Morphological and Genetic Variability of Natural Syrian Carob (Ceratonia Silique L.)

Hafez Mahfoud[*1], Tala Ameen[**2], Fadi Kazngi[***3], Samer Nasser[***4]

** Researcher - department of biotechnology. General Commission for Scientific Agricultural Research-Latakia-Syria*

***Professor, Department of Forestry and Ecology, Faculty of Agriculture, Tishreen University, Latakia Syria*

**** Postgraduate student, Department of Forestry and Ecology, Faculty of Agriculture, Tishreen University, Lattakia, Syria.*

Abstract

Morphological and genetic variation of 24 carob accessions geographically dispersed all over the Syrian coast were investigated. In order to establish genetic and morphological relationships among the studied accessions, 18 randomly amplified polymorphic DNA (RAPD) primers, along with measurements from 13 morphological traits of carob pods and seeds were used. For the RAPD analysis, the 18 primers yielded 169 bands from which 105 were polymorphic. The cophenetic correlation coefficient between the similarities and (UPGMA) phenograms were 39% for the RAPDs and 88% for the morphological traits.In general, morphological analysis were found to be in consistent with the molecular data, and both methods generated enough polymorphism to discriminate the Syrian carob resulted in the separation of the 24 accessions into three groups (morphological data) and two groups (RAPD marker).

Keywords

Carob, RAPD, Syrian coast, Morphology, Genetic diversity.

I. INTRODUCTION

Carob *(Ceratonia siliqua* L.) is an angiosperm, leguminous and evergreen forest or semi forest tree belonging to the order Rosales, family Fabaceae (Leguminouceae) ([1], [2]). It is native to the Mediterranean basin including NE Syria and is broadly cultivated in different parts of the worldwide.

Carob tree is well adapted to survive and grew under different environmental stress conditions in the Mediterranean region especially during long arid and dry summer ([3], [4], [5]). The use of carob tree in Syria has been increasing in recent years. It is used in reforestation of arid and degraded areas as well as for ornamental purposes since all its parts have value in several areas ([4], [6]), especially Carob bods and seeds which used in food and pharmacological industry [7]. Moreover, the locust bean gum (E 410) extracted from the endosperm of Carob seeds is used widely in food and pharmaceutical industry ([3], [8]).

Several authors investigated and characterized different selections of natural and cultivated carob trees in different Mediterranean countries depends on morphological characters ([9], [10], [11], [12]) chemical contents ([13], [14]) and genetic variation ([1], [9], [10], [15], [16]).

Morphological characters of pods and seeds are the most valuable and quantitative marker widely used to identify Carob varieties ([4], [17]). Carob pods are characterized by a high sugar content (48-56%) ([7], [18]), and is a good source of protein, fiber and minerals ([14], [19]), the different rates of carob chemical composition are depending on the geographical origin, climate conditions and mainly on the genotype ([3], [11]).

However, in spite of the great interest to carob and their use in different applications, only one unpublished molecular study included few accessions from a small part of NW-Latakia (Nasser et al., in press), and few ecological and propagation studies are available on Syrian carob ([20], [21]). So that, an intensive investigation on Syrian carob is needed. Based on the above considerations, the aim of our study is to assess morphological characteristics and genetic variation of natural Syrian carob.

II. MATERIALS AND METHODS

A) Sampling and experimental method for morphological-and chemical analysis

Twenty-four carob accessions were chosen from various geographic sites of Syrian coast where they grow naturally. Altitude and latitude of every location of habitat were determined by GPS locator (Table 1).

B) *Morphological parameters*

One hundred pods were collected during the stage of maturity 2016 from different parts of each carob tree and the following parameters have been measured using an analytical digital balance and vernier caliper (weight, length, width, thickness, color, shape, texture and the number of seeds/pod). Pod width and thickness (cm) was assessed by taking the average of three measurements (top, middle, and bottom of pod).
For seed characterization, 100 seed from the same ripe pods were collected and measured (Length, wide, thickness and seeds weight (1000 seed)).

C) *Genetic analysis*

For molecular analysis, total DNA was extracted from fresh leaves collected of each tree using CTAB methods [22]. Eighteen RAPD primers (VBC biotech - Germany) were used for screening all the accessions and revealing the genetic diversity (Table 4).

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).

D) *Statistical Analysis*

The morphological and molecular results were analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYS) - version 2.02 [23]. Similarity index was estimated using the Jaccard's coefficients and the dendrograms were generated by the unweighted pair group method with an arithmetic mean (UPGMA).

Table 1: Accessions used in the present study including information about the origin of materials, altitude, latitude and rainfall.

Nr.	Accession	Origin	Latitude N	Latitude E	Altitude (m)
1	WK1	Wade Kandel-W Latakia	35°47'13''	35°57'71''	25
2	WK2	Wade Kandel-W Latakia	35°41'94''	35°51'28''	15
3	WK3	Wade Kandel-W Latakia	35°42'24''	35°51'61''	08
4	UT1	Um Altueur-W Latakia	35°45'36''	35°48'02''	102
5	UT2	Um Altueur-W Latakia	35°45'36''	35°47'74''	41
6	UT3	Um Altueur-W Latakia	35°46'41''	35°46'60''	23
7	DK1	Al dkaka-W Latakia	35°54'06''	35°60'18''	140
8	DK2	Al dkaka-W Latakia	35°54'06''	35°62'18''	82
9	DK3	Aldkaka-W Latakia	35°54'06''	35°58'33''	56
10	BA1	Albasit- NW Latakia	35°68'55''	35°53'62''	04
11	BA2	Albasit-NW Latakia	35°72'49''	35°54'66''	16
12	BA3	Albasit-NW Latakia	35°72'22''	35°54'14''	24
13	SN1	Alsanubar-E Latakia	35°26'72''	35°88'63''	25
14	SN2	Alsanubar-E Latakia	35°32'66''	35°84'63''	44
15	SN3	Alsanubar-E Latakia	35°30'71''	35°86'41''	35
16	GI1	Giboul-Jablah	35°28'52''	36°08'11''	540
17	GI2	Giboul-Jablah	35°28'58''	36°08'18''	512
18	GI3	Giboul-Jablah	35°26'78''	36°07'51''	489
19	HA1	Jblaiah-Alhaffah	35°35'72''	36°10'95''	307
20	HA2	Jblaiah-Alhaffah	35°35'39''	36°03'44''	350
21	HA3	Jblaiah-Alhaffah	35°35'46''	36°13'23''	375
22	KR1	Bkramah-Alkardaha	35°42'32''	36°06'11''	331
23	KR2	Bkramah-Alkardaha	35°45'53''	36°06'52''	311
24	KR3	Bschlamah-Alkardaha	35°46'12''	36°06'41''	276

III. RESULTS AND DISCUSSION

A) Morphological characteristics

Results of morphological traits of carob pods and seeds (except pod color, texture and shape, seed color) are presented in Table 2.

The morphological characters related to the size of carob pods (length, width and thickness) are variable from one accession to another. The pod length varied between 12.87 cm (BA3) to 22.32 cm (SN1), width varied between 1.98 cm (SN3) to 3.12 cm (UT1), and thickness varies between 0.58 cm (WK2) to 1.26 cm (HA2). The bod weight was also varied between the accessions 8.99 g (BA3) to 26.33 g (SN1). The total number of seeds per pod varies between 8.26 (WK1) to 16.11 (DK1).

In addition to, the morphological characteristics of the seeds (length, width, thickness, weight) were also varied among the studied accessions. Values corresponding to the length, width, and thickness varied respectively 0.81 cm (GI1) to 1.14 cm (KR1), 0.62 cm (HA3) to 0.83 cm (BA1) , 0.37 (WK1, DK1 and DK3) to 0.46 cm (SN1 and GI3), and the 1000 seed weight was also varied between 178.12 g (UT3) to 278.33 g (SN1).

Table. 2: Morphological characterization (pod and seed) of Syrian carob (*C. siliqua* L.)

Accession	Pod					seed			
	Length (cm)	Width (cm)	Thickness (cm)	Weight (g)	Seeds number/ pod	Length (cm)	Width (cm)	Thickness (cm)	Weight (g)1000 seeds
WK1	21.53	2.88	1.09	18.61	8.26	1.05	0.73	0.37	231.14
WK2	19.52	2.56	0.58	19.56	8.88	1.03	0.74	0.38	218.17
WK3	20.14	2.61	0.81	24.77	9.40	0.99	0.77	0.40	196.17
UT1	14.22	3.12	1.15	14.87	10.14	1.04	0.70	0.38	242.24
UT2	18.33	2.39	0.82	12.54	13.78	1.07	0.73	0.38	222.25
UT3	16.55	2.15	0.92	9.55	11.11	0.98	0.74	0.41	178.12
DK1	18.02	2.21	0.62	11.64	16.11	0.94	0.66	0.37	228.00
DK2	17.22	2.35	0.69	16.11	14.56	0.89	0.72	0.41	225.96
DK3	17.68	2.78	1.18	21.46	13.57	0.97	0.72	0.37	240.41
BA1	18.69	2.49	0.98	17.38	11.35	1.13	0.83	0.42	244.11
BA2	18.24	2.45	0.78	15.02	10.02	1.01	0.79	0.43	178.89
BA3	12.87	2.33	0.72	8.99	11.15	0.91	0.69	0.45	180.45
SN1	22.32	2.62	1.02	26.33	13.15	1.03	0.79	0.46	278.33
SN2	16.68	2.02	0.92	15.12	11.08	0.95	0.73	0.45	211.47
SN3	18.75	1.98	0.81	12.97	14.63	0.98	0.72	0.41	201.62
GI1	16.17	2.51	1.12	15.88	12.44	1.07	0.72	0.41	182.15
GI2	18.11	2.54	0.91	23.65	12.38	0.81	0.65	0.43	180.14
GI3	17.45	2.42	0.97	21.79	13.66	0.84	0.67	0.46	239.14
HA1	13.45	3.02	0.88	22.14	9.02	0.97	0.74	0.45	217.17
HA2	15.11	2.58	1.26	14.78	9.10	0.97	0.74	0.45	225.25
HA3	15.73	2.26	0.61	10.12	11.14	1.07	0.62	0.43	183.75
KR1	15.89	2.45	0.75	12.55	13.12	1.14	0.78	0.42	229.12
KR2	17.87	2.32	0.79	13.98	13.88	1.08	0,74	0.42	221.54
KR3	18.54	2.29	0.90	14.27	11.47	0.88	0.69	0.41	232.17

Depends on the results of 13 morphological traits of carob bod and seed (table 2) a special descriptors for wild Syrian carob has been achieved (table 3).

The dendrogram obtained with the 13 morphological traits of pods and seeds, considering the 24 *C. siliqua* .L accessions, shows the formation of three groups, with an average dissimilarity of 0.88 (Figure 1). The first group consisted of nine accessions of which 6 from Latakia, two from Alhaffa and one from Gablah, whereas the second represents 11 accessions (7 froma Latakia, one from Alhaffah and all Alkardaha accession). The third groud represent four accessions, two accessions from Latakia and two from Gablah. Variables such as pod shape, thickness and size, seed number per pod and seed weight, as well as chemical content of pod and seed explained the largest portion of the total variance observed.

Table 3: Descriptors for morphological and chemical characterization of Syrian carob.

Code	Traits/descriptors	Score code - descriptors state
1	**Pod**	
1-1-	weight (g)	<10.48 very low, 10.48-15.60 low, 15.61-20.73 intermediate, 20.73-25.95 high, >25.95 very high.
1-2-	length (cm)	<15 very short, 15-16.99 short, 017-18.99 intermediate, 19-20.99 long, >20.99 large
1-3-	wide	<2 very small, 2-2.32 small, 2.33-2.65 intermediate, 2.66 - 2.98 wide, >2.98 very wide.
1-4-	thickness	<0.64 very low, 0.64 -0.82 low, 0.83-1.01 intermediate, 1.02-1.20 high, >1.20 very high.
1-5-	color	1 hell brown, 2brown, 3 dark brown
1-6-	seeds number	<9.25 low, 9.25-14.75 intermediate, >14.75 high.
1-7-	texture	1 smooth, 2 rough
1-8-	shape	1 straight, 2 slightly curved, 3 curved.
2	**Seed**	
2-1-	Length (mm)	<0.82 very short, 0.82-0.91 short, 0.92-1.01 intermediate, 1.02-1.11 long, >1.11 very long.
2-2-	Wide (mm)	<0.58 very small, 0.58-0.66 small, 0.67-0.75 intermediate, 0.76 - 0.84 wide, >0.84very wide.
2-3-	thickness	<0.4 low, 0.4-0.44 intermediate, >0.44 high
2-4-	seeds weight (1000 seed)	<176.60 very low, 176.60-198.65 low, 198.66-220.71 intermediate, 220.72-242.79 high, >242.79 very high.
2-5-	color	1 brown, 2 dark brown

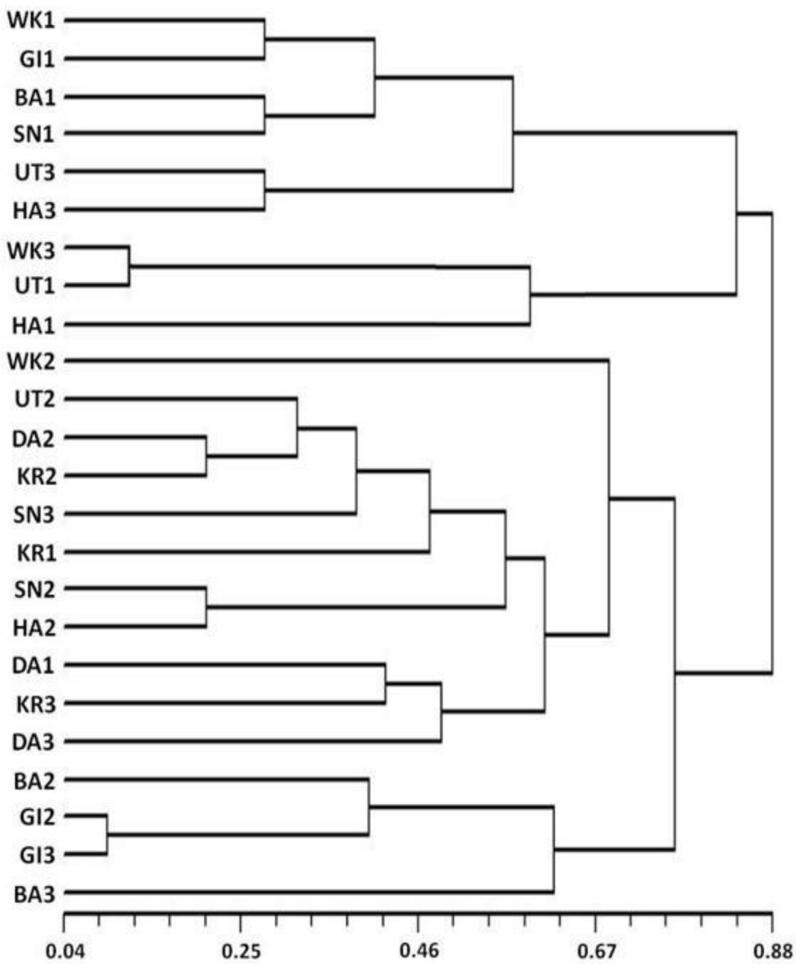

Figure 1. UPGMA dendrogram of Syrian carob generated by morphological characteristics of pods and seeds.

B) Genetic characteristics

The eighteen RAPD primers detected a total of 169 amplification bands, varying from 6 to 13 bands per primer, of which 105 were polymorphic (61.85%) (table. 4).

The dendrogram based on UPGMA analysis of the genetic dissimilarity matrix (Figure 2) show that all the 24 accessions were clustered into two major groups. The first one consisted of ten accessions of which nine from Latakia and one accession from Alhaffah (Jiblaiah HA1, whereas the second represents 14 accessions (all Alkrdaha and Gablah accession, 5 accessions from Latakia and 2 from Alhaffah), each of the main groups could be further classified into two subgroups.

Table 4: Codes and sequences of the RAPD primers used and number of total and polymorphic bands produced from the PCR reactions of the 24 carob Accessions.

	Primer	Sequence (3` - 5`)	Total bands	Polymorphic bands	Percent polymorphism
1	OPA-04	3´-CAATCGCTCG-5´	8	5	62.5
2	OPA-08	3´-CGTTCCTGCA-5´	9	5	55.55
3	OPAC-2	3´-GGATAGCGCT-5´	7	5	71.42
4	OPAC-5	3´-ATGTGGCACC-5´	8	5	62.5
5	OPAD-1	3´-TTAGAGGTCC-5´	12	7	85.33
6	OPAF-10	3´-CGTAGCGCCT-5´	11	7	63.63
7	OPAF-14	3´-GGTGCGCACT-5´	13	7	53.84
8	OPAO-15	3´-GAAGGCTCCC-5´	6	4	66.66
9	OPCA-01	3´GAAGCACGTG- -5´	9	6	66.66
10	OPCA-02	3´AACGGTCATA- -5´	10	7	70
11	OPCA-05	3´ATGCCCTCTC- -5´	9	5	55.55
12	OPCA-06	3´ACAGGTCATG- -5´	7	4	57.14
13	OPCA-09	3´CAACGGTCCTA- -5´	10	6	60
14	OPF-16	3´GGAGTACGTG- -5´	8	4	50
15	OPJ-01	3´CCCGGCTATA- -5´	11	8	72.72
16	OPK-12	3´TGGCCCTCTA- -5´	9	7	77.77
17	OPK-14	3´ACGCGGTCTG- -5´	10	6	60
18	OPN-08	3´CTTCGCTCTA- -5´	12	7	58.33
Sum			169	105	62.13

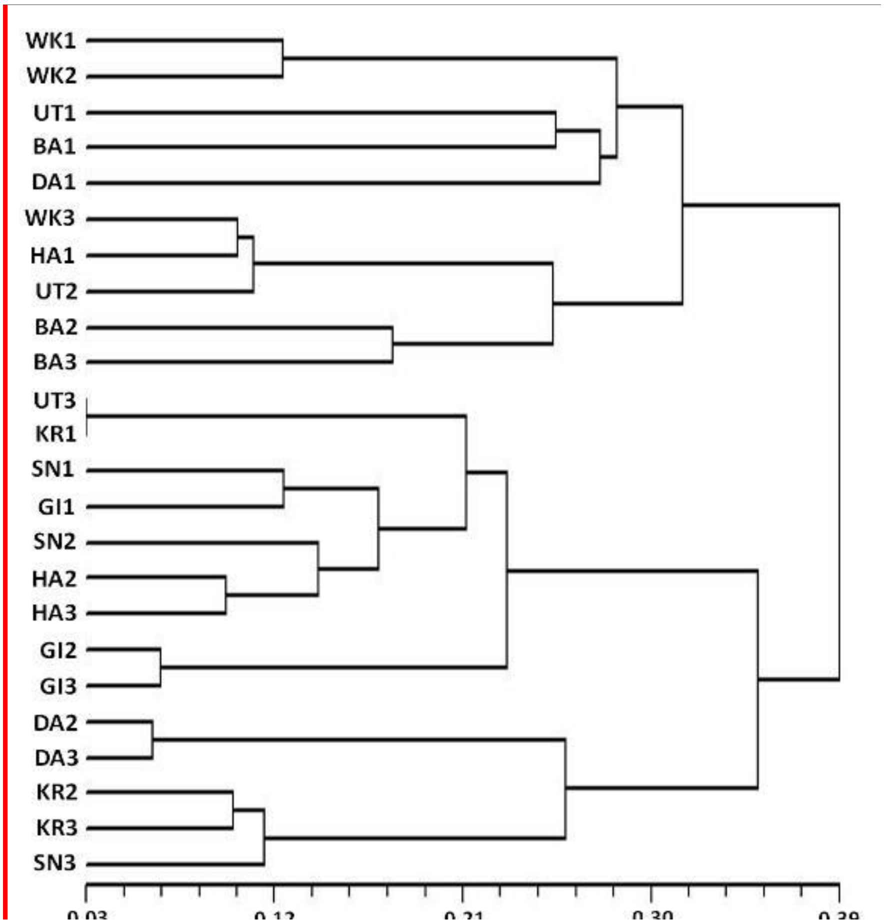

Figure 2. UPGMA dendrogram of Syrian carob generated by RAPDs.

IV. DISSCUSION

In the present study the morphological and genetic diversity of *C. siliqua* .L were assessed for wild carob trees geographically dispersed all over the Syrian coast. Both morphological and molecular methods generated enough polymorphism to discriminate the Syrian carob resulted in the separation of the 24 accessions into three groups (morphological data) and two groups (RAPD marker).

The morphological characters of carob pods and seeds was used to assess genetic variation within different carob populations by several authors ([4], [24], [25]), most of the studied characters showed significant difference, the type and geographical origin of carob trees has been taken as the source of variation. Which is in contrast to our results revealed by morphological characters and supported by genetic analysis. Furthermore, the morphological differences between accessions from the same locality such as Alsanubar or Um Altueur accessions cannot be explained by environmental conditions, differences are primarily caused by genetic factors, which is confirmed by RAPD analysis.

Although different rates of correlation between the morphological and genetic similarities were observed, some accessions from the same locality (eg. HA2 and HA3) were separated in two distinct groups in morphological analysis and clustered together in the genetic analysis, while other accessions from different localities show high correlation between morphological and genetic analysis. These results agreed with those of [9] who found that some accessions from different localities (about 700 Km) were clustered together in the phylogenetic tree.

The analysis of RAPD profiles revealed a high degree of genetic diversity within Syrian carob accessions. Overall, in most cases we note that morphological relationship between carob accessions are closely related to their molecular relationship. Several authors have found such high levels of morphological and genetic differentiation in different Mediterranean countries ([4], [9], [12], [15]).

Our study detect no correlation between pod size and seeds number per pod in contrast with the results obtained by several authors ([4], [6], [9]). Furthermore, we found that some Syrian accessions have a promising industrial interest because some of the accessions produce pods with a high weight (26.33g) comparing with Moroccan carob (15.69g) [9], and (18.19g) [4], as well as Lebanese carob 3.61-5.62 [24]. In addition to a high average number of seeds per pod observed in some accessions (e.g. DKI: 16.11) compared with Lebanese carob (14.10) [24], Moroccan carob (15.43) [4], and Croatian carob (11.05) [25].

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the Syrian General Commission for Scientific Agricultural Research (GCSAR). We thank Dr. Magda Mofleh and Dr. Mohammad Salhab for always being available with valuable help and support.

REFERENCES

[1] S. N. Talhouk, P. Van Breugel, R. Zurayk, A. Al-Khatib, J. Estephan, A. Ghalayini, N. Debian, and D. Lychaa,. "Status and prospects for the conservation of remnant semi-natural carob Ceratonia siliqua L. populations in Lebanon," For. Ecol. Manage., vol. 206, pp. 49-59. 2005.

[2] K. D. Ekinci, D. Yılmaz, and C. Ertekin, "Effects of moisture content and compression positions on mechanical properties of Carob pod (Ceratonia siliqua)," African Journal of Agricultural Research., vol. 5(10), pp. 1015-1021. 2010.

[3] I. Battle, and J. Tous, "Carob tree. Ceratonia siliqua L. Promoting the conservation and use of underutilized and neglected crops," Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute (eds), Rome, Italy., 92 p. 1997.

[4] R. El-Kahkahi, R. Zouhair, M. Ait Chitt, and R. Errakhi, "Morocco carob (Ceratonia siliqua L.) populations: Morphological variability of Pods and Kernel," Int. J. Pure App. Biosci., vol. 2(4), pp. 38-47. 2014.

[5] G. Sahin, and N. Tasligil, "Agricultural Geography Analysis of Carob Tree (Ceratonia siliqua L.) from Turkey," Turkish Journal of Agriculture- Food Science and Technology. vol. 4(12), pp.1192-1200. 2016.

[6] E. Albanell, G. Caja, and J. Plaixats, "Characterization of carob fruits (Ceratonia siliqua L.) cultivated in Spain for agro industrial use," Int. Tree Crops J., vol. 9, pp. 1-9. 1992.

[7] P. D. Markis, and P. Kefalas, "Carob Pods (Ceratonia siliqua L.) as a Source of Polyphenolic Antioxidants," Food Technol. Biotechnol., vol 42 (2). pp. 105-108. 2004.

[8] C. Sandolo, T. Coviello, P. Matricardi, and F. Alhaique, "Characterization of polysaccharide hydrogels for modified drug delivery," Eur. Biophys. J., vol. 36(7), pp. 693-700. 2007.

[9] I. Konate, F. A. Maltouf, and E. B. Berraho, "Diversity analusis of Moroccan Carob (Ceratonia siliqua L.) accessions using phenotypic traits and RAPD markers," Acta Botanica Malacitana., vol. 23, pp. 79-90. 2007

[10] P. Barracosa, P. Lima, M. B. Lima, and A. Cravador, "Analysis of genetic diversity in Portuguese Ceratonia siliqua L. cultivars using RAPD and AFLP markers," Scientia Horticulturae., vol. 118, pp. 189-199. 2008.

[11] M. M. Sidina, M. Hansali , N. Wahid, A. Ouatmane, A. Boulli, and A. Haddioui, "Fruit and seed diversity of domesticated carob (Ceratonia siliqua L.) in Morocco," Scintia Horticulturae., vol. 123, pp. 110-116. 2009.

[12] S. Naghmouchi, M. L. Khouja, A. Romero, J. Tous, and M. Boussaaid, "Tunisian carob (Ceratonia siliqua L.) populations: Morphological variability of pods and kernel," Scintia Horticulturae., vol. 121(2), pp. 125-130. 2009.

[13] S. Gruendel, B. Otto, A. L. Garcia, K. Wagner, C. Muelle, M. O. Weickert, W. Heldwein, and C. Koebnick, "Carob pulp preparation rich in insoluble dietary fibre and polyphenols increases plasma glucose and serum insulin responses in combination with a glucose load in humans," Br. J. Nutr., vol 98 (1), pp. 101-105. 2007.

[14] L. Custódio, E. Fernandes, A. L. Escapa, A. Fajardo, R. Aligue, F. Albericio, N. R. Neng. J. M. Nogueira, and A. Romano, "Antioxidant and cytotoxic activities of carob tree fruit pulps strongly influenced by gender and cultivar," J. Agric. Food Chem. vol. 59, pp. 7005-7012. 2011.

[15] M. Afif, C. Messaoud, A. Baoulila, H. Chougrani, A. Bejaoui, M. N. Rejeb, and M. Boussaid, "Genetic structure of Tunisian natural carob tree (Ceratonia siliqua L.) populations Inferred from RAPD markers," Ann. For. Sci., vol. 65(710). 2008.

[16] S. La Malfa, S. Curro, B. A. Douglas, M. Brugaletta, M. Caruso, and A. Gentile, "Genetic diversity revealed by EST-SSR arkers in carob tree (Ceratonia siliqua L.)," Biochemical Systematics and Ecology., vol. 55, pp. 205-211. 2014.

[17] G. Macleod, and M. Forcen, "Analysis of volatile components derived from the carob bean ceratonia silique," Phytochemistry., vol. 31(9). pp. 3113-3119. 1992.

[18] M. Khlifa, A. Bahloul, and S. Kitane, "Determination of chemical composition of carob pod (Ceratonia siliqua L.) and its morphological study," J. Mater. Environ. Sci., vol. 4(3), pp. 348-353. 2013.

[19] F. A. Ayaz, T. S. Hülya, J. C. Pedr, A. Manuel, C. G. Carlos, and S. Miroslav "Determination of Chemical Composition Of Anatolian Carob Pod (Ceratonia Siliqua L.): Sugars, Amino And Organic Acids, Minerals And Phenolic Compounds," Journal of Food Quality., vol. 30, pp. 1040-1055. 2007.

[20] T. Ameen, H. Mahfoud, and S. Nasser, "Effect of seed size on some quantitative indicators of growth and development of seedlings of Carob tree (Ceratonia siliqua L.)," Tishreen University Journal for Research and Scientific Studies - Biological Sciences Series. vol. 37(4), pp. 117-132. 2015.

[21] T. Ammen, H. Mahfoud, and F. Kazngi, "The effect of some biological and genetic indicators of Scions on the success of grafting ceratonia siliqua L. seedlings and on development of their subsequent growth," Tishreen University Journal for Research and Scientific Studies - Biological Sciences Series., vol. 38(4), pp. 56-66. 2016.

[22] G. Kahl, "The Dictionary of Gene Technology," Wiley-VCH, Weinheim. 2001.

[23] F. J. Rohlf, "Numerical taxonomy and multivariant analysis system. NTSYS version 2.11a.Applied Biostatistics Inc," New York, Stoy Book., N.Y., USA, 231.

[24] A. Haddarah, A. Ismail, A. Bassal, T. Hamieh, I. Ioannou, and M. Ghoul, M. "Morphological and chemical variability of Lebanese carob varieties," European Scientific Journal., vol. 9 (18), pp. 353-369. 2013.

[25] S. Srecec, D. Kremer, K. Karlovic, P. T. Volf, R. Erhatic, Z. Augustinovic, I. Kvaternjak, S. Bolaric, D. Dujmovic, V. Dunkic, N. Bezic, and M. Randic, "Comparison of Morphological Characteristics of Carob Tree (Ceratonia siliqua L.) Pods and Seeds of Populations Collected from Two Distant Croatian Islands: Drvenik Mali and Mali Lošinj," Agriculturae Conspectus Science. vol. 8. pp. 61-64. 2016.