



## ORIGINAL ARTICLE

### A Study of *Carpinus orientalis* Mill. Populations Based on Morphological Characteristics in the Hyrcanian Forests, N. Iran

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#### ABSTRACT

Natural distribution range of *Carpinus orientalis* Mill. is high and middle altitudes of the Hyrcanian forests, north Iran. The morphological characteristics of *C. orientalis* and its distribution were studied by using 32 populations selected from four provinces of north and northeastern Iran. A great variation was observed in the quantitative characteristics including ratio of petiole percentage to total leaf length, petiole length and distance between the base and the widest point of the lamina and also in qualitative characteristics including fruit bract and fruit shape. The results indicated that leaf length, number of paired veins per leaf and maximum of petiole length percentage to total leaf length are the main morphological characteristics for grouping variables among the populations of *C. orientalis* in the Hyrcanian forests of Iran.

**Key words.** *Carpinus orientalis*, cluster analysis, Hyrcanian forest, morphological characters

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#### INTRODUCTION

The genus *Carpinus* L. now belongs to Betulaceae family [1, 2, 3]. The Betulaceae are a well-defined family with six living genera and 130 species [4]. Determination of the taxa in the genus *Carpinus* in Iran is one of the most controversial issues among the researchers; for example we can see this claim in the recent botanical literatures such as Sabeti [5] and Browicz [6]. However, two good species namely *C. betulus* L. and *C. orinetalis* Mill. are the main species in Iran, adjacent regions [7, 8, 9] and also in Europe [1, 2]. Intraspecific delimitation of *Carpinus orientalis* has been variously treated in different investigations. Some taxonomists believe the occurrence of two subspecies namely subsp. *orientalis* and subsp. *macrocarpa* Willk. [6], whereas others do not believe in any intraspecific classification within the species, but instead they consider them as two separate species [5].

*Carpinus orientalis*, a small and slow-growing deciduous tree/shrub, is different from its relative species, *C. betulus* due to having shorter and more base-branched trunks as well as growing mainly on rocky and poor sites [5,10]. This species is distributed in SE Europe [9], Turkey, Caucasus and northern Iran, from west to easternmost of Hyrcanian forest especially in north of Khorassan province [6]. It also disjunctly occurs in Semnan province [11]. According to the Flora Iranica [6], *C. orientalis* subsp. *orientalis* is distributed through northern Iran and also as a relatively small patch in Azerbaijan (Ali Bolaghi) and Khorasan-e Shomali (Bojnourd), while subsp. *macrocarpa* Willk. is distributed only in northern Iran (Gilan, Mazandaran and Golestan). The latter subspecies is an endemic taxon in the Iranian Hyrcanian forest zone and Talish in Azerbaijan Republic [6].

The aim of this research is to study the morphological variability of *C. orientalis* populations based on live and herbarium specimens in the range of five Iranian provinces (Gilan, Mazandaran, Golestan, Semnan and Khorasan-e Shomali).

#### MATERIALS AND METHOD

In this research, 32 populations from five provinces i.e. Khorasan-e Shomali, Golestan, Mazandaran, Gilan and Semnan were studied. Live specimens were collected from four provinces in different sites and

elevation ranges. As a result, five trees from each site were selected and to prevent sampling trees from same parents or pedigree, a minimum distance of 100m on average was considered between each tree [12]. All samples were collected during May to September 2011 to obtain both reproductive (flowers and fruits) and vegetative parts. The specimens were determined according to the available literatures [5, 6]. In the other hand, we studied herbarium specimens from Herbarium of Research Center of Agricultural and Natural Resources of Gorgan, FUMH, TARI and HNBG (Herbarium of Nowshahr Botanical Garden) that were considered as seventeen populations (Table 1).

Table1. Localities and the site data of *Carpinus orientalis*. \*New collections: housed in herbarium of University of Mazandaran

Code	Province	Locality	Altitude (m a.s.l.)	Herbarium and Herbarium number
ori1-1	Golestan	Tangrah, near road, 55°57'17.18" E, 37° 22' 8.62" N	650	1514*
ori1-5	Khorasan-e Shomali	Darkesh, Gollak region, 56° 44'49.0" E 37°24'45.9" N	1413	1515*
ori 1-6	Khorasan-e Shomali	Darkesh, Kanigandi, 56°43'49.5" E, 37°25'45.5" N	1300	1521*
ori 1-10	Khorasan-e Shomali	Darkesh, Piazkuh, Geji, 56°43'34.23" E, 37°25'56.14" N	1400	1522*
ori 1-11	Khorasan-e Shomali	West of Bojnurd, Ghorkhud, 6km on road Kastan-Chalbash,Sulehbakh valley, 56° 20'43.1" E, 37°28'39.1" N	1325	FUMH 41089
ori 2-1	Golestan	Gorgan, Fazelabad, Mohammad Abad valley, 54°48'57.60" E, 36° 44'35.6" N	918	1517*
ori 2-3	Golestan	Golestan National Park, Golzar Camping, 55°53'56.81" E, 37° 22'23.67" N	693	1527*
ori 2-5	Golestan	Golestan National Park, Tangeh Gol, 55°53'56.81" E, 37°22' 23.67" N	600	1528*
ori 2-6	Golestan	Marave Tappeh	1200	4711 (Gorgan Herbarium)
ori 2-7	Golestan	Derazno	2200	353 (Gorgan Herbarium)
ori 2-8	Golestan	Kordkuy, Derazno	2400	4640 (Gorgan Herbarium)
ori 2-9	Golestan	Marazkuh	2200	3299 (Gorgan Herbarium)
ori 2-10	Golestan	Golestan National Park, Tangeh Gol, 55°53'56.81" E, 37° 22' 23.67" N	800	3499 (Gorgan Herbarium)
ori 2-12	Golestan	Golestan National Park	840	TARI 76084
ori 2-13	Golestan	Golestan National Park, Abshar	700	TARI 14270
ori 2-14	Golestan	Gorgan: 22km from Azadshahr to Shahrud	300	TARI 76116
ori 2-15	Golestan	Gorgan: 22km from Azadshahr to Shahrud	700	TARI 76117
ori 3-6	Mazandaran	Sari, Kiasar forest	1000	1523*
ori 3-7	Mazandaran	Sari, Kiasar forest	1400	1524*
ori 3-1	Mazandaran	Noor,Vaz forest	1900	HNBG 8677
ori 3-2	Mazandaran	Sari, Bula, Poshtnat	2400	HNBG 8676
ori 3-10	Mazandaran	Nowshahr, Botanical garden	-21	1518*
ori3-11	Mazandaran	Chalus, Kandovan Road, Naharkhoran Region	1261	1519*
ori 3-12	Mazandaran	Chalus, Kandovan road, Valiabad. Region	1700	1520*
ori 3-15	Mazandaran	Ramsar, 18 Km on road to Jenat-Rudbar	1040	TARI 21584
ori 3-16	Mazandaran	Siahbisheh	2500	TARI 76687
ori 3-17	Mazandaran	Near Veresk	1630	TARI 75994
ori 4-1	Gilan	Rasht, Rostamabad, Ammarlu, Sibon, Arbonav, Chichal	1600	1525*
ori 4-3	Gilan	Rasht, Rostamabad, Ammarlu, Sibon, Arbonav, Chichal	1800	1526*
ori4-4	Khorasan-e Shomali	Jozak, 56°44'53.5" E, 37° 25' 10.6" N	1296	1516*
ori 5-1	Semnan	Shahmirzad,Parvar,Tangkavard,	1500	TARI 72680
ori 5-2	Semnan	Parvar to Hikoh, Sheli	1800	TARI 72650

Twenty eight morphological characters including 13 quantitative data (Table 2) and 15 qualitative data (Table 3) were selected. The selection of main characteristics was based on Danquash *et al.*, [12]; Yoo and Wen, [1]; Kovacic and Nikolic, [13]. Regarding quantitative and qualitative characters, five repeats of

individuals of each population were collected and from each individual, three samples of each character were studied in live specimens. For herbarium dried specimens, the available materials (sometimes with only one or two sheets) were also included in our analyses. For all measurement, the maximum sizes and the most abundant repetitions were considered for quantitative and qualitative characters of each population respectively. Finally, characters were utilized to run a cluster analysis with Ward method using PAST software [14]. A principal component analysis (PCA) were also used to show populations on bi-dimensional graphs using PAST software [14].

## RESULTS

The dendrogram of hierarchical classification of morphological characters of 32 populations showed that Valiabad and Vaz forest populations have the lowest similarity to the other populations and were separated at a linkage distance 36 (Fig. 2). However, the two cited populations are not located close to each other in the ordination diagram. Vaz population is distinguished by its minimum leaf length, minimum leaf width, number of vein pairs per leaf and maximum petiole percentage to total leaf length. Likewise, Valiabad population is distinguished by its longest petiole and also fruit bract length (Fig. 4, 5). The rest of studied populations can be divided to two separate clusters at the linkage distance 25 (Fig. 2). The first cluster includes Fazelabad, Nowshahr, Azadshahr, Veresk, Marazkuh, Tangeh Gol, Tang-e Kavard, Gollak and Kanigandi whose populations share similar petiole length, and number of vein pairs per leaf. The second cluster is divided into three groups (Fig. 2). The first group including Geji, Derazno Tangeh Gol, Arbonav, Golzar and Siahbisheh with similar middle lobe and number of vein pair per leaf and the second group including Abshahr, Sulehbakh, Maravetappeh, Ramsar, Azadshahr, Kiasar, Sheli, Arbonav, Poshtnat and Golestan national park populations whose petiole and inflorescence length are similar. The last group including three populations of Poshtnat, Jozak, Tangrah which share similar bract length and bract width.

The principal component analysis (PCA) resolved four principal components (PC) which have Eigen values of 30.61, 4.20, 2.10 and 1.09, respectively which explain four variances, 73.11 %, 10.03 %, 5.02 % and 2.59 %. The variable which gave the highest contribution to the first principal components (PC1) is petiole percentage to the total leaf length (RPLP) (Fig. 3).

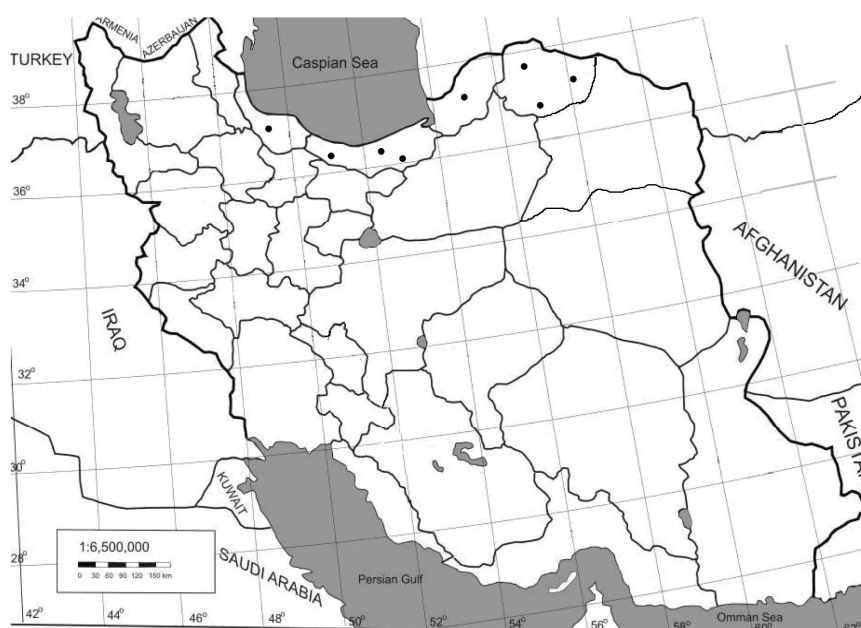
The second component (PC2) is related to the number of vein pairs per leaf (NVPL) and leaf length (LL). Other important characteristics for separation of the populations on this axis are leaf width (LW), petiole length (PL) and distance between the widest point of the lamina to the base (WPLB). All these characteristics are negatively correlated with length of inflorescence (LI) and serrated margins on the middle lobe of bracts (SMLB) (Fig. 3). The PCA ordination plots showed a clear separation between Valiabad and Vaz forest populations and the other studied populations.

Table 2. Quantitative characteristics including minimum, maximum and average of measured characteristics of *Carpinus orientalis*.

no	Character	Minimum	Maximum	Average
1	Leaf length	4.6	11	6.7
2	Leaf width	2.1	4.8	3.45
3	The widest part of leaf base	1	4	2.51
4	Petiole length	0.6	4	1.26
5	PL/LL	0.10	0.52	0.19
6	LW/LL	0.25	0.87	0.48
7	$PL*100/(LL+PL)$	9.38	34.29	15.70
8	Number of vein pairs per leaf	11	17	14
9	Length of inflorescence	1	3.5	2.03
10	Fruit bract length	0.9	3.5	2.34
11	Fruit bract high latitudes	0.2	1	0.65
12	Fruit bract bottom width	0.1	2.3	1.22
13	Number of lobes on fruit bracts	1	3	1.31

Table 3. Qualitative characteristics and their states in the studied materials of *C. orientalis*.

Characters	Character status	Populations
Stipule	absent	Some individuals
	present	Most individuals
Leaf margin trichomes	absent	8677,8676,14270,1516,1515,1518,1527
	present	Rest individuals
Fruit bracts	symmetrical	Some individuals
	asymmetrical	Most individuals
Serrate margin on the middle lobe of bracts	without margin	1518, 3499
	one side only	8677,21584,76687,1523,1571,1516,1515,1514,1527,4711,41089
	both side	Rest populations
Nutlet shape	ovate	Most individuals
	oval	Some individuals
Leaf margin	serrated	all
Upper leaf surface trichomes	absent	1518,8676
	present	Rest populations
Lower leaf surface trichomes	absent	1518,8676
	present	Rest populations
Bract of nutlet	not enclosing	76117,1523,1524
	somewhat	1518,1520
	enclosing	Rest populations
Trichome of the inner surface of the fruit	present	all
Trichome of the outer surface of the fruit	present	all
Setose trichomes at the base of bracts	present	all
Lamina tip	acute	Most individuals
	acute rarely acuminate	Some individuals
	acute rarely Rounded	21584,76687,75994,76116,1519,1527,1528,1522,3499,41089
	rounded rarely acute	353,4640,3299
Basal lamina	rounded	Some individuals
	rounded rarely cordate	Some individuals
	cordate	353,3299
The lamina	ovate	Some individuals
	ovate rarely elliptical	Some individuals
	elliptical rarely ovate	72650,1524,1517,1522
	elliptical	1518

Fig 1. Geographical location of studied specimens of *Carpinus orientalis* populations in the Hyrcanian forests

## DISCUSSION

Some previous studies e.g. Browicz [6] and Sabeti [5] indicated that the most important characteristics for delimitation of intraspecific units of *Carpinus orientalis* are the size of leaf and fruit bracts. The occurrence of large leaf and fruit bract fit with subspecies. *macrocarpa*. One of our studied herbarium sheet (ori2-12: Golestan National Park) was previously identified as *C. macrocarpa* (= *C. orientalis* subsp. *macrocarpa*). It is however seen close to Chichal population (ori4-1) in the cluster analysis. The specimen has only leaf length similar to characters already described for subsp. *macrocarpa* and fruit bract length has similarity with characters of subsp. *orientalis*. In the current study, leaf length of all studied populations and herbaria specimens varies between 4.6-11 cm, leaf width varies between 2.1-4.8 cm and fruit bract length varies between 0.9-3.5 cm. Based on these measurements, leaf length of populations such as ori3-10 (Nowshahr), ori3-12 (ValiAbad), ori4-3 (Chichal), ori3-16 (Siabhisheh) and ori2-7 (Derazno) fits within the size variation considered for subsp. *macrocarpa*, whereas fruit bract length does not fit with the latter subspecies. Likewise, fruit bract lengths of ori2-10 (Tangeh Gol), ori2-6 (Marav-e Tappeh), ori2-15 (Shahrud) and ori1-6 (Kanigandi) fit with subsp. *macrocarpa*, while leaf length does not fit. Therefore, no studied populations represent two above mentioned characters simultaneously.

High variations among the quantitative morphological characteristics were observed within *Carpinus orientalis* populations. The most important characters with large variability include petiole percentage to total leaf length (29.3–38.9), petiole length (0.4–6 cm), distance from the base of the leaf to the point of maximum width (1–4) and number of paired veins per leaves (11–17). Latter characters were previously employed for separation of other woody species (e.g. Danquash *et al.* 2011). The number of fruit bracts and the number of vein pairs per leaf have been also used for delimitation of *Carpinus* sections [1]. Number of vein pairs per leaf, leaf width, catkin length, petiole length, nutlet length and width have been also utilized for separation of different populations of *Carpinus betulus* in Iran and Poland [15, 16].

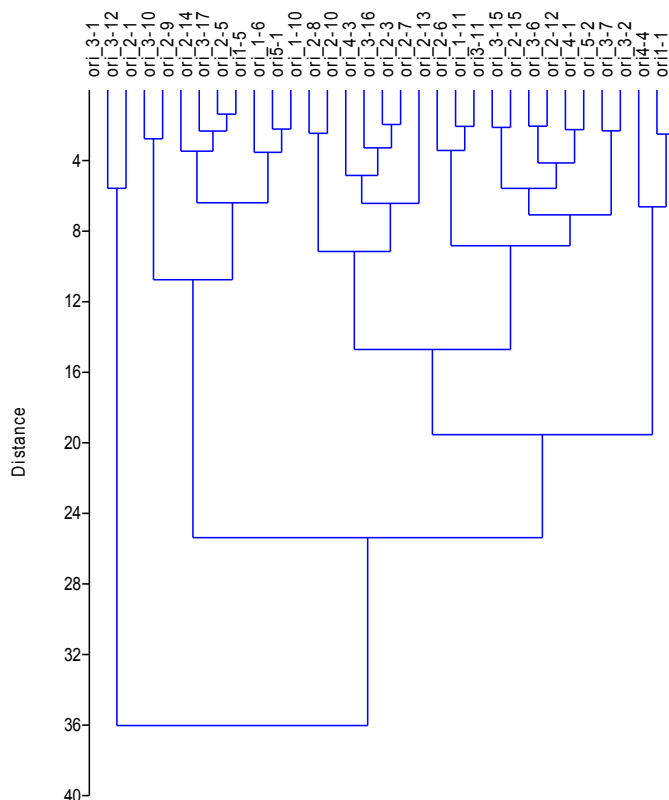


Fig. 2. A cluster analysis of 32 populations of *Carpinus orientalis* using Ward method. Abbreviations were given in Table 1.

In addition, a large variability was observed on the qualitative characteristics. Most studied populations had asymmetrical fruit bracts. However, a few of them possess symmetrical fruit bracts. The symmetry of fruit bract and leaf margin trichoms were considered as important features in the genus *Carpinus* [1]. Yoo & Wen [2] used nutlet shapes and their position regarding fruit bracts for separation of *Carpinus* and *Ostrya* genera.



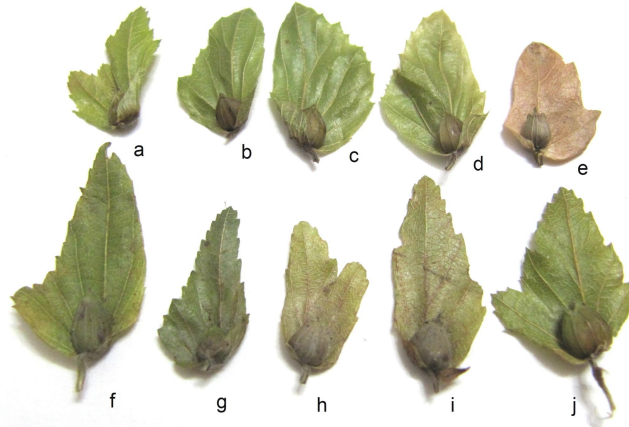


Fig. 5. Fruit bracts variation in different populations of *Carpinus orientalis* (a) ori1-10, (b) ori1-1, (c) ori2-3, (d) ori2-5, (e) ori3-12, (f) ori4-3, (g) ori4-1, (h) ori3-6, (i) ori3-7, (j) ori1-6.

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