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HIPPOPHAE RHAMNOIDES L.: FRUIT AND SEED MORPHOLOGY AND ITS TAXONOMIC PROBLEMS IN TURKEY

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Abstract

Hippophae rhamnoides, which comprises of 9 subspecies, has an extremely wide distribution but fragmentally in Eurasia. *H.rhamnoides* L. subsp. *caucasica* is the only known species growing in Turkey. In this paper, morphological traits of seed and fruit considered as diagnostic characters of *H.rhamnoides* L. subsp. *caucasica* in Turkey (Sivas, Trabzon, Ilgaz, Ürgüp) were analyzed in order to show whether there is taxonomical problems and try to reconstruct the relationship among the taxon of different regions, to test whether there is a significant association between the morphological characters measured and its environ. Photographs, fruit and seed dimension (length and width) and shape are recorded, using performed ANOVA, Duncan test, UPGMA cluster analysis, climatic diagrams. UPGMA cluster analysis showed that subsp. *caucasica* samples of Trabzon-Ilgaz regions form a branch and Sivas 94-Ürgüp another branch, while Sivas 96 samples form a complete different group. Climatic diagrams for study areas revealed that the differences, variations in the fruit and seed characters were not significantly correlated with ecological conditions. All results of this study imply that another taxon or taxa of *H. rhamnoides* is likely present in Turkey and also the specimens of these different regions can be differentiated on the basis of these features.

Introduction

The genus *Hippophae*, which belongs to the family Elaeagnaceae, is distributed between 27°-69° N latitude and 7°W to 122°E longitude (Rousi, 971; an *et al*., 989, Yu *et al*., 989). According to the last records, Lian *et al*., (2003a) described a new subspecies for *H. rhamnoides* (*Hippophae rhamnoides* subsp. *wolongensis* Y.S.Lian, K.Sun, & X.L.Chen) and therefore, this genus has reached 7 species and 9 subspecies (Sun *et al*., 2003).

Hippophae rhamnoides L., has an extremely wide distribution but fragmentally in Europe and Asia, from China, Mongolia, Russia, Kazakistan, Turkey, Romania, Switzerland, France to Britain and north to Finland, Norway and Sweden (Rousi, 1971; Rongsen, 1997; Lian *et al*., 2000; Bartish *et al*., 2000b, 2002), whereas *Hippophae rhamnoides* L., subsp. *caucasica* Rousi is the only known species growing in Turkey among the *Hippophae* L., taxa (Rousi, 1971; Browicz, 1986; Mc Kean, 1982). This plant has been distributed over Turkey at mainly North and East regions from the sea level up to high elevations of about 3000 m (Mc Kean, 1982).

During the last decades, many studies have been undertaken on this plant, concentrating on its agricultural, nutritional, medical and ornamental values (Süleyman *et al*., 2001, 2002; Gümüştekin, 2003; Gentili & Huss-Danell, 2002; Geetha *et al*., 2002; Gao *et al*., 2003; Rosch *et al*., 2003; Yang 2002; Yao & Tigerstedt, 1992; Öner & Abay, 2001). However, in spite of many molecular, taxonomic and phylogenetic studies, which

were made to identify the taxonomic problems (Bartish *et al*., 1999, 2000 a, 2000 b; Sun *et al*., 2002, 2003; Lian, 1988; Lian *et al*., 2003a, 2003b; Liu & He 1978; Yao & Tigerstedt, 1994; Yu *et al*., 1989), there are still taxonomical problems on the *H. rhamnoides*.

Several studies on *Hippophae rhamnoides* L., were performed in Turkey (Rousi 1971; Mc Kean, 1982; Bottema *et al*., (1995); Aras-Tayhan, 1995a, 1995b, 1997; Merev, 1998; Süleyman *et al*., 2001, 2002; Gümüştekin, 2003). Rousi (1971) reported that some Turkish specimens have unusually small leaves which are silvery on both surfaces, and also stem and spine characters, thus bearing a certain resemblance to subsp. *turkestanica* and this resemblance probably results from adaptation to aridity. However, subsp. *caucasica* specimens from the Bulgarian coast of the Black Sea represent a transition to subsp. *carpatica*.

It is reported that some morphological characteristics of pollen of subsp.*caucasica* in Turkey showed different features and also pollen grains collected from Trabzon showed hybride features; dimesions of the seeds from Trabzon and Sivas were similar, but their surface ornemantations of testa were higly different and some critical wood anatomical characteristics were different in wood specimens taken from different sites and based on these results, it was concluded that these differences were not from ecological conditions and there were taxonomical problems on *Hippophae rhamnoides* L., in Turkey (Aras-Tayhan, 1995a, 1995b, 1997). Aras-Tayhan also discussed that there would probably be different taxon, site races or taxa of *H. rhamnoides* in Turkey.

Rousi (1971) pointed that there is considerable racial variation within subsp. *rhamnoides,* which, however, is difficult to classify taxonomically, because of its clinal nature. In addition, Rousi (1971) reported that fruit characteristics vary considerably such as other morphological characteristics within subspecies and within populations of *H. rhamnoides.* However, he pointed out that fruit dimension and especially its shape were characteristic features in taxonomy.

On the basis of Rousi's findings, Trofimov (1961, 1967) clasified *H. rhamnoides* under four groups according to seed characters, and concluded with good reason that seed characters could be used as one of the main characters in its taxonomic division. Rousi (1965, 1971) stated that seed characteristics would succesfully be useful in racial diversitiy of the taxon.

In the present study, we investigated fruit and seed morphology by using main characters in the taxonomic division of *H. rhamnoides*, 1) to clarify taxonomically important of fruit and seed dimension, 2) to address whether there are taxonomical problems in *H. rhamnoides* which was collected from different geographical regions, 3) to try to reconstruct the relationship within the taxon, 4) to test whether there is a significant association between the morphological characters measured in the *H. rhamnoides* and its environ.

Material and Method

In this study, the mature seed and fruits of *H. rhamnoides* L., all collected from natural populations from Sivas (1), Sivas (2), Trabzon, Ilgaz and Ürgüp were used as research materials. Information about the sampling sites and their locations are given in Table 1 and Fig. 1.

Table 1.Some characteristics of the sampling sites.

Sampling sites	Latitude/ longitude	Altitude	Annual precipitation (mm)	Annual temperature (°C)	Climate type	Sampling date
Trabzon Esiroğlu Beldesi Değirmendere basin	$40^0$52'N $39^0$45'E	75 m	798	14.4	Semi-humid	1995
Sivas Sincan stream	$39^0$54'N– $37^0$59'E	2000 m	794	2.8	Humid	1994-1996
Ilgaz Bolu-Kastamonu Çamkur junction	$40^0$56'N– $33^0$36'E	1000 m	520	9.1	Semi-humid	1996
Ürgüp	$38^0$40'N– 34^o56'E	1060 m	383	10.2	Semi-dry	2003

Fig. 1. The sampling sites.

In the study, 30 measurements for fruit and 50 for seed dimensions (length and width) were performed by using a stereomicroscope with 0.01 mm sensitivity. The obtained results from our measurements were given comparatively with the results of subsp. *caucasica*, subsp. *turkestanica* and subsp. *carpatica* from Rousi (1971) and subsp. *caucasica* from Mc Kean (1982). Photography of fruit and seed was in transmitted light using a Wild Heerbrug microscope and a Pixelink digital camera. Digital images were adjusted (converted to grey-scale, brightness and contrast) in Adobe Photoshop.

Morphological distances between pairs of populations were calculated using average taxonomic distance. Cluster analysis (CA) was on the average taxonomic distance with the clustering method of Unweighted Pair Group Average (UPGMA), using the program MVSP v.3.12 a (Kovach, 2000). In addition, we performed ANOVA and Duncan test in SPSS to test differences in mean between independent samples.

Thornthwaite method (Erinç, 1962; Ardel *et al*., 1969) was used to find water balance and climate type of the sites and to interpret the statistical results in respect of ecological conditions.

Table 2. Mean and standard deviations of fruit and seeds from 5 different sites and their comparison with several subspecies from different sites.

subsp. *caucasica* from	FRUIT		SEED		
	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length/ width ratio
Trabzon	7.45- 8.63- 9.55	4.86- 5.65- 6.51	3.03- 4.15- 5.3	1.13- 2.04- 3.12	2.68- 2.03- 1.70
Sivas-94	6.17- 7.35- 8.34	4.16- 5.04- 5.86	2.86- 4.11- 5.47	1.75- 2.04- 2.31	1.63- 2.01- 2.37
Sivas-96	8.09- 9.16- 9.87	5.72- 6.21- 6.89	4.19- 5.64- 6.77	1.83- 2.31- 2.81	2.29- 2.44- 2.41
Ilgaz	6.86- 8.30- 9.63	4.85- 6.71-7.86	3.38- 4.72- 5.7	1.27- 2.14- 2.53	2.66- 2.58- 3.17
Ürgüp	5.5- 6.89- 7.7	4.85- 5.58- 6.59	3.03- 4.27- 5.3	1.62- 2.45- 3.54	1.87- 1.74- 1.50
subsp. *caucasica**	6-9	3-7	-	-	-
subsp. *caucasica***	7-8	6-7	3.5- 4.31- 5.3	1.7- 2.21- 3.0	1.5- 2.00- 2.8
subsp. *turkestanica***	6-10	6-8	2.7- 3.61- 4.2	1.5- 2.08- 2.5	1.5- 1.81- 2.1
subsp. *carpatica***	6-8	5-7	3.3- 4.21- 5.3	2.0- 2.27- 2.6	1.6- 1.87-2.3

*= From Mc Kean (1982); **= From Rousi (1971)

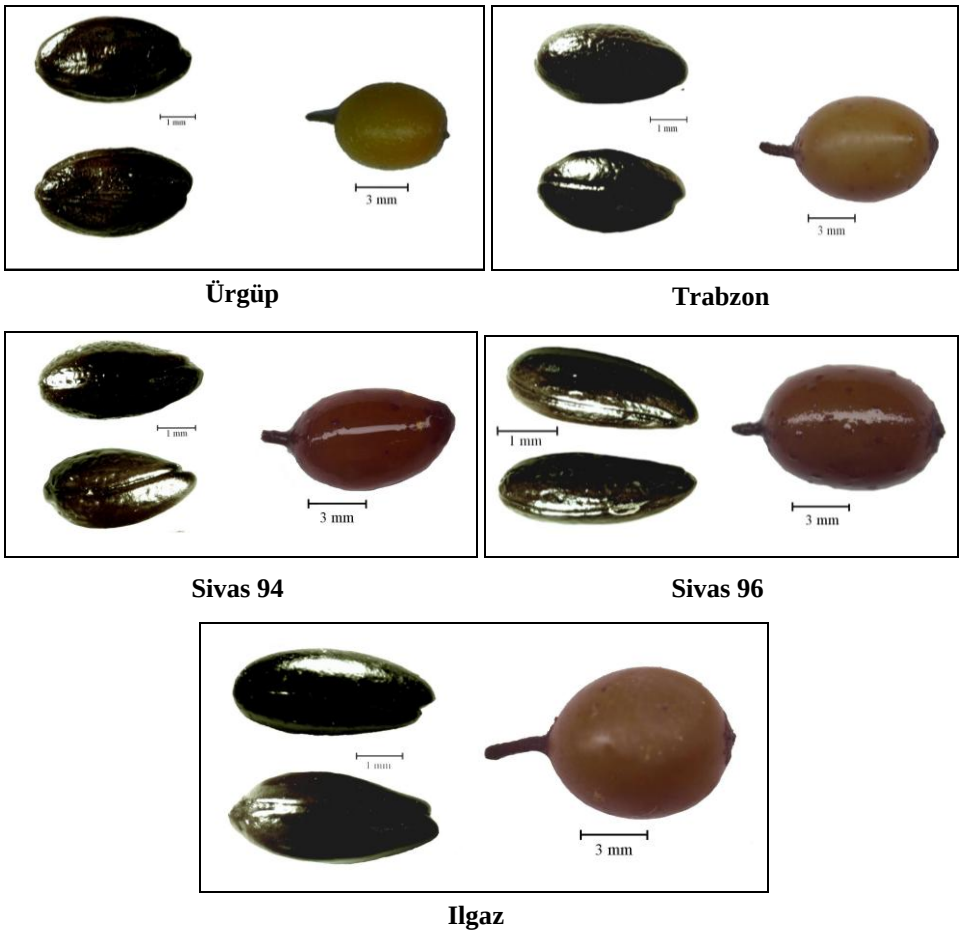

Fig. 2. Seeds (left ones in each cell) and fruits (right ones) of *Hippophae rhamnoides* subsp. *rhamnoides* from five different sites. Scale bars are 1 mm for seeds, and 3 mm for fruits.

Results

Results obtained from macromorphological studies of fruit and seed are presented in Table 2. In order to compare the measurements belonging to subsp.*caucasica*, subsp.*carpatica* and subsp.*turkestanica* offered by Rousi (1971) and subsp.*caucasica* by Mc Kean (1982) is also given. Shapes of fruits and seeds are shown in Fig. 2. Regarding size and according to our results, within those specimens belonging to subsp. *caucasica* collected from different regions in Turkey, fruit size ranges between 6.179.87 mm in length, 4.16 -7.86 mm in width and the shape of Ilgaz and Trabzon samples is elliptic, Ürgüp's is widely elliptic, Sivas 94's is elliptic-ovate and Sivas 96's is cylindrical. Seed size ranges between 2.86- 6.77 mm in length and 1.13–3.54 mm in width. The seed shapes are ovate-lanceolate in Ilgaz and Sivas 96, ovate in Sivas 94 and Trabzon and elliptic in Ürgüp. Only seeds of Ürgüp regions are flattened in shape and this case also made them quite different from the rest.

According to the ANOVA results for fruit length and width (Table 3a and 3c), F-value for fruit length was 84.137 ($p<0.000$) and for fruit width 41.675 ($p<0.000$). These results showed that fruit dimensions were statistically different among the sites. As can be seen from the Duncan Test results, fruit dimensions showed different groups (Table 3b and 3d).

Table 3. Results of ANOVA and Duncan test: A) ANOVA for fruit length; B) Duncan Test for fruit length; C) ANOVA for fruit width; D) Duncan test for fruit width.

A
ANOVA
Fruit length

	Sum of squares	df	Mean square	F	Sig.
Between Groups	103.837	4	25.959	84.137	0.000
Within Groups	44.737	145	0.309		
Total	148.574	149			

B
DUNCAN TEST
Fruit length

SITES*	N	Subset for alpha = 0.05				
		1	2	3	4	5
URFL	30	6.8910				
SI94FL	30		7.3530			
ILFL	30			8.3010		
TRFL	30				8.6260	
SI96FL	30					9.1630

C
ANOVA
Fruit width

	Sum of squares	df	Mean square	F	Sig.
Between Groups	49.065	4	12.266	41.675	0.000
Within Groups	42.678	145	0.294		
Total	91.743	149			

D
DUNCAN TEST
Fruit width

SITES**	N	Subset for alpha = .05			
		1	2	3	4
SI94FW	30	5.0443			
URFW	30		5.5757		
TRFW	30		5.6500		
SI96FW	30			6.2120	
ILFW	30				6.7107

*= URFL: Ürgüp, SI94FL: Sivas 94, ILFL: Ilgaz, TRFL: Trabzon, SI96FL: Sivas 96 fruit lengths
**= SI94FW: Sivas 94, URFW: Ürgüp, TRFW: Trabzon, SI96FW: Sivas 96, ILFW: Ilgaz fruit widths

ANOVA and Duncan Test results for seed dimensions can be seen in Table 4. ANOVA results (Table 4a and 4c) showed that mean dimensions of seeds were statistically different in various sites. F-values were 100.522 ($p<0.000$) for seed length and 23.926 ($p<0.000$) for seed width. These findings showed that seed dimensions were statistically different more than at the 0.001 confidence level. This difference was seen as different groups in Table 4b and 4d.

Table 4. Results of ANOVA and Duncan test: A) ANOVA for seed length; B) Duncan Test for seed length; C) ANOVA for seed width; D) Duncan test for seed width.

A
ANOVA
Seed length

	Sum of squares	df	Mean square	F	Sig.
Between Groups	82.087	4	20.522	100.119	0.000
Within Groups	50.218	245	0.205		
Total	132.305	249			

B
DUNCAN TEST
Seed length

SITES*	N	Subset for alpha = 0.05		
		1	2	3
SI94SL	50	4.1068		
TRSL	50	4.1516		
URSL	50	4.2678		
ILSL	50		4.7186	
SI96SL	50			5.6372

C
ANOVA
Seed width

	Sum of squares	df	Mean square	F	Sig.
Between Groups	6.296	4	1.574	23.926	0.000
Within Groups	16.117	245	0.066		
Total	22.413	249			

D
DUNCAN TEST
Seed width

SITES**	N	Subset for alpha = .05			
		1	2	3	4
SI94FW	50	2.0354			
URFW	50	2.0402			
TRFW	50		2.1622		
SI96FW	50			2.3066	
ILFW	50				2.4460

*= SI94SL: Sivas 94, TRSL: Trabzon, URSL: Ürgüp, ILSL: Ilgaz, SI96SL: Sivas 96 seed lengths
**= TRSW: Trabzon, SI94SW: Sivas 94, ILSW: Ilgaz, SI96SW: Sivas 96, URSW: Ürgüp seed widths

Water balance diagrams of the sampling sites can be seen in Fig. 6. Water deficit occurs between July-September in Trabzon, July-October in Ilgaz and Ürgüp, and July-August in Sivas. Climate types of these sites were given below:

- Trabzon; semi-humid, mesothermal, water deficit in summer and at the middle level,
- Ilgaz; semi-humid, mesothermal, water deficit in summer and at the high level,
- Ürgüp; semi-dry, mesothermal, no water exceed or very less,
- Sivas; humid, microthermal, water deficit in summer and at the middle level.

Discussion and Conclusion

In the genus *Hippophae*, *H. rhamnoides* is the only one with wide distribution in Eurosia. It was found to be extremely heterogeneous and problematic (Rousi 1971; Lian 1988; Lian *et al*., 2000).

As pointed out by Rousi (1971), subspecies of this species were not easily distinguished. However, in the taxonomic division of it, racial diversity of seed features can succesfully be used (Rousi, 1971; Trofimov, 1961, 1967). Rousi (1971) reported that fruit features within subspecies and within populations of *H. rhamnoides* were very variable, however, fruit length and shape were characteristic; subsp. *caucasica*, which was represented by a very small material, had characteristic fruit shapes of its own; size of subsp. *rhamnoides* fruits is larger than in the other subspecies and also their cylindirical shape also made them quite different from the rest.

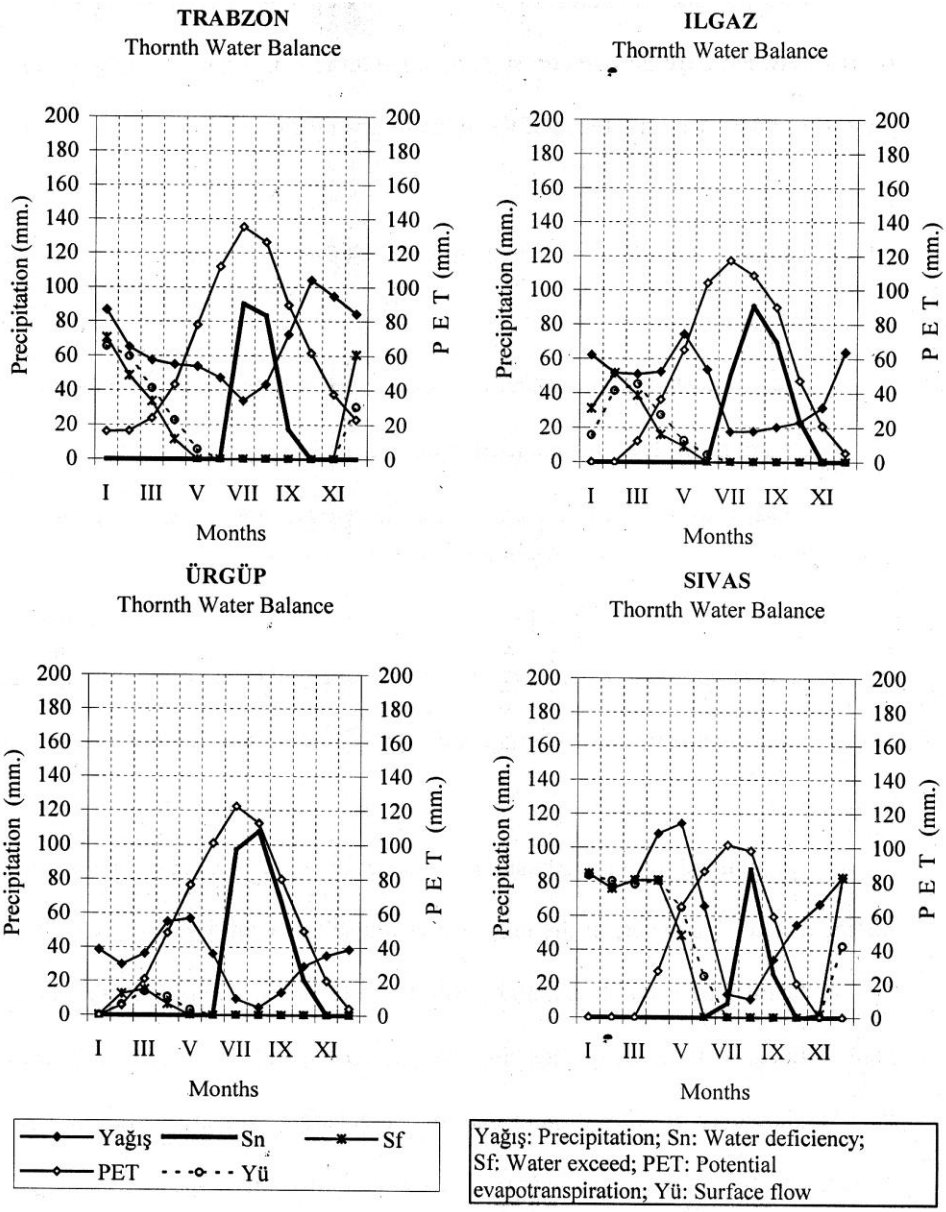

Fig. 3. Climatic diagrams for the sampled sites.

The ANOVA result showed that the F values are higher in fruit and seed lengths (84.137 for fruit length and 100.119 for seed length). In the UPGMA cluster analysis, three clades can be seen (Fig. 3): Trabzon-Ilgaz (dissimiliriate 0.718), Sivas 94-Ürgüp (0.549) and Sivas 96. Based on the results we can conclude that at least the seed and fruit collected from Sivas 96 were statistically different from those in other sites.

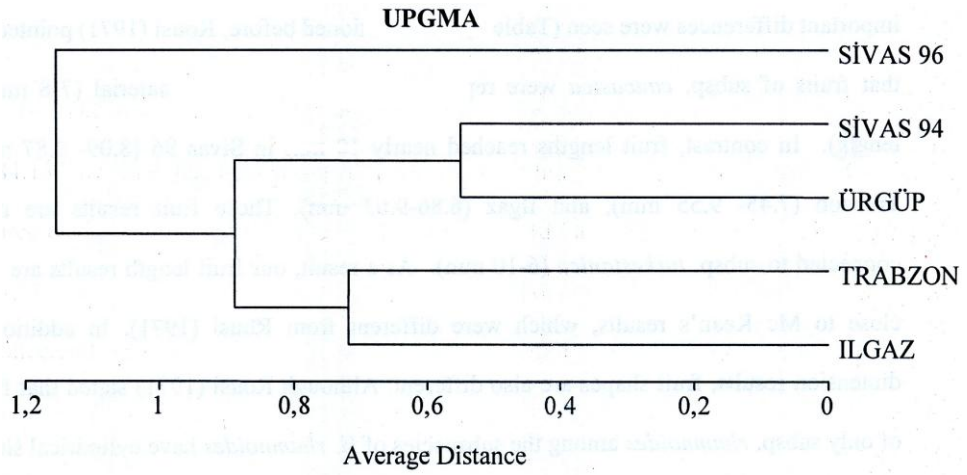

Fig. 4. Dendrograms from cluster analysis for seed dimensions.

Due to having a great horizontal and vertical distribution area, ecological amplitude of subsp.*caucasica* is very wide. Rousi (1971) stated that the differences in some characters of ssp., *caucasica* in Turkey could be caused from ecological conditions such as aridity. In the climate diagrams (Fig. 3) prepared to see whether the cause of the variations in fruit and seed dimensions are really climatic conditions (especially aridity), specimens from Sivas 94 (2000 m a.s.l.) and Ürgüp (1060 m a.s.l.), which are ecologically different sites, were morphologically close to each other. Similar results could be seen in specimens from Trabzon (75 m a.s.l.) and Ilgaz (1000 m a.s.l.). However, it is quite intriguing that specimens from Sivas 96 (2000 m a.s.l.) and Sivas 94, in which conditions are the same, located at the furthest clades in the Cluster Analysis. These data showed that the reasons for the variations in the fruit and seeds are not ecological conditions, but probably stem from taxonomical problems of *H. rhamnoides*.

Our results when compared with those of Rousi (1971) and Mc Kean (1982), important differences were seen (Table 2). As mentioned before, Rousi (1971) pointed out that fruits of subsp. *caucasica* were represented by a very small material (7-8 mm in length). In contrast, fruit lengths reached nearly 10 mm in Sivas 96 (8.09- 9.87 mm), Trabzon (7.45- 9.55 mm), and Ilgaz (6.86-9.63 mm). These fruit results are more connected to subsp. *turkestanica* (6-10 mm). As a result, our fruit length results are very close to Mc Kean's results, which were different from Rousi (1971). In addition to dimension results, fruit shapes are also different. Although Rousi (1971) stated that fruits of only subsp. *rhamnoides* among the subspecies of *H. rhamnoides* have cylindrical shape, in Sivas 96 fruits have also the same shape.

As for seed length, those of Sivas 96 are statistically different with 4.19-6.77 mm length from other ones. Those of Ürgüp, Trabzon and Sivas 94 are very close to subsp. *caucasica*. As can be seen from Rousi's results for fruit and seed length (Table 2), subsp. *caucasica* and subsp. *carpatica* are very close to each other. From Rousi's (1971) results of fruit and seed length, it is seen that results of subsp. *caucasica* and subsp. *carpatica* are very close, revealing that not only subsp. *caucasica*, but also subsp. *carpatica* should exist in Turkey.

On the other hand, it can be seen that two conclusions arise from the results of our investigation: either 1) subsp. *caucasica* has a greater morphological variation than mentioned so far, or 2) subsp. *caucasica* is not the only subspecies, and probably there is another taxa of *H. rhamnoides*. However, in previous reports (Aras-Tayhan 1995a, 1995b, 1997) a lot of characters of subsp. *caucasica* were found to be significantly different in samples collected from different regions Furthermore, especially hybrid features of pollen grains collected from Trabzon support the idea that there is another taxa *H. rhamnoides* other than subsp. *caucasica*.

Differences in fruit and seed features, as diagnostic characters, require taxonomic and phylogenetic studies by using molecular markers and taxonomic revision should be done in and around Turkey to solve the problems of the classification of *H. rhamnoides* and its subspecies.

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