 2009). Seaweed extracts are produced because of the antimicrobial activity pertaining to seaweeds against yeast, bacteria, and moulds; while the influence of these extracts on cell metabolism results in increased plant growth, quality and yield via the initiation of antioxidant molecule synthesis which could enhance plant growth as well as plant resistance towards stress (Zhang & Schmidt, 1999).

Phenotypic is an approach for determining rootstocks and genotypes on the basis of observations; however, this depends on ecological influences. Furthermore, it is a sluggish process because of the long juvenile span of trees such as apricot (Welsh et al., 1991). Unlike morphological properties, molecular markers are now being used for genetic diversity studies, as they are not impacted by environmental changes. In order to enhance the production quality of fruit, molecular markers need to be identified, which can also define the genetic make-up pertaining to the cultivars (Rowland & Levi, 1994; Mariniello et al., 2002). A database should be created that can act as an information source regarding control of product and nursery (Koller et al., 1993; Iannelli et al., 1998).

The molecular techniques are common methods used (Kalapchieva et al., 2020; Khazraji et al., 2021). A molecular approach is ideal for certification since morphological attributes could be influenced by ecological conditions. An extensively utilised genotyping technique is random amplified polymorphic DNA (RAPD) (Sirijan et al., 2020). RAPD technique can be defined as the amplification of discrete regions pertaining to the genome by PCR containing arbitrary sequence’s short oligonucleotide primers. As this methodology entails no previous knowledge of the genome which is being examined. It could be utilised for various species through universal primers. Because of their speed and efficacy, some simple methods pool together several benefits of AFLP and SSR (Wang et al., 2017).

The diversity of apricots has long been investigated in terms of morphological, pomological, and phenological attributes (Guerriero & Watkins, 1984). Of late, DNA-based markers have been extensively utilised for clarifying the genetic relationship between the apricot accessions (Romero et al., 2003; Barakat et al., 2012; Yilmaz & Paydas-Kargi, 2012). Lately, AFLP (Krichen et al., 2006;
Yuan et al., 2007; Volkova et al., 2020), ISSR (Alhasnawi et al., 2017; Alhasnawi, 2019; Bhadkaria et al., 2020; Xiang et al., 2020), SSR (Maghuly et al., 2005; Chio et al., 2020; Cmejlova et al., 2020) and SRAP (Uzuni et al., 2010; Xiang et al., 2020). Methodologies have often been utilized in apricots to portray diverse genotypes pertaining to varied ecogeographical groups. The RAPD methodology formulated by Williams et al. (1990) has been regularly utilized in apricots for evaluating genetic variability and associations between cultivars (Kumar et al., 2001; Mariniello et al., 2002).

This study intended to ascertain: genetic relationships and genetic diversity of Prunus armeniaca L. by utilising the PCR-based RAPD method; secondly, for genotypes, ParARF3 gene expressions were exposed under two concentrations of marine algae extracts (5 and 10 ml.L\(^{-1}\)) amongst apricot genotypes (Zinni, Baia, Labeeb-1, Zaghenia and Kaisy) that were grown in Iraq.

Materials & Methods

Collection of plant material

Leaves from five Apricot trees (Prunus armeniaca L.) genotypes (Baia, Zaghenia, Zinni, Labeeb-1 and Kaisy) were collected from Iraq’s nursery of agriculture directorate in Najaf.

DNA extraction from fresh leaves of apricots

Three hundred grams of fresh apricot leaves were crushed and ground to fine powder using liquid nitrogen, then DNA was extracted according to the instructions of Genomic DNA mini Kit (Geneaid–Taiwan). Nanodrop spectrophotometer was using to measure the purity and quantity of DNA which then stored at –20 °C until further applications.

PCR Amplification

RAPD- PCR technique was used to amplify the DNA by using six primers (OPE A-10, OPE G-14, U-17, OPE K-02, OPA-18 and M 32) as shown in table (1). The PCR condition include an initial denaturation at 94°C for 1.5 minute, then 45 cycle of denaturation at 94°C for 1 min., Annealing at 36°C for 1 min., extension at 72°C for 2 min. Then one cycle of final extension at 72°C for 7 min.

Table (1): Six primers (OPE A-10, OPE G-14, U-17, OPE K-02, OPA-18 and M 32) were used in the current study.

| Primer (RAPD) sequences 5’ to 3’ | Molecular Primers |
|---------------------------------|-------------------|
| GGATGAGACC                     | OPE G-14          |
| GTGATCGCAC                     | OPE A-10          |
| GTCTCCGCAA                     | OPE K-02          |
| CTGCCAGCAT                     | U-17              |
| CTGCCAGCAT                     | M 32              |

DNA Electrophoresis

Electrophoresis (100 V) was carried out for 30 minutes to separate amplified products in agarose gels (1.5%) and then stained by ethidium bromide. A photographic record was acquired under UV-illumination.

Data analysis

Only unblemished and repeatable application products were recorded as 0 for absent bands and 1 for the ones that were present. The precise bands suitable for ascertaining genotype and species were first named with a primer number, and then the rough size of the amplified fragment in base pairs. Analysis of the amplified products was done through
pairwise comparison of the genotypes on the basis of the proportion of common fragments, and a similarity matrix was computed (Nei & Li, 1979). The 0 or 1 data matrix was generated and utilised for computing the genetic distance and similarity by utilising ‘Simqual’, an NTSYS-PC subprogram (numerical taxonomy-and multivariate analysis system program) (Rohlf, 2000). With regards to the genetic distance matrix, construction of a dendrogram was done by making use of an unweighted pair group method with arithmetic mean (UPGMA) cluster analysis that employed Molecular Evolutionary Genetics Analysis (MEGA) version 2.0 (Erdogan-Orhan & Kartal, 2011).

**ParARF3 gene expression for five apricot genotypes under marine algae extract treatments**

Two concentrations of marine algae extract (5 and 10 ml.L⁻¹) were used for apricot genotypes, and the results were ParARF3 gene expression amongst apricot in genotypes were kept in Marine Alga (*Enteromorpha intestinalis* + *Cladophoropsis gerloffii*) Extracts treatments 50 and 100 ml.L⁻¹ for the five apricot genotypes (Kaisy, Labeeb-1, Zinni, Baia, and Zagheni).

**Gene expression measurement**

cDNA synthesis and total RNA extraction: Total RNA was isolated from samples of various treatment leaves based on uses (Total RNA Isolation kit, Bioneer, South Korea). The RNA quality was confirmed based on the intact ribosomal bands seen, followed by agarose gel electrophoresis. The DNase I Mix/ Bioneer Korea (DNase I, MnCl₂, yellow core buffer) was used to remove DNA from RNA samples. The cDNA Syntheses kit, Bioneer, South Korea) with Oligo (dT) 15 primer was employed to synthesise first strand cDNA from 16µl of total RNA, followed by the manufacturer's instructions, and gel electrophoresis was employed for quantification.

**Quantitative Real Time PCR (qRTPCR):**

qRT-PCR employing a Mini Option's System real-time PCR as well as GO Taq Master Mix-SYBR Green kit QPCR/ Bioneer \ South Korea were used for gene expression analyses. To produce a product of 1113 bp, primers were designed for gene specific amplification pertaining to RNA apricot genotypes, and these primers are available in Gen Bank (www.ncbi.nlm.gov/Gen Bank/EMBL/DDBJ) - the primers sequences ParARF3 gene (Auxin response factor 3). There were two parts to the primers for QRTPCR amplification (Forward 5'-CTGAACAGTGTGACGAGGA-3) (Reverse5-AACAAACGCGAAGAGGTT-3), T.m (59.75-59.89), GC %55. In plate, PCR reactions were conducted in duplicate. Reaction mix (22.5µl per well) included 2.5µl forward and reverse primers, 12.5µl Master Mix SYBR Green, 2.5µl of cDNA and 7.5µl DEPC-D.W. The thermal cycling scenarios comprised an initial denaturation stage of 95°C for 10min, followed by 40 cycles of 95°C for 30s, 60°C for 1min, and 72°C for 30s. The melting curve analysis was employed to monitor the specificity of the PCR amplification and in the final step. To normalise, housekeeping *Pa26sRIP* gene (*Pa26sRIP* Genebank accession no. AFOO3997) was employed as endogenous reference (Forward5-AACGCAGGTTGTCTTAAGATGAG-3) (Reverse 5-GCTGCCACAA GCCAGTTA TCC-3), T.m(60.04-59.82), GC %55. The real-time PCR data was analysed using GeneX programs.
Results & Discussion

Fig. (1) demonstrated the results pertaining to isolate total DNA from the leaves of the studied apricot genotypes, which were filtered and then transferred to agar gel (1.5%), at voltage 100V for 30 min, in order to isolate the DNA from this apricot.

Fig. (1): Represents the isolated total DNA quality extracted of the apricot genotypes [1; Kaisy, 2; Labeeb-1, 3; Zinni; 4; Baia, and 5; Zagheni] on agarose gel (1.5%) and voltage 100 V for 30 minutes

Polymorphisms and monomorphisms detected by polymorphism and this attribute has been considered in the current work (Fig. 2).

One of the major and significant attributes of the RAPD method is identifying high degrees

Fig. (2): RAPD profiles of the five apricot genotypes amplified with Molecular Markers, (M; molecular weight marker), apricot genotypes: (1; Kaisy, 2; Labeeb-1, 3; Zinni; 4; Baia, and 5; Zagheni) on agarose gel (1.5%) and voltage (100V) for 30 minute.
Table (2): Number of amplicons, polymorphic, monomorphic amplicons, and percentage of monomorphism, polymorphism as revealed by Molecular markers among the five apricot genotypes.

| Molecular Primers | Primer (RAPD) sequences 5’ to 3’ | No. of total amplified fragments | No. of Unique Fragments Bands | Unique Fragments Bands Percentage (%) | No. of Polymorphic Fragments Bands | Polymorphism Fragments Percentage (%) | No. of Monomorphic Fragments Bands | Monomorphic Fragments Percentage (%) |
|-------------------|----------------------------------|---------------------------------|-------------------------------|---------------------------------------|-----------------------------------|---------------------------------------|-----------------------------------|--------------------------------------|
| OPE G-14          | GGATGAGACC                       | 9                               | 1                             | 11.11                                 | 3                                 | 33.33                                 | 5                                 | 55.55                                |
| OPE A-10          | GTGATCGCAC                       | 13                              | 1                             | 7.69                                  | 3                                 | 23.07                                 | 9                                 | 69.23                                |
| OPE K-02          | GTCTCCGCAA                       | 14                              | 3                             | 21.42                                 | 3                                 | 21.42                                 | 8                                 | 57.14                                |
| U-17              | CTGCCAGCAT                       | 13                              | 3                             | 23.07                                 | 3                                 | 23.07                                 | 7                                 | 53.04                                |
| M 32              | CTGCCAGCAT                       | 15                              | 3                             | 20                                    | 2                                 | 13.33                                 | 10                                | 66.66                                |
| OPA-18            | AGGTGACC                         | 16                              | 3                             | 18.75                                 | 3                                 | 18.75                                 | 9                                 | 56.25                                |
| Total             |                                  | 80                              | 14                            | 17                                    | 2.83                              | 22.16                                 | 47                                | 59.64                                |
| Average           |                                  | 13.33                           | 2.33                          | 17                                    | 2.83                              | 22.16                                 | 7.83                             | 59.64                                |
Investigation of seven primers was done with the DNA of the five apricot genotypes. Of the seven tested primers, six produced easily storable and reproducible RAPD profiles with several amplified DNA-fragments ranging from 9 to 16 (table 2). Primer OPN-16 returned negative results. The overall quantity of fragments generated by the six primers was 80 at an average of 13.33 fragments/primer. As demonstrated in table (2), primer OPA-

**Apricot genotypes fragment numbers from RAPD markers**

On comparing the apricot genotypes, RAPD-marker data demonstrated that high fragment number was seen in Zaghenia genotype, reaching to 68 fragments band, while Baia genotype showed lesser fragment number reaching to 60 fragments band (Table 3). 18 amplified the maximum number of amplicons to reach to 16, while primer OPE G-14 allowed amplification of minimum number of fragments reaching to 9. The number of unique fragments ranged from 1 to 3 at an average of 2.33 fragments/ primer. The highest unique percentage band depicted in U-17 touched 23%. The overall quantity of polymorphic bands touched 17 at an average of 2.83 fragments/ primer. The number of monomorphic lied in the range of 5 to 10 and was a total of monomorphic 47 with an average of 7.83 fragments/ primer. The highest number of monomorphic bands was 10, acquired through primer M32.

| Genotype    | No. of total fragment |
|-------------|------------------------|
| Zaghenia    | 68                     |
| Baia        | 60                     |
| Zinni       | 65                     |
| Labeeb      | 62                     |
| Kaisy       | 62                     |

**Genetic distance among apricot genotypes**

Data pertaining to RAPD markers included 5 apricot genotypes containing reproducible primers that were employed for genetic distance as well as similarity value coefficient. Between Zaghenia and Zinni, a maximum genetic distance value was seen reaching 0.8 along with less similarity value reaching 20%. While between Kaisy and Baia, a minimum genetic distance value was seen reaching 0.44721 along with high similarity value reaching 55.3% (Table 4).
Table (4): Genetic distance among apricot genotypes.

| Genotypes    | Kaisy | Labeeb-1 | Zinni   | Baia    | Zaghenia |
|--------------|-------|----------|---------|---------|----------|
| Zaghenia     | 0.52915 | 0.6      | 0.8     | 0.56569 | 0        |
| Baia         | 0.44721 | 0.52915 | 0.74833 | 0       | 0.56569  |
| Zinni        | 0.72111 | 0.7746   | 0       | 0.74833 | 0.8      |
| Labeeb-1     | 0.4899  | 0        | 0.7746  | 0.52915 | 0.6      |
| Kaisy        | 0      | 0.4899   | 0.72111 | 0.44721 | 0.52915  |

Genetic relationships as revealed by RAPD markers and using dendrograms

For ascertaining the genetic relationships between five apricot genotypes, the scoring data were utilised to calculate the similarity matrices. For the cluster analysis, these genetic similarity matrices were employed to construct a dendrogram used for cluster analysis and UPGMA analysis. According to the cluster tree analysis (Fig. 3), the genotypes were generally split into two core groups A and B. Zinni is also a group that included one apricot genotype, whose genetic similarity reached 20% when compared with the other genotypes in B group. The [B group] was split into two sub-clusters B1 and B2 with genetic similarity touching 44%. The first sub-cluster [B1] comprised just Labeeb-1 genotype. The second sub-cluster [B2] was split into two sets [B2A and B2B]. The first set [B2A] comprised Kaisi and Baia genotypes with high genetic similarity touching 52%, and the second set [B2B] comprised only Zaghinia genotype.

As per fig. (4), success pertaining to RNA extraction from apricot genotypes leaves, which were introduced to Marine Alga Extracts treatments, by employing SV Total RNA Isolation kit/ Bioneer-South Korea, was seen to be highly efficient and yielded RNA concentration in the range of 72 to 79 (ng. μl⁻¹) with purity reaching 1.8 to 2 (OD260/280). The synthesised cDNA for RNA of apricot genotypes exposed to Marine Alga Extracts treatments was a success due to the use of cDNA syntheses kit/ Bioneer - South Korea that was specific as well as highly efficient (Fig. 5).
Fig. (3): Dendrogram for the five apricot genotypes (1; Kaisy, 2; Labeeb-1, 3; Zinni; 4; Baia, and 5; Zagheni) constructed from RAPDs-data using UPGMA (Unweighted Pair-group Arithmetic Average) and similarity matrices computed according to treatments.

Fig. (4): Represents extraction total RNA of the apricot genotypes (1; Kaisy, 2; Labeeb-1, 3; Zinni; 4; Baia, and 5; Zagheni) under 50 and 100 ml.L-1 of marine alga extracts treatments on agarose gel (1.5%) and voltage (100V) for 20 min.

Fig. (5): Synthesis cDNA for RNA of the apricot genotypes (1; Kaisy, 2; Labeeb-1, 3; Zinni; 4; Baia, and 5; Zagheni) under 50 and 100 ml.L-1 of marine alga extracts treatments on agarose gel (1.5%) and voltage (100V) for 20 min.
The ParARF3 gene that is responsible for Auxin response factor 3 plays a key role in the synthesis of auxin. The expression ParARF3 gene patterns in genotypes were kept in marine Alga Extracts treatments 50 and 100 ml.L\(^{-1}\) and were seen to be amongst apricot genotypes. For both marine alga extracts 50 and 100 ml.L\(^{-1}\), the high relative gene expression was seen to appear in Zaghenia genotype, which reached 38.55 and 48.54-fold, respectively. The ParARF3 relative gene expression pertaining to Zinni genotypes was second as well as convergent with that of gene expression for Zaghenia genotype results, which were seen to reach 36.10 and 44.13-fold, respectively. In ParARF3 relative gene expression, Labeeb-1 genotype was found to be the lowest, which was exposed to marine Alga Extracts treatments 50 and 100 ml.L\(^{-1}\) and reached 18.01 and 30.1-fold, respectively. The results from Baia and Kaisy genotypes lied in between the lowest and highest ParARF3 value gene expression exposed to marine alga Extracts treatments 50 and 100 ml.L\(^{-1}\) that reached 22.12, 26.13 and 31.38, 36.39-fold, respectively (Fig. 6).

![Fig. (6): The expression ParARF3 gene patterns in genotypes were kept in marine alga extracts treatments 50 and 100 ml.L\(^{-1}\) for the five apricot genotypes (1; Kaisy, 2; Labeeb-1, 3; Zinni; 4; Baia, and 5; Zagheni).](image)

**Discussion**

The RAPD method has been extensively utilised in research on wild plants (Brakea *et al.*, 2014; Rao *et al.*, 2020; Sirijan *et al.*, 2020). According to the observations, a high genetic distance was noted between Zaghenia and Zinni genotypes and a low genetic distance between Kaisy and Baia, with high similarity. This genetic data could be utilised for instituting family trees and the genetic distance to common descendants (Morales *et al.*, 2011).

This study offers a constructive tool for the genetic characterisation of apricots Baia and Kaisy, typical yields stemming from centuries of selection. The molecular markers signify a valuable tool to certify genuineness and traceability in the agri-food chain for
ascertaining production of every variety and for regulating the compliance of plant material during the different nursery phases.

RAPD markers have been already successfully employed for differentiation at a molecular level for apricot genotypes (Shanguan et al., 2012). The RAPD profile only discloses the screening of a very tiny portion of the entire plant genome. Therefore, corroboration of the generated RAPD profile is essential and this could be achieved by utilising various molecular marker systems (Khor et al., 2020). These outcomes might signify the ability of the studied molecular method to distinguish among the apricot genotypes, quite beneficial in choosing the parental genotypes utilised in hybridisation breeding programs. Furthermore, the RAPD markers, by effectively distinguishing between apricot genotypes, could be utilised for diversity and characterisation study of apricot’s genetic resources.

By identifying various key auxin biosynthetic genes, auxin biosynthesis can be monitored by analysing changes in gene expression in response to different signals or in various mutant backgrounds (Zhao, 2010). The adaptations of gene expression driven by tissue or cell-specific promoters using gene editing technology or classic genetic methods and the key genes involved in auxin biosynthesis might be directly regulated by transcription factors (Cao et al., 2020).

**Conclusion**

The _ParARF3_ gene that is responsible for Auxin response Factor 3 pattern was found to be the same for RAPD marker and, amongst genotypes, genetic similarity was highest between Zaghenia and Zinni apricot genotypes. As per the results, _ParARF3_ gene is responsible for Auxin response factor 3, which showed lowest genetics similarity amongst Leebeb-1 genotype as well as others too. The results were the same even for RAPD marker. Thus, it could be that the Zaghenia and Zinni genotypes grew up together exposed to semi-conditions, while the other genotypes grew up exposed to different conditions as well as adaptation pertaining to that conditions. Comprehension of genetic diversity in apricots is vital for planning and usage of breeding programs, germplasm collection and conducting of molecular research.

**Conflict of interest**

Authors declare that there is no conflict of interests.

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تحديد التنوع الوراثي باستخدام الواسم الجزيئي RAPD والتعبيرات الجيني للجين ParARF3 في بعض أصناف المشمش المزروعة في العراق

المستخلص: تم في هذه التجربة استخدام تقنية الواسعات الجزيئية الـ RAPD لتحديد العلاقة بين التعبير الجيني لجين ParARF3 في بعض أصناف المشمش (Zaghenia، Baia، Zinni، Kaisy، Labeeb) التي عُرفت بتشكيل مختلفة من مستخلص الطحالب البحرية. تم استعما...