Genetic Diversity among Carob Accessions from NW-Latakia (Syria) as detected by RAPDs

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Abstract:

Fifteen RAPD primers were used to assess the genetic diversity and structure of 26 Syrian natural *Ceratonia siliqua* L. (Carob) accessions from four locations in NW-Latakia province. Atotal of 102 bands were obtained, 63 of which (61.74%) were polymorphic, with an average of 6.8 bands/primer. The percent of polymorphic bands within accessions from each location was calculated and it was highest within Al-Dakaka accessions (50.56%) while lowest was recorded in Um-Altueur (44.57%).

The dendrogramme produced by Jaccard's coefficient and the UPGMA clustering method applied to the RAPD analysis shows four main groups with coefficient of dissimilarity values ranged from 0.05 to 0.25. The maximum dissimilarity percentage i.e. 25% was found between two accessions from Um Al-Tueur (T3, T8) and all other studied accessions, while the minimum similarity i.e. 5% was found between D2 and D3 from Al-Dakaka location.

Key words: Carob, Genetic diversity, RAPD, NW-Latakia.

1. Introduction:

Ceratonia siliqua L. commonly known as Carob is leguminous evergreen forest or semi forest tree belongs to the Fabaceae (Leguminouceae) family (Battle, 1997; Talhouk et al., 2005; Ekinci et al., 2010). The plant is native to coastal regions of Mediterranean basin and southwest Asia and is broadly cultivated in North Africa, USA, Australia for human and animal consumption (Albanell et al., 1996; Afif et al., 2008). It is also considered to be an important component of vegetation for economic and environmental reasons (Karababa and Coşkuner, 2013). Carob grows in arid and warm bioclimatic with rainfall less than 200 mm in temperate and sub-humid bioclimatic zones, some Carob trees shows tolerance to low temperature in winter (-2°) (El Kahkahi et al., 2014). In addition its response to fire, common in the Mediterranean region, to which Carob is quite more tolerant than other forest species (Sahin and Tasligil, 2016).

Carob tree is considered a fruit, medical and forest trees more efficient; since all its parts (leaves, flowers, fruits, wood and roots) are useful and have value in several areas (El Kahkahi et al., 2014). Currently, the demand for this plant was increased mainly for seed as well as fruit products because of its pharmaceutical, dietetic, food and cosmetic uses (Batle, 1997; Tsatsaragkou et al., 2012; Youssef et al., 2013, Moreira et al., 2017).

Several studies addressing genetic diversity and differentiation among natural carob populations in the Mediterranean countries were achieved by different molecular markers. Some studies used RAPD (Random Amplified Polymorphic DNA) markers to assess genetic diversity among endangered natural Carob populations in Lebanon (Talhouk et al., 2005), Morocco (Konaté et al., 2007), Tunisia (Afif et al., 2008), and Portugal (Barracosa et al., 2008). AFLP (Amplified Fragment Length Polymorphism) markers have been used to estimate intra-specific genetic

diversity among several populations of Carob from Portugal (Barracosa et al., 2008), and from Italy, Spain and Turkey (Caruso et al. 2008).

(La Malfa et al., 2014) used the microsatellites marker EST-SSR (Expressed Sequence Tags-Simple Sequence Repeat) to assess the level of genetic diversity among 71 Carob cultivars and accessions collected from (Italy, Malta and Spain).

The main Syrian populations of carob is located in the regions of Syrian Cost and NW-Syria, few Carob trees could be found in other different parts of Syria (Edleb and Hama), under a rainfall ranging between 350 and 800 mm/year. Populations are associated with Mastic (*Pistacia lentiscus*), Olive (*Olea europea*), and grow on calcareous, sandy or low-clayey soils at altitudes varying from sea-shore to 350 m. Carob tree has an economic and environmental importance in Syria, it is used in reforestation of arid and degraded areas, as well as for food and ornamental purposes. However, in spite of the great importance of carob tree and their use in different applications, only very few studies are available on Syrian carob based on Biological properties (Ameen et al., 2015), morphological and chemical composition for some varieties (Ameem et al., 2017).

For this reason, our study aimed to assess the genetic variation in some Syrian carob accessions using random amplified polymorphic DNA (RAPD) to improve current understanding of the Carob genetic diversity and population structure.

2. Materials and methods:

2.1. Location selection and plant Material:

A total of twenty six natural Carob tree accessions from 4 locations in North- Latakia province (Syria) during 2015 and 2016 were evaluated in this work (Table 1).

Table 1: Location, Altitude and studied accessions of Syrian Carob.

Location	Altitude	Accessions
Wade Kandel	0-50	K1, K2, K3, K4, K5, K6
Um-Altueur	0-120	T1, T2, T3, T4, T5, T6, T7, T8
Al-Dakaka	60-140	D1, D2, D3, D4, D5
Al-Basset	0-20	B1, B2, B3, B4, B5, B6, B6, B7

2.2. DNA extraction and RAPD amplification:

For molecular analysis, total DNA was extracted from fresh leaves using CTAB methods (Khal, 2001) as follows:1g of fresh leaves were cut finely then ground in 5 ml of the lysis buffer: [50 mM Tris HCl, pH8.0, 1.4 M NaCl, 20 mM EDTA(pH8), 2% CTAB, 2% (w/v) PVP-40 (polyvinylpyrrolidone) and 1% (v/v) b-mercaptoethanol], then incubated at 65°C for 30 min. followed by two chloroform/isoamyl alcohol (24:1) extractions. Every washing was followed by a centrifugation at 10.000 X g for 10 min. The last aqueous supernatant was recovered in fresh tubes and precipitated in 2/3 volumes of cold isopropanol at -20°C. One hour later, the suspension was centrifuged at 10 000 x g for 10 min, the pellet was twice washed with cold ethanol (70%), airdried and redissolved in 100 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4), then digested with 1 µL of RNAase (10 µg/ml) and incubated at 37°C for 30 min. The DNA

concentration was determined by using spectrophotometer at 260 nm (1 OD260 = 50 ng/µL), and stored at 4C.

Fifteen RAPD primers (VBC biotech - Germany) were used for screening all the accessions and revealing the genetic diversity (Table 2).

The protocol for RAPD analysis was performed in a volume of 25-µl containing 2.5 mM MgCl2, 25 mM dNTPs, 10 µM primers, 15 ng template DNA and 1.5 units of Taq polymerase (Vivantis - Malaysia). The amplification reactions were performed in a Biometra (Flixe-gene - Germany) thermocycler and consisted of an initial 2 min denaturation step at 94°C, followed by 45 cycles of 30 sec at 94°C, 1 min at (32 -34)°C and 2 min at 72°C. A final extension of 10 min at 72°C completed the amplification. The PCR products were separated in 1.2% agarose gels. Gels were stained with ethidium bromide, visualized with a UV trans illuminator (CAMAG Reprostar3 - Switzerland).

Table 2: List of selected RAPD primers for polymorphic DNA generations.

	Primer Name	Sequence (3` - 5`)	Temperature
1	OPA-06	3´-GGTCCCTGAC-5´	34
2	OPAL-16	3´-CTTTCGAGGG-5´	32
3	OPAF-14	3´-GGTGCGCACT-5´	34
4	OPAK-06	3´-TTGGCGAGATA-5´	34
5	OPAN-09	3´-GGGGGAGATG-5´	34
6	OPAO-15	3´-GAAGGCTCCC-5´	34
7	OPAU-02	3´-CCAACCCGCA-5´	34
8	OPAX-02	3´-GGGAGGCAAA-5´	32

9	OPBB-06	3´-TTCCCGTGAG-5´	34
10	OPBC-11	3´-TTTTGCCCCC-5´	32
11	OPB-18	3´CCACAGCAGT- -5´	32
12	OPC-14	3´-TGCGTGCTTG-5´	32
13	OPF-16	3´GGAGTACGTG- -5´	32
14	OPJ-01	3´CCCGGCTATA- -5´	32
15	OPK-12	3´TGGCCCTCTA- -5´	34

2.3. Statistical Analysis:

To calculate RAPD polymorphism, a binary data matrix was made based on the marker data. RAPD markers were scored for the presence (1) or absence (0) of amplified bands for each of 26 accessions. Similarity index was estimated using the Jaccard's coefficients. Cluster analysis was performed using the unweighted pair group method with an arithmetic mean (UPGMA), and the dendrograms were drawn using NTSYS software version 2.02 (Rohlf, 2002).

3. Results:

From a set of 18 primers screened, 15 produced clear RAPD patterns consisting of a total of 102 scorable markers, 63 of which (61.74%) were polymorphic, with an average of 6.8 bands / primer (Table 3). The number of fragments per primer was ranger from 4 (OPAK-06) to 11 (OPAF-14) from wich 2 and 5 polymorphic respectively (Figure 1). Screening of the entire set of samples was repeated with two of the primers to assess repeatability of RAPD profiles, and identical RAPD patterns were obtained, no population-specific bands were observed.

Different numbers of RAPD bands were found between Carob accessions from different localities. Most of the loci were revealed to be highly polymorphic whereas very few accessions-specific polymorphisms were identified. Um Altueur accessions provide the highest number of RAPD bands (89) of which (45) were polymorphic, while the lowest number of total bands (82) were found within Al-Dakaka accessions from which 39 were polymorphic (Table 4)

Table 3: Characteristics of amplified fragments obtained from 15 primers for RAPD analysis of Carob accessions.

	Primer Name	Total bands	Monomorphic bands	Polymorphic bands	Polymorphic percent %
1	OPA-06	6	3	3	50
2	OPAL-16	7	3	4	57.14
3	OPAF-14	11	6	5	45.45
4	OPAK-06	4	2	2	50
5	OPAN-09	7	3	4	57.14
6	OPAO-15	4	1	3	75
7	OPAU-02	5	2	3	60
8	OPAX-02	6	2	4	66.66
9	OPBB-06	8	2	6	75
10	OPBC-11	10	3	7	70

11	OPB-18	7	2	5	71.42
12	OPC-14	7	4	3	42.85
13	OPF-16	8	2	6	75
14	OPJ-01	5	2	3	60
15	OPK-12	7	2	5	71.42
Sum		102	39	63	61.74

Table 4: Characteristics of Carob accessions per location obtained from RAPD analysis.

Location	Total bands	Polymorphic bands	Monomorphic bands	Polymorphic percent %
Wade Kandel	82	43	39	47.56
Um-Altueur	83	46	37	44.57
Al-Dakaka	89	44	45	50.56
Al-Basset	86	47	39	45.34

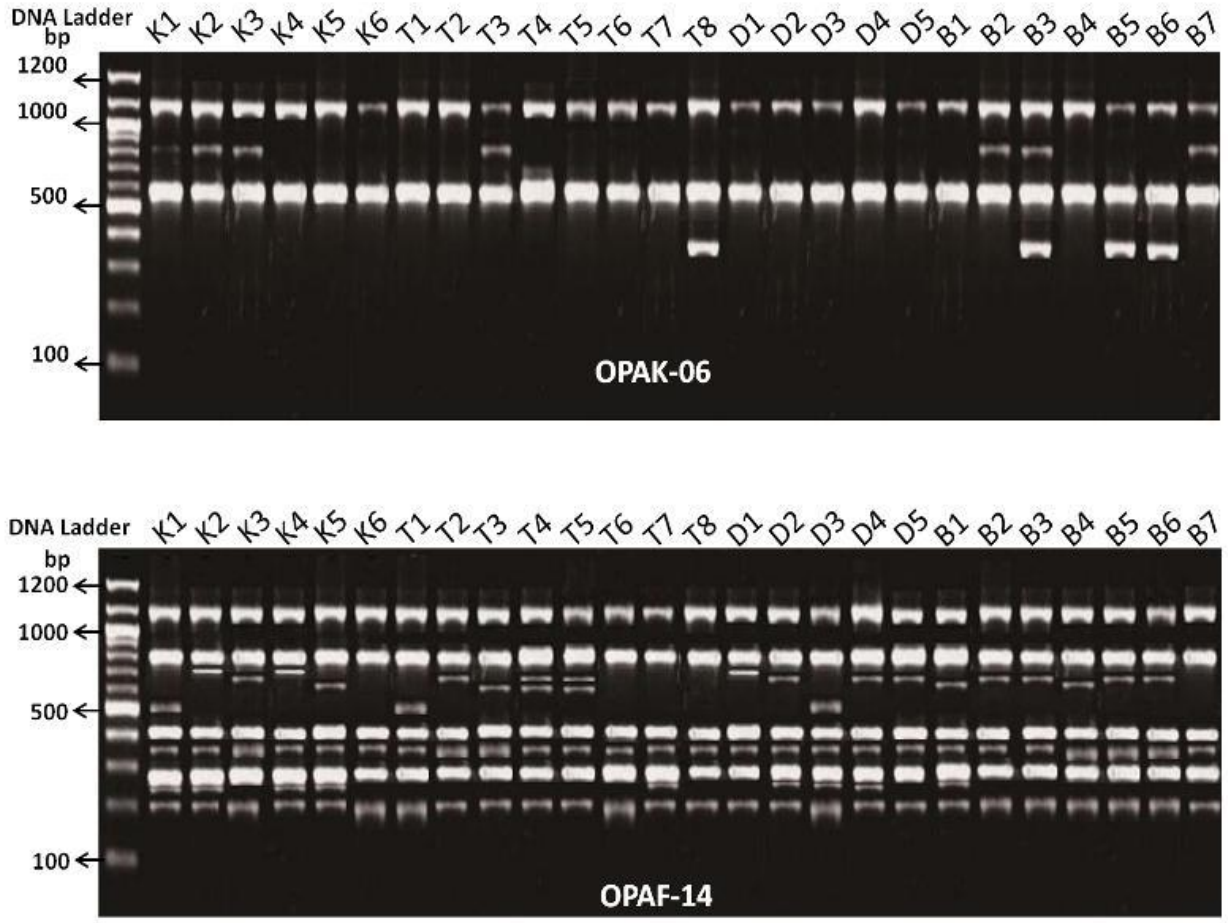

Figure 1. Example of amplification products from primers OPAK-06 and OPAF-14

Associations among the Carob accessions revealed by UPGMA cluster analysis are presented in figure 2. The cluster analysis indicates that the accessions of Carob were grouped roughly into four distant groups, one minor group having only tow accessions (K1 and K2), second major group containing most of the studied accessions (18) from all locations. The third group represent four Accessions from Al-Dakaka, while only two accessions from Um-Altueur (T3, T8) formed the fourth minor group.

The value of dissimilarity coefficient ranged from 0.05 to 0.25. Accessions D2 and D3 represent lowest average dissimilarity coefficient value (0.05), while The maximum dissimilarity

coefficient value (0.25) was found between (T3, T8) and all other studied accessions. In some cases, genetic affinities occurred between populations from different locations. For example, populations K5 and B2 clustered close together on the UPGMA analysis. These populations were also genetically closer to some Um-Altueur (T1, T5, T6) and wade Kandel (K3, K4, K6) accessions. on the opposite side, some accessions from the same locality tended to be relatively distinct genetically, such as T3 and T8 from all other Um-Altueur accessions.

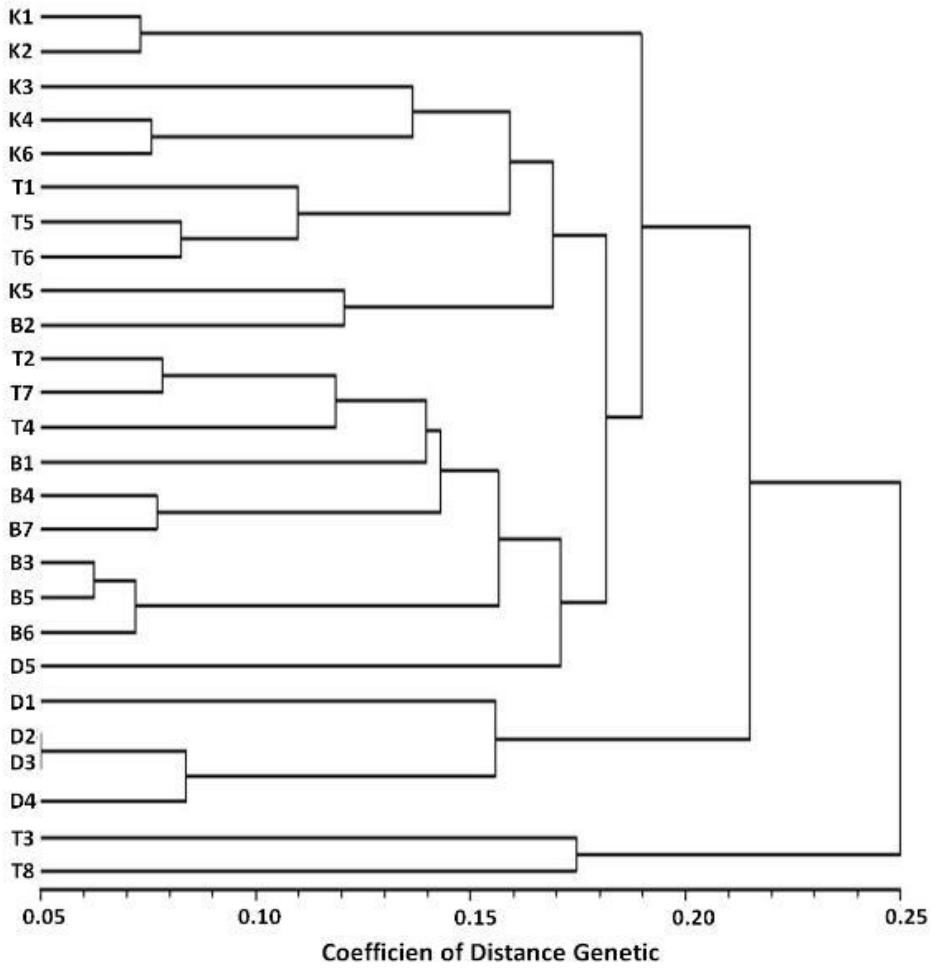

Figure 2. Dendrogram generated by UPGMA cluster analysis showing genetic diversity among the different Carob accessions.

4. Discussion:

Although all the studied accessions are located in a small size area with similar geographic and environmental conditions (about 40Km between Al-Dakaka and Al-Basset), the dendrogram established from RAPD data of our Carob accessions, showed a promiscuity of accessions and no correlation between their clustering and their location. This is consistent with many previous studies which indicated a significant genetic variation among natural Carob populations scattered in different geographic and bioclimatic zones (Afif et al., 2008), or between planted cultivars in one area e.g. Algarve–Portugal (Barracosa et al., 2008). Also our study is in contrast with (Konate et al., 2007) who revealed a rough distribution of Moroccan Carob accessions according to their geographical origin, and found that, two geographically distant accessions (about 700Km) were clustered together in RAPD dendrogram.

Natural Syrian carob populations from Latakia province maintain a genetic diversity as revealed by RAPDs estimates. The level of variation was lower than that reported for Lebanon Carob populations (Talhouk et al., 2005), Tunisian natural populations (Afif et al., 2008), and Portuguese *Ceratonia siliqua* cultivars (Barracosa et al., 2008). It could be explained by the predominantly low number of the studied accessions in closely related locations with similar geographical and environmental condition in our study, compared with a high number of studied populations in different geographical zones in the above mentioned studies.

Genetic differentiation can be increased as all studied accessions distributed in a small area with the same environmental conditions due to direct or indirect human impacts in the form of habitat loss, overexploitation, pollution, or introduced predators, competitors or diseases, more than the impact of rapid environmental changes, hybridization, and adaptation which cause genetic differentiation between closely populations.

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