BIODIVERSITY ASSESSMENT FOR SOME ALMOND GENOTYPES CULTIVATED IN LIBYA USING SRAP AND ISSR

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Increasing the domestic consumption level is a very important issue as far as the future of hazelnut is considered (Sagra and Ozalp, 1988; Yucel, 1990). Velasco *et al*. (2016) stated that the domesticated almond [*Prunus dulcis* (L.) Batsch] and peach [*P. persica* (Mill.) D. A. Webb] originated in Asia and was independently domesticated. While inter-fertile, they possess alternate mating systems and differed in a number of morphological and physiological traits.

Kodad and Socias Company (2008) determined oil and fatty acid content in the kernel oil of some almond genotypes developed in an almond breeding program. They reported that considerable variation between genotypes was found for all parameters. Oil content ranged from 48% to 67% of the total kernel dry weight. Fatty acid composition was also varied among these genotypes with significant differences. Oleic acids, ranged from 63% to 78%, and linoleic acid ranged from 12% to 27%, which were the major fatty acids.

Sorkheh *et al*. (2016) assessed the levels of variation in oil content and fatty acid composition among wild almond genotypes (*Prunus scoparia* L.). To identify genotypes with desirable traits in terms of oil quantity, quality and industrial utilization. They reported that oleic and linoleic fatty acids showed high variability among accessions, ranging from 232.4 to 359.6 g/kg oil and from 190.7 to 348.8 g/kg oil, respectively. Total unsaturated fatty acid fraction was higher than total saturated fatty acid. These results could contribute to select wild almond genotypes as genetic sources for oil production.

Worldwide, many markers have been used to identify almond genotypes such as isozymes (Viruel *et al*., 1995), AFLP (Martins *et al*., 2001), ISSR (Martins *et al*., 2003), RAPD (Gouta *et al*., 2008) and SNP (Wu *et al*., 2008).

Zhaobin *et al*. (2016) studied biodiversity among four Chinese wild almonds and two cultivars (*Amygdalus communis* L.) using SRAP marker. They

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found that a relatively high level of genetic diversity (h = 0.3363, I = 0.5075) among species analyzed as evidenced by the fact that 161 out of a total of 162 bands amplified with fourteen SRAP primer combinations revealed polymorphism among species under investigation. AMOVA analysis revealed a high level of genetic differentiation among populations (T= 0.4474) but The UPMGA cluster analysis indicated that *A. communis* and *A. mongolica* were more closely related.

Guenni *et al*. (2016) used (SRAP) markers to study the genetic diversity of Tunisian pistachio. Forty three *Pistacia vera* accessions were screened. A total of 78 markers was revealed (95.12%) with an average polymorphic information content of 0.850. The results suggested that there was strong genetic differentiation, which characterized the local resources. High gene flow among groups was explained by the exchange of plant material among regions. Analysis of molecular variance revealed significant differences within groups and showed that 73.88% of the total genetic diversity occurred within groups, whereas the remaining 26.12% occurred among groups.

Simsek *et al*. (2017) characterized molecularly some Cyclamen genetic resources collected from different regions of Turkey, using 14 SRAP primer pairs. They reported that among the generated 216 bands, showing 100% polymorphism. All data were scored and UPGMA dendrograms were constructed with similar results in SRAP marker system, i.e., different species from nine provinces of Turkey were separated from each other in the dendrograms with the same species being clustered together.

Jia *et al*. (2011) used molecular markers to assess the genetic diversity of pecan plants cultivars and to identify the pedigrees of fine. A total of 77 samples grown in China were studied, including 14 introduced cultivars, 12 domestic seedling breeding cultivars, and 49 fine pecan plants, together with *Carya cathayensis* and *Juglans nigra*. A total of 77 ISSR primers were screened; 10 ISSR primers were selected, yielding a total of 94 amplified bands (100% polymorphic) in the range of 140-1950 bp for the ISSR markers. Genetic diversity analyses indicated Chinese-grown pecan cultivars and fine plants had significant diversity at the DNA level. The dendrogram constructed with ISSR showed very weak grouping association with morphological characters. The results showed that ISSR technique was suitable for genetic diversity analyses and the identification of pecan resources.

Liu *et al*. (2015) used inter-simple sequence repeat (ISSR) markers to evaluate genetic diversity among 22 sweet kernel apricot accessions and 12 cultivars in China. They reported that the nine used ISSR primers amplified 67 allelic variants with 50 polymorphic bands (74.63%). There was a relatively distant genetic relationship between the 34 samples, where their genetic similarity coefficient was between 0.62 and 0.99. The UPGMA dendrogram constructed separated the geno-

types into three main clusters.

Wang *et al*. (2016) investigated the genetic variation in 49 individuals including 40 cultivars, nine species of wild lily (*Lilium* spp). Genetic diversity and interrelation-ships were assessed through analysis of phenotypic characteristics and ISSR molecular markers. Quantitative characters were selected to analyse phenotypic variation, with results indicated greater variability in petiole length as compared to other characters. ISSR makers demonstrated that both cultivars and wild species possess high levels of genetic diversity. Specifically, the genetic diversity of wild lily was higher than cultivars.

Naik *et al*. (2017) characterized 15 accessions of *Costus pictus*, collected from different regions of India, using 20 ISSR primers. These primers produced 177 loci, of which 77 were polymorphic with an average of 3.85 loci per primer. The similarity coefficients ranged from 0.86-0.96 for ISSR. The UPGMA dendrogram generated using these data showed low level of divergence among the accessions from South and West regions. Further, accession-specific bands were also revealed by ISSR markers which might be contributed to specific trait. The results indicated that ISSR marker tools can be used in determine the genetic relationship between the accessions. It may be concluded that data of hereditary differences appeared among the *C. pictus* accessions could be utilized for their conservation and breeding programs.

MATERIALS AND METHODS

Plant materials

Fresh fruits of eighteen different genotypes of almonds were collected from well grown almond trees at Tarhuna Research Station, Libya; namely; Bezotta (1), Amazitto (2), Grabillo (3), Avola (4), Castilla (5), Santuoro (6), Mondorilla Bianca (7), Mondorilla ladolcsi (8), Rachil (9), Fragillia (10), Montiron (11), Fragillia de bagilia (12), Isabella (13), Branchibatta (14), Ansperabilla (15), Faccinado (16), City Bianca (17) and Romana (18).

Physical parameters of the fruits

The length, diameter, fruit fresh and dry weights, number of fruits/tree and the yield/year were measured using fruits.

Chemical components

I- oil content

Oil contents in the seeds of the eighteen almond genotypes were extracted according to Roncero *et al*. (2016) using petroleum ether.

II- Oleic Acid contents

Oleic acid content was estimated in the extracted oil of the eighteen almond genotypes according to Aryee *et al*. (2009) using a mixture of ethanol and diethyl ether (1/1 v/v).

III- Protein contents

Protein was extracted and determined using Esteban *et al*. (1985) protocol

using 0.1 M concentration of sodium hydroxide.

Statistical analysis

The data of the eighteen almond genotypes were calculated, using Excel software program.

Molecular studies

DNA extraction

Genomic DNA was extracted according to Junghans and Metzallat (1990) with some modifications. Briefly, 0.5 g leaves tissue was ground in liquid nitrogen and homogenized in 300 µl of freshly prepared and autoclaved extraction buffer (1.4 M NaCl, 100 mM Tris-HCl, 20 mM EDTA, 0.2% ß-mercaptoethanol, pH 8.0). DNA pellet was precipitated in 500 µL cooled iso-propanol. DNA pellet was washed twice with 70% ethanol and dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8). DNA quality was checked on a 1.2% agarose gel. Samples were stored at 4°C, until used for SRAP and ISSR analysis.

SRAP analysis

SRAP technique was performed according to Li and Quiros (2001) using the following primers (Table 1).

ISSR analysis

The ISSR primers were synthesized by Metabion, Germany. ISSR-PCR amplification was done according to Sankar and Moore (2001). The sequence of the nine used ISSR primers are shown in Table (2).

Band scoring and data analysis

Similarity indexes and dendrogram of the eighteen almond genotypes were performed on the bases of the banding patterns of SRAP and ISSR independently and combined using SPSS computer program to study the genetic relationships among these eighteen almond genotypes.

RESULTS AND DISCUSSIONS

Assessment of physical characters of the fruit

Different fruit parameters were measured to assess the variation among the eighteen almond genotypes. The results (Table 3) indicated that the highest fruit length was recorded in Avola (41.91 cm) while, the lowest was (29.17 cm) in Rachil genotype. The highest fruit diameter was recorded in Avola (26.66 cm) while, the lowest was (19.38 cm) in Fragillia genotype.

In fruit fresh weight, the highest (16.67 g) was recorded in Faccinado while the lowest (7.69 g) in Fragillia. In fruit dry weight, the highest (7.5 g) was recorded in Faccinado and Castilla, while the lowest 3.29 g in Fragillia genotype.

The highest in number of fruits/tree (260) was recorded in Fragillia genotype, while the lowest (26) in genotype Castilla. The highest in yield/tree (2099.9 and 2099.12 g/tree) was recorded in Fragillia

de bagilia and Isabella genotypes, respectively, while the lowest 300 g/tree was recorded in Santuoro genotype.

Assessment of the biochemical characters

Some chemical components were estimated such as total oil contents, oleic acid contents and protein contents (Table 4). The results in (Table 4) and Fig. (1 a, b and c) showed that, oil contents ranged from (52.63 mg/ g), the highest in genotype montiron to (27.77 mg/g and 27.83 mg/g seeds), the lowest which was recorded in Mondorilla Ladolcsi and Mondorilla Bianca, respectively. In oleic acid contents, it ranged from (0.67 µg/g oil) in genotype mondorilla ladolcsi, the highest, to (0.22 µg/g oil) in genotype city Bianca, the lowest. For protein contents, it ranged from (26.40 µg/g seeds) in genotype Santuoro, the highest, to (17.58 µg/g seeds) in genotype Montiron, the lowest in protein contents.

These results were in partial agreement with the findings of Ahrens *et al*. (2005) who reported that almond was an important commercial and medicinal fruit, due to its protein, fat, mineral matter, fibre and vitamin E content. Moisture content, fat content, protein content and ash content of almond species such as Carmel, Te-as and Nonpareil which varied within the ranges of 3.05-4.33%, 43.37-47.50%, 20.68-23.30% and 5.35-7.45%, respectively.

Mahesh (2006) analyzed the chemical composition and moisture sorption of commercially important edible nut seeds and reported that moisture ranged from 1.47-9.51%, while proteins ranged between 7.50-21.56%, lipid (42.88-66.71%), ash (1.16-3.28%), total soluble sugars (0.55-3.96%), tannins (0.01-0.88%), and phytate (0.15-0.35%) contents varied considerably among different kinds of nuts. Regardless of seed type, lipids were mainly composed of mono- and polyunsaturated fatty acids (>75% of the total lipids). Fatty acid composition analysis indicated that oleic acid (C18:1) was the main constituent of monounsaturated lipids in all seed samples. With the exception of macadamia, linoleic acid (C18:2) was the major polyunsaturated fatty acid. In the case of walnuts, in addition to linoleic acid (59.79%).

Beyhan *et al*. (2011) determined the fatty acid composition of Picantili, Ferraduel, Drake and Nonpareil commercial almond species (*Prunus amygdalus* L.). They reported that the amounts of oleic acid, linoleic acid, palmitic acid and stearic acid, respectively, were found to be higher than the other fatty acids in all genotypes. They concluded that almond has beneficial effects on human health since it contains a high level of unsaturated fatty acids. These are oleic, linoleic, palmitic and stearic acids. The amounts of these fatty acids were found to be higher than the other fatty acids in all genotypes. These fatty acids were similar in amounts with little changes between genotypes.

Biodiversity assessment at the molecular level

The diversity and overlapping of morphological characters in the old and recent descriptions has led to considerable complications in the taxonomy of almond. In this investigation genetic diversity was examined among eighteen different genotypes of almond using two different molecular techniques, these were sequences related amplified polymorphism (SRAP) and inter simple sequences repeats (ISSR).

Sequence-related amplified polymorphism (SRAP)

The results of twenty used SRAP primer combinations are showed in (Table 5) and Fig. (2 a, b, c and d). Sequence-related amplified polymorphism (SRAP) aimed for the amplification of open reading frames (ORFs). The used primers produced a total of 98 bands. Out of these produced bands, 77 were polymorphic and 21 monomorphic. Out of the polymorphic bands there were 12 bands generated overall the 20 used primers which could be considered as cultivar specific markers, six out of them could be used as cultivar specific positive markers and six could be used as cultivar specific negative markers.

It is possible to conclude that the discrimination between these cultivars at the molecular level using SRAP technique revealed a total of 98 amplified fragments which were produced with a mean of 4.9 amplicons per assay using the same twenty primer pair combinations and the eighteen Libyan almond genotypes. These results were in agreement with the findings of Li and Quiros, (2001) that developed dominant marker technique, sequence-related amplified polymorphism (SRAP). PCR-based molecular analysis is simple, inexpensive, and effective for producing genome-wide fragments with high reproducibility and versatility. Daniel (2014) reported that SRAP markers were developed, and used to amplify coding regions of DNA with primers targeting open reading frames. SRAP markers have been used primarily for agronomic and horticultural purposes, developing quantitative trait loci in advanced hybrids and assessing genetic diversity of large germplasm collections.

Similarity indices according to SRAP banding patterns

On the bases of the banding patterns overall the eighteen almond genotypes and the 20 used SRAP primer combinations similarity indices were developed using SPSS computer program which was based on the Jaccard's coefficient (Table 6). The similarity values were further used to construct a phonetic dendrogram revealing the genetic relationships based on UPGMA (un-weighted pair group method using arithmetic averages). The results revealed that the two most closely related genotypes were Ansperabilla and Faccinado with similarity value of 0.819 followed by Isabilla and Faccinado with similarity value of 0.812. These results revealed that these three genotypes were the most similar ones among the eighteen almond genotypes

under investigation. On the other hand, the two most distantly related genotypes were Avola and Castilla, with similarity value of 0.423.

Consensus tree according to SRAP banding patterns

On the base of the banding patterns overall the eighteen almond genotypes and the 20 used SRAP primer combinations dendrogram was developed using SPSS computer program based on the Jaccard's coefficient. The results of dendrogram on the bases of SRAP banding patterns (Fig. 3).

The eighteen almond genotypes were grouped into two main clusters as shown in Fig. (3). The first main cluster was divided into two sub-clusters, the first sub-cluster included almond genotypes Romana and the second sub-cluster included Castilla, Rachil, city Bianca, Isabella, Ansperabilla and Faccinado. On the other hand the second main cluster was divided into five sub clusters. The first sub-cluster included almond genotype Branchiate. The second sub-cluster included Amazitto, Mondorilla Bianca and Fragillia. The third sub-cluster included Grabillo, Santuoro and Mondorilla Ladolcsi. The fourth sub-cluster included almond genotypes Montiron and Fragillia de bagilia. The last sub-cluster included almond genotypes Bezotta and Avola. The results showed that genotypes Avola and Romana were the two most distantly related genotypes. On the other hand, Bezotta and Mondorilla Bianca were the most closely related genotypes. The results were in partial agreement with the findings of Chen *et al*. (2012) who studied genetic diversity and genetic relationships among *Lonicera macranthoides* cultivars using SRAP. They reported that 58 SRAP primer combinations amplified 591 bands with 347 (55.46%) polymorphic bands. Genetic distance ranges was 0.1071-0.2611 using SRAP. SRAP analyses revealed a middle level of genetic diversity in *L. macranthoides* cultivars. They concluded that SRAP markers can be effectively applied to genetic analysis in plants.

Liang *et al*. (2014) studied the genetic diversity and relationships among 80 accessions of chicories and endives using SRAP markers. They stated that the polymorphic rate was 96.83%, and the average polymorphic information content was 0.323, suggesting the rich genetic diversity of chicory.

Simsek *et al*. (2017) used 14 SRAP primer pairs to characterize plants of cyclamen species collected from different parts of Turkey on the molecular level. The results showed that a total of 216 bands were generated, showing 100% polymorphism. The number of bands detected by a single primer pair ranged from 9 to 22 (average of 15.4). Data were scored and UPGMA dendrogram was constructed. They concluded that, different species from nine provinces of Turkey were separated from each other in the dendrogram with the same species being clustered together.

Inter simple sequences repeats (ISSR)

Nine ISSR primers were used to detect the variation among the eighteen almond genotypes under investigation. These primers produced a total of 111 bands. Out of which 109 were polymorphic and two monomorphic with an average of 12.33 bands/ primer. These nine ISSR primers generated a total of 19 cultivar specific markers, 14 out of them were positive markers while 5 was cultivar negative markers. The results of twenty used ISSR primer are shown in Table (6) and Fig. (4 a and b).

Similarity indexes according to ISSR banding patterns

The results revealed that, the two most closely related genotypes were Ansperabilla and Faccinado with similarity value of 0.683 followed by Romana and Faccinado with similarity value of 0.629. These results revealed that these three genotypes were the most similar ones among the eighteen almond genotypes under investigation. On the other hand, the two most distantly related genotypes were City Bianca and Castilla, with similarity value of 0.184.

Consensus tree according to ISSR banding patterns

The similarity values were further used to construct a phonetic dendrogram revealing the genetic relationships based on UPGMA (un-weighted pair group method using arithmetic averages). The results of similarity indexes on the base of ISSR banding patterns were shown in Table (7) and Fig. (5).

The results showed that the dendrogram included two main clusters. The first main cluster included genotype Fragillia only. On the other hand, the second main cluster was divided into two sub clusters. The first sub-cluster included almond genotypes Castilla and Mondorilla Ladolcsi. The second sub-cluster was divided into two classes; the first class included almond genotypes Romana, City Bianca, Isabella, Ansperabilla, Faccinado, Branchibatta and Fragillia de bagilia. The second class included Bezotta, Amazitto, Grabillo, Avola, Santuoro, Mondorilla Bianca, Rachil and Montiron.

These results were in partial agreement with the findings of Chatti *et al*. (2010) who used 48 ISSR markers to detect polymorphism and to establish genetic relationships among Tunisian fig tree cultivars. The data showed considerable genetic diversity, and the tested markers discriminated all fig genotypes under investigation.

Zhan *et al*. (2015) analyzed genetic similarities among olive cultivars in China using ISSR technique. Thirty-two samples were collected from Xichang. They reported that nine ISSR primers generated 85 reproducible bands of which 78 (91.8%) were polymorphic. Based on the data, genetic similarity between cultivars ranged from 0.57 to 0.83. Cluster analysis revealed that 32 cultivars were clustered into six groups, which supports similar morphology such as use, oil content and

fruit weight but not similar geographical origins. The data also allow the identification of unknown cultivars and cases of synonyms. Our results were also in agreement with Liu *et al*. (2015) who used ISSR markers to evaluate genetic diversity among 22 sweet kernel apricot accessions and 12 cultivars in China to provide information on how to improve the utilization of kernel apricot germplasms. The results showed that 9 ISSR primers amplified 67 allelic variants with 50 polymorphic bands (74.63%). There was a relatively distant genetic relationship between the 34 samples, where their genetic similarity coefficient was ranged between 0.62 and 0.99. The dendrogram constructed using ISSR data of the two marker systems separated the genotypes into three main clusters.

Similarity indices according to combined banding patterns of SRAP and ISSR

On the bases of the banding patterns overall the eighteen almond genotypes and the data combination SRAP and ISSR, similarity indices were developed (Table 8). The results revealed that, the two most similar genotypes i.e. the two most closely related genotypes were Ansperabilla and Faccinado with similarity value of 0.758. On the other hand, the two most distantly related genotypes were Avola and Castilla, with similarity value of 0.369.

Consensus tree according to combined data

On the bases of the banding patterns overall the eighteen almond genotypes and the data combination of the twenty SRAP primers and the nine used ISSR primers dendrogram was developed using SPSS computer program which based on the Jaccard's coefficient (Fig. 5).

The results showed that the dendrogram included two main clusters. The first main cluster included genotypes Fragillia, Rachil, Castilla, Amazitto, Mondorilla Bianca, Mondorilla Ladolcsi, Grabillo and Santuoro. On the other hand, the second main cluster was divided into two sub clusters. The first sub-cluster included almond genotypes Avola and Montiron. The second sub-cluster included almond genotypes Romana, city Bianca, Isabella, Ansperabilla, Faccinado, Branchibatta, Fragillia de bagilia and Bezotta. These results were in partial agreement with the findings of Simsek *et al*. (2017) who characterized some plant materials of the genus Cyclamen (family *Myrsinaceae*) contains about 20 species, which considered as genetic resources collected from different regions of Turkey using RAPD, ISSR and SRAP.

It is possible to conclude that the phylogenetic analysis based on PCR using genomic DNA revealed variations among the different isolates or genotypes. However, further genetic analyses could be used as complementary tools to the morphological characterisation in order to elucidate the phylogenetic relationships among different genotypes. Molecular techniques could generate data to resolve unknown relationships among different genotypes. Molecular techniques could

also estimate allele sharing (AS) between pairs of individuals resulting from the same breeding program.

SUMMARY

This work aimed to assess the biodiversity of eighteen almond genotypes grown in Libya on the bases of some agronomical, biochemical and molecular characteristics. The agronomical traits were: fruit length, fruit diameter, fruit fresh and dry weights, number of fruits/tree and total yield/tree. The biochemical traits were: total oil, linoleic acid and protein contents. In this context, it was very important to establish an accurate DNA fingerprints for cultivar characterization in order to identify the genetic diversity among these genotypes. For this purpose, 20 combinations of SRAP and 9 primers of ISSR molecular markers were applied to discriminate the 18 almond (*Prunus Amygdalus*, *dulcis* L.) genotypes. SRAP generated polymorphic and unique bands for all genotypes. SRAP generated 98 bands, out of these, 22 were common bands while 14 were unique ones used as molecular markers, eight bands out of these unique bands could be considered as positive markers and six as negative markers.

ISSR generated 111 bands, out of which, there were three common bands and 19 unique ones, fourteen bands out of them could be considered as positive markers and five as negative markers for a particular genotype.

Similarity indices generated shuffling in the arrangement in the cultivar relations according to the two used molecular techniques (SRAP and ISSR), as in SRAP, the two most closely related cultivars were Ansperabilla and Faccinado with similarity value of 0.819, while it was 0.683 between genotypes Ansperabilla and Faccinado as revealed from ISSR data. On the other hand, the two most distantly related cultivars, according to SRAP data, were Avola and Castilla, with similarity value of 0.423, while it was 0.184 between City Bianca and Castilla genotypes according to ISSR data. Dendrogram was conducted for the eighteen genotypes under investigation using the two used molecular techniques. The results revealed different distances in their genetic relationships among the eighteen almond genotypes under investigation.

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Table (1): The used SRAP primers and their sequences.

Primer code	Forward primers 5→3	Primer code	Reverse primers 5→3
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGACA	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGACG	Em7	GACTGCGTACGAATTCAA
Me8	TGAGTCCAAACCGGACT	Em8	GACTGCGTACGAATTCTG
Me9	TGAGTCCAAACCGGAGG	Em9	GACTGCGTACGAATTCAG
Me10	TGAGTCCAAACCGGAAA	Em10	GACTGCGTACGAATTCAT
Me11	TGAGTCCAAACCGGAAC	Em11	GACTGCGTACGAATTCTA
Me12	TGAGTCCAAACCGGAGA	Em12	GACTGCGTACGAATTCTC
DN06	TGAGTCCAAACCGGTAA	Em13	GACTGCGTACGAATTCTT
DN07	TGAGTCCAAACCGGTCC	Em14	GACTGCGTACGAATTGAT
DN08	TGAGTCCAAACCGGTGC	Em15	GACTGCGTACGAATTGTC
DN09	TGAGTCCAAACCGGTCA	Em16	GACTGCGTACGAATTCGA
DN10	TGAGTCCAAACCGGGCT	Em 17	GACTGCGTACGAATTAGC
DN11	TGAGTCCAAACCGGTAG	Em 18	GACTGCGTACGAATTGAG
DN12	TGAGTCCAAACCGGTGT	Em 19	GACTGCGTACGAATTGCC

Table (2): The used ISSR primers and their sequences.

No.	Primer code	Primer sequence	No	Primer code	Primer sequence
1	807	$(AG)^8$ T	5	HB 8	$(GA)^6$ GG
2	17898A	$(CA)^6$ AC	6	HB10	$(GA)^6$ CC
3	HB01	$(CAA)^5$	7	HB11	$(GT)^6$ CC
4	HB04	$(GACA)^4$	8	HB12	$(CAC)^3$ GC
9	HB15	$(GTG)^3$ GC			

Table (3): Some fruit phenotypic traits for the eighteen almond genotypes under investigation.

Plant name	Fruit length	Fruit diameter	Fruit fresh weight	Fruit dry weight	Number of fruits/ tree	Total fruit yield/ tree
Bezotta	40.67	26.04	12.05	5.50	166	2000.30
Amazitto	33.67	25.91	10.08	3.57	119	1199.52
Grabillo	37.12	24.72	12.66	5.32	158	2000.28
Avola	41.91	26.66	13.07	5.85	153	1999.71
Castilla	39.57	23.60	15.38	7.50	26	399.88
Santuoro	37.57	22.16	10.00	3.83	30	300.00
Mondorilla Bianca	30.81	23.71	14.55	5.27	55	800.25
Mondorilla ladolcsi	34.17	21.23	12.14	4.75	140	1699.60
Rachil	29.17	19.43	10.50	3.70	181	1900.50
Fragillia	30.54	19.38	7.69	3.29	260	1999.40
Montiron	35.13	24.30	10.61	5.53	132	1400.52
Fragillia de bagilia	30.66	19.77	9.13	3.48	230	2099.90
Isabella	37.41	26.65	13.81	6.58	152	2099.12
Branchibatta	31.76	20.40	12.86	4.21	140	1800.40
Ansperabilla	30.44	24.01	9.62	4.01	156	1500.72
Faccinado	38.27	23.61	16.67	7.50	120	2000.40
City Bianca	32.86	21.14	10.42	3.88	192	2000.64
Romana	37.93	24.99	15.63	6.56	128	1995.66

Table (4): Some chemical contents in almond seeds of the 18 different genotypes.

Cultivar name	Oil contents Mg/g ground seeds	Oleic acid µg/g seed oil	Protein contents Mg/g ground seeds
Bezotta	41.40	0.50	22.66
Amazitto	42.78	0.50	23.38
Grabillo	33.42	0.42	21.11
Avola	42.51	0.49	21.45
Castilla	49.16	0.50	24.12
Santuoro	36.68	0.52	26.40
Mondorilla Bianca	27.83	0.52	20.05
Mondorilla ladolcsi	27.77	0.67	23.30
Rachil	35.03	0.44	23.67
Fragillia	44.41	0.32	22.95
Montiron	52.63	0.28	17.58
Fragillia de bagilia	34.30	0.34	18.15
Isabella	41.99	0.37	21.00
Branchibatta	44.25	0.32	25.78
Ansperabilla	49.50	0.25	22.05
Faccinddo	36.57	0.23	22.70
City Bianca	44.55	0.22	19.14
Romana	45.80	0.35	22.31

Table (5): Polymorphism in banding patterns of the 18 almond using SRAP.

Primer combination	Number of bands	Number of common bands	% of polymorphism	No. of positive markers	No. of negative markers
DN7FX EM17R	2	1	50.00	-	-
DN9FX EM19R	5	1	80.00	-	-
ME4FXEM6R	5	1	80.00	-	1
ME6FXEM7R	8	2	80.00	-	2
ME5FXEM7R	10	2	80.00	1	1
ME1FXEM8R	5	1	80.00	-	-
ME5FXEM8R	5	1	80.00	1	-
ME2FXEM8R	6	2	67.00	1	-
ME5FXEM11R	9	2	67.00	1	-
ME6FXEM11R	4	-	100.00	-	-
ME11FXEM11R	3	1	67.00	-	-
ME6FXEM12R	7	1	85.70	-	-
ME8FXEM16R	3	2	33.30	-	-
ME5FXEM20R	4	1	75.00	-	1
ME9FXEM20R	3	1	66.70	-	-
ME7FXEM1R	4	1	75.00	1	-
ME6FXEM6R	4	1	75.00	-	-
ME7FXEM7R	5	1	80.00	-	-
ME10FXEM10R	2	1	50.00	-	-
ME6FXEM18R	4	-	100.00	1	1

Table (6): Similarity indexes among 18 almond genotypes using SRAP technique.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	4.444																
3	4.444	4.440															
4	4.444	4.444	4.400														
5	4.441	4.444	4.444	4.044													
6	4.441	4.444	4.444	4.444	0.634												
7	4.414	4.444	4.444	4.444	4.414	4.444											
8	4.404	4.444	4.444	4.400	4.441	4.444	0.754										
9	4.041	4.444	4.441	4.004	4.444	4.410	4.444	4.444									
10	4.444	4.444	4.441	4.444	4.404	4.441	4.444	4.441	0.628								
11	4.444	4.444	4.440	4.414	4.444	4.444	4.444	4.444	4.441	4.440							
12	4.444	4.414	4.444	4.444	4.441	4.444	4.444	4.440	4.444	4.444	0.741						
13	4.444	4.444	4.444	4.044	4.444	4.444	4.444	4.444	4.444	4.440	4.404	4.444					
14	4.444	4.404	4.444	4.440	4.444	4.440	4.444	4.444	4.444	4.404	4.444	4.400	0.569				
15	4.444	4.444	4.444	4.444	4.404	4.440	4.444	4.444	4.414	4.444	4.440	4.400	4.444	4.444			
16	4.444	4.400	4.444	4.044	4.440	4.444	4.444	4.440	4.444	4.441	4.444	4.440	4.411	4.414	0.819		
17	4.444	4.444	4.441	4.044	4.444	4.444	4.444	4.444	4.444	4.444	4.444	4.444	4.414	4.444	4.441	4.444	
18	4.444	4444	4.441	4.444	4.444	4.444	4.444	4.444	4.411	4.414	4.441	4.444	4.414	4.441	4.444	4.414	0.649

Table (7): Polymorphism revealed from the banding patterns of the 18 almond genotypes using ISSR.

Primer combination	Number of bands	Number of common bands	% of polymorphism	No. of positive markers	No. of negative markers
807	14	-	100.00	3	1
17898A	16	-	100.00	4	-
HB1	19	-	100.00	4	-
HB4	10	-	100.00	2	1
HB8	15	-	100.00	1	-
HB10	4	1	75.00	-	-
HB11	8	-	100.00	-	-
HB12	11	-	100.00	-	1
HB15	14	1	92.86	-	2

Table (8): Similarity indexes among 18 almond genotypes using ISSR technique.

	1	4	4	0	4	4	4	4	4	14	11	14	14	10	14	14	14
4	4.404																
4	4.044	4.444															
0	4.444	4.444	4.044														
4	4.444	4.444	4.414	4.444													
4	4.041	4.414	4.444	4.044	4.004												
4	4.440	4.444	4.044	4.040	4.440	4.041											
4	4.444	4.400	4.444	4.444	4.444	4.444	4.440										
4	4.444	4.044	4.044	4.041	4.44	4.044	4.004	4.444									
14	4.414	4.144	4.444	4.444	4.440	4.444	4.444	4.414	4.404								
11	4.444	4.444	4.404	4.440	4.444	4.014	4.004	4.444	4.044	4.444							
14	4.440	4.440	4.444	4.444	4.444	4.414	4.444	4.404	4.444	4.444	4.044						
14	4.014	4.444	4.440	4.044	4.444	4.440	4.444	4.400	4.444	4.444	4.044	4.044					
10	4.444	4.440	4.440	4.441	4.444	4.414	4.404	4.444	4.444	4.444	4.044	4.041	4.044				
14	4.044	4.444	4.044	4.044	4.444	4.444	4.004	4.440	4.044	4.414	4.044	4.444	4.400	4.044			
14	4.044	4.444	4.044	4.444	4.144	4.440	4.041	4.441	4.044	4.444	4.001	4.044	4.414	4.044	4.444		
14	4.040	4.444	4.444	4.040	4.140	4.440	4.444	4.404	4.044	4.444	4.044	4.404	4.444	4.044	4.444	4.444	
14	4.444	4.444	4.014	4.044	4.414	4.441	4.004	4.410	4.044	4.444	4.044	4.444	4.404	4.044	4.404	4.444	4.444

Table (9): Similarity indexes among 18 almond genotypes according to the combined data.

	1	4	4	0	4	4	4	4	4	14	11	14	14	10	14	14	14
4	4.441																
4	4.441	4.444															
0	4.444	4.444	4.044														
4	4.004	4.044	4.444	4.444													
4	4.404	4.401	4.441	4.444	4.444												
4	4.440	4.444	4.441	004.4	4.414	4.444											
4	4.441	4.444	4.414	4.044	4.441	004.4	4.444										
4	4.040	4.044	4.444	4.044	4.444	4.444	4.414	4.044									
14	4.044	4.414	4.444	4.004	4.044	4.444	004.4	4.404	4.004								
11	4.044	4.441	4.041	4.444	4.044	4.404	4.444	4.044	4.444	4.414							
14	4.440	4.044	4.044	4.044	4.041	4.044	4.044	4.004	4.040	4.004	4.444						
14	4.044	4.040	4.440	4.044	4.014	4.044	4.410	4.044	4.444	4.400	4.440	4.444					
10	4.044	4.444	4.044	4.044	4.044	4.044	4.440	4.040	4.040	4.414	4.444	4.444	4.414				
14	4.440	4.044	4.444	4.044	4.044	4.444	4.444	4.444	4.444	4.404	4.444	4.444	4.444	4.444			
14	4.044	4.044	4.444	4.444	4.001	4.410	4.444	4.440	4.444	4.444	4.411	4.444	4.414	4.400	4.444		
14	4.401	4.004	4.444	04.44	4.004	4.044	4.444	4.400	4.440	4.044	4.044	4.444	4.410	4.441	4.444	4.414	
14	4.041	4.004	4.040	4.044	4.041	4.044	4.444	4.444	4.441	4.004	4.444	4.404	4.444	4.410	4.441	4.444	4.444

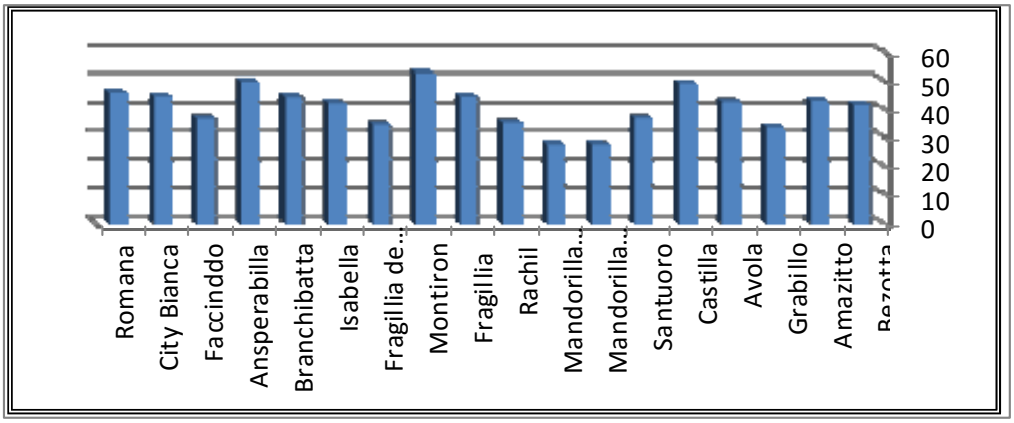


Fig. (1 a): Oil contents in almond seeds of the 18 genotypes under investigation.

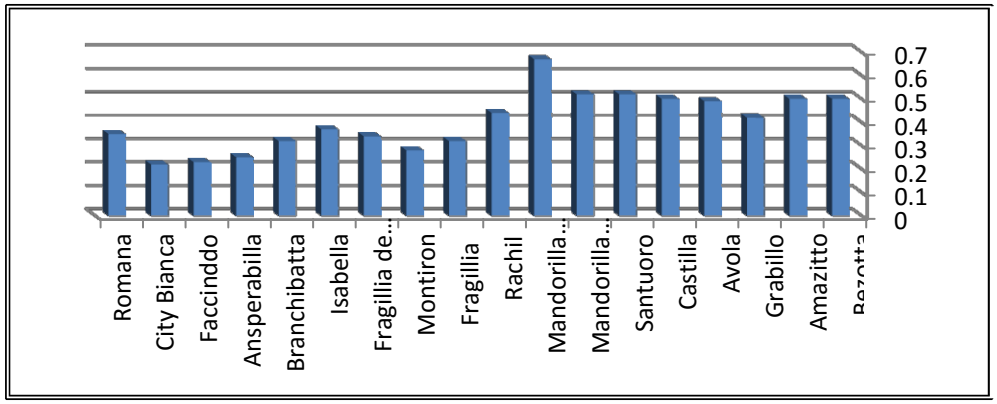


Fig. (1 b): Oleic acid contents in almond seeds of the 18 genotypes under investigation.

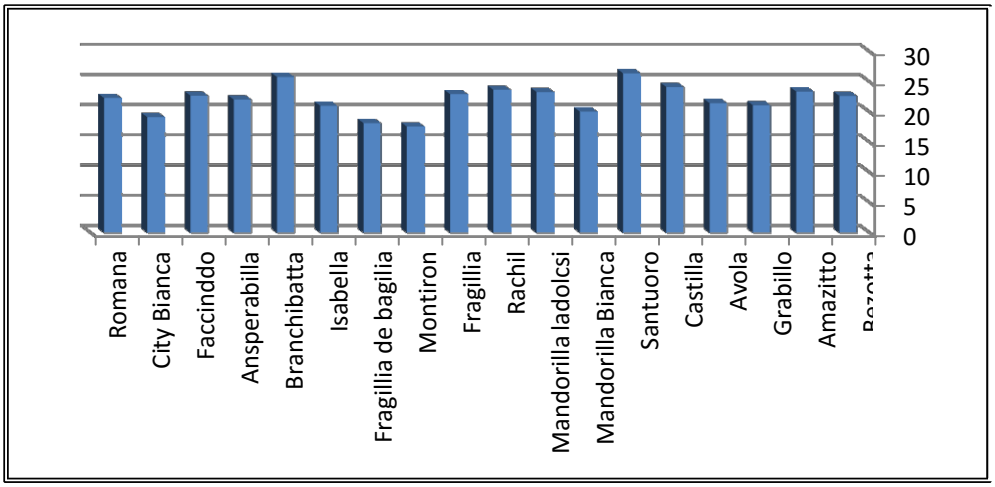
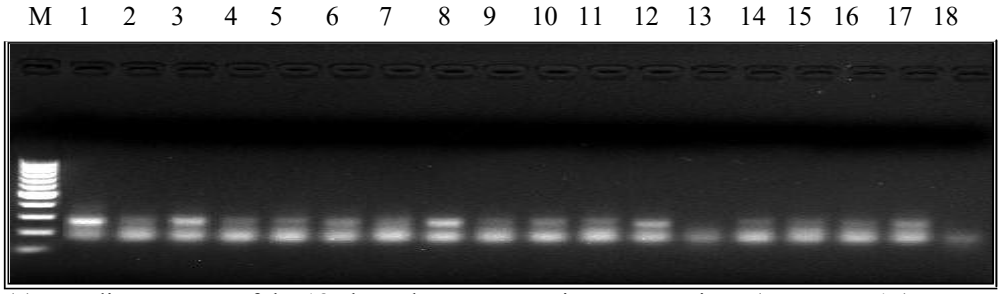
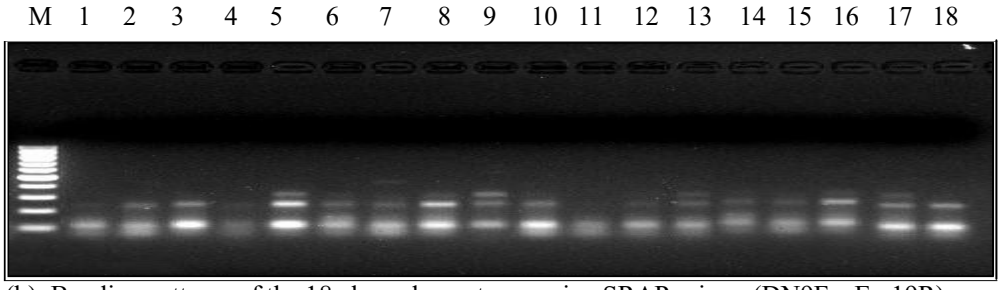


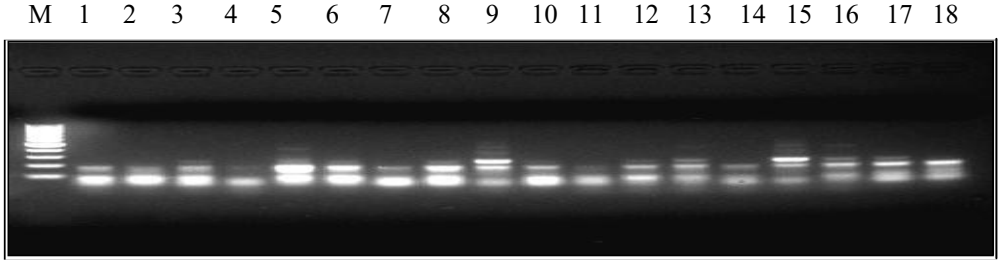
Fig. (1 c): Protein contents in almond seeds of the 18 genotypes under investigation.



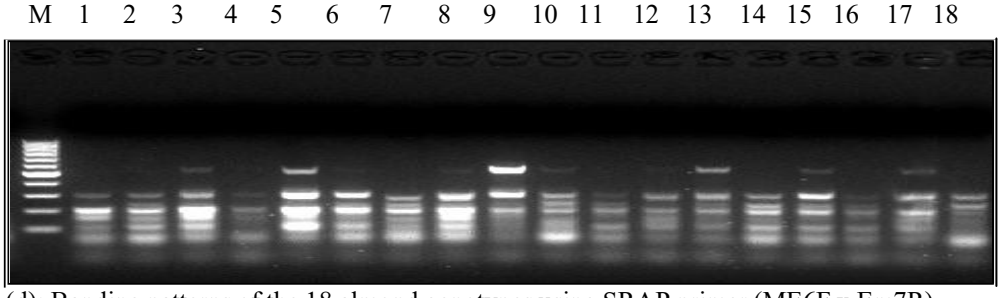
(a): Banding patterns of the 18 almond genotypes using SRAP primer (DN7 x Em17).



(b): Banding patterns of the 18 almond genotypes using SRAP primer (DN9F x Em19R).



(c): Banding patterns of the 18 almond genotypes using SRAP primer (ME4F x Em6R).



(d): Banding patterns of the 18 almond genotypes using SRAP primer (ME6F x Em7R).

Fig. (2): Banding patterns of Some SRAP combinations and the 18 almond genotypes.

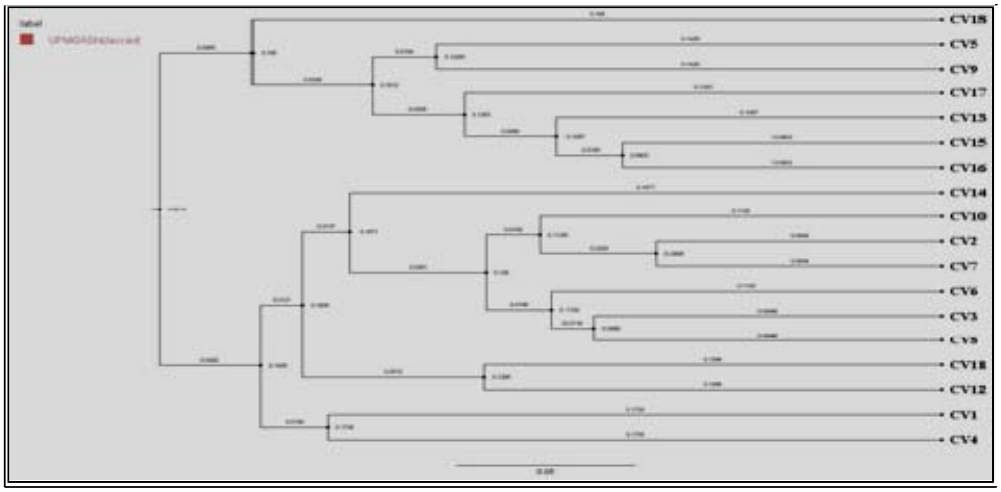
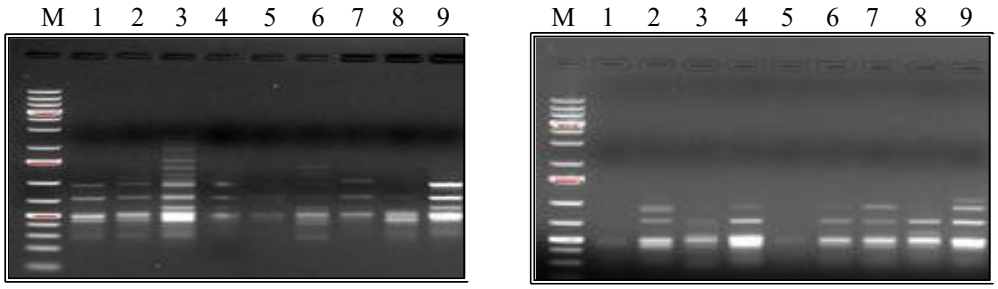
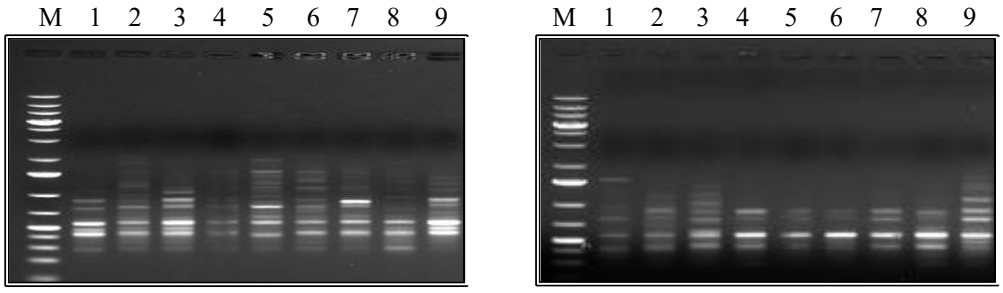


Fig. (3): Consensus tree with UPGMA method among 18 almond genotypes collected from Tarhuna Research Station, Libya using twenty SRAP primers.



(a): ISSR primer (HB10)



(b): ISSR primer (HB10)

Fig. (4): Banding patterns of some ISSR and the 18 almond genotypes.

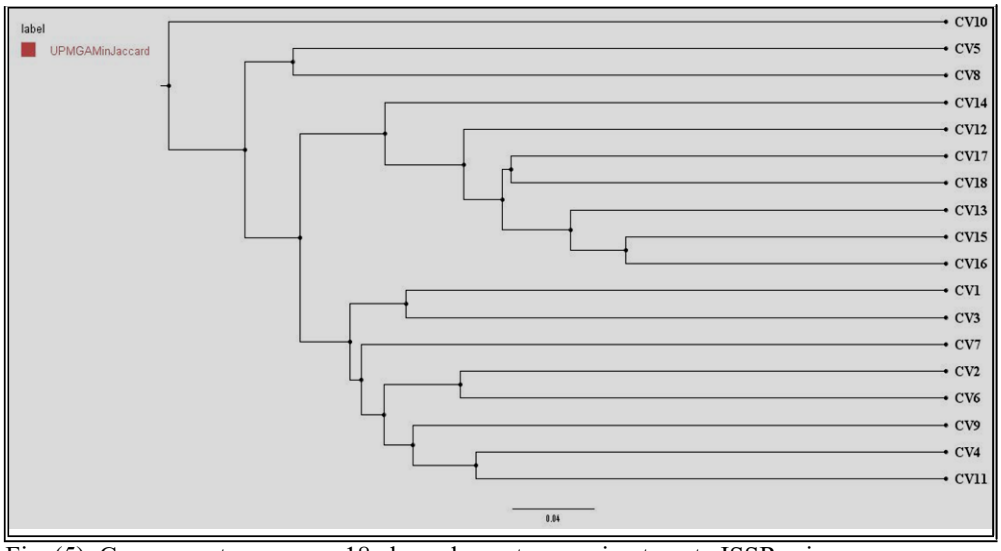


Fig. (5): Consensus tree among 18 almond genotypes using twenty ISSR primers.

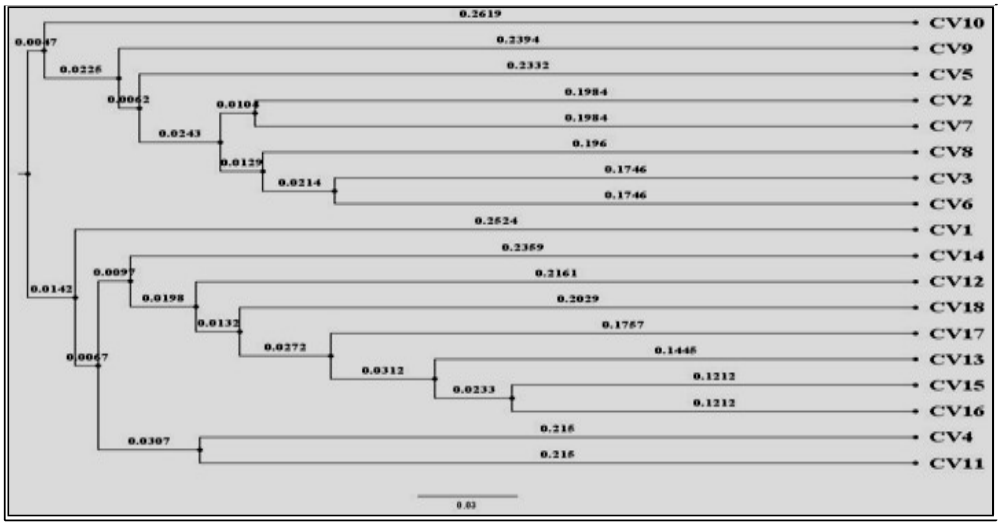


Fig. (6): Consensus tree among 18 almond genotypes according to the combined data.