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IMPACT OF TUBER LOCATION ON MORPHOLOGICAL CHARACTERISTICS OF *CYCLAMEN*

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Abstract

In *Cyclamen genus*, there are differences at some degree of morphological characters among closely related wild species with disjunct distributions. Two hundred and fiveTurkish landraces of cyclamen coming from different areas containing different temperature, altitudes, soil and vegetation, relative humidity and light intensity were evaluated for 12 quantitative morphological traits of flowers and leaves such as petal length, petal width, flower number/plant, pedicel length, basal corolla ring diameter, pistil length, stamen length, leaf number/plant, petiole length, lamina length, lamina width, plant growth habit, in *Cyclamen mirabile*, *Cyclamen alpinum* and *Cyclamen hederifolium.* According to the location of species, all three species showed morphological differentiation in two traits (pedicel length and petiole length). *C. mirabile* showed high levels of morphological differentiation from other species (in eight traits out of 12, as 66,7%), followed by *C. hederifolium* (in six traits out of 12, as 50%) and *C. alpinum* (in four traits out of 12, as 33,3%).

Key words: *Cyclamen mirabile*, *Cyclamen alpinum*, *Cyclamen hederifolium*, Morphological differentiations.

Introduction

More than 500 geophyte species grow naturally in Turkey and the bulbs of the majority of these are exported. The genus Cyclamen consists of 22 species (Grey-Wilson, 2002), and is distributed in and near the Mediterranean region especially Greece and Turkey. Cyclamen species have been used as ornamentals (primarily as indoor, winter blooming potted plants and to a less extent as outdoor plants) for the last 400 years or so (Ishizaka, 2008).

Anderberg (1994) and Grey-Wilson (2002) made subgenus classification in the genus, whereas the classifications did not coincide in some points between the reports. Anderberg *et al.* (2000) made a molecular phylogenetic study of the genus Cyclamen by DNA sequence, and suggested four monophyletic subgroups from the sequence data and morphological data in the genus.

The Mediterranean basin is a major centre of plant endemism (Medail & Quezel, 1997) and population differentiation (Thompson, 1999). The occurrence of high numbers of endemic species, particularly on islands and in mountain ranges in the Mediterranean region, attests to the high levels of geographic differentiation that occurs in the flora. Many species have disjunct distributions such that geographically isolated populations may also exhibit high levels of differentiation. The extent to which such differences among populations compares with differences among what are suggested to be different but closely related endemic taxa in the Mediterranean flora is an issue which has recently attracted attention (Debussche & Thompson, 2002). This issue is particularly important in order to identify and delimit taxa which merit conservation status (Olfelt *et al.*, 2001). In fact, for only a few endemic and protected species we do have information concerning levels of population differentiation (e.g. Affre & Thompson, 1997; Affre *et al*., 1997; Freville *et al*., 1998; Petit *et al*., 2001) and amounts of morphological variation among closely related sister taxa (Debussche & Thompson, 2002).

There was little study focusing on the impact of the tuber location of cyclamen on its morphologicial characteristics. However, there is argument and some empirical evidence in the literature suggesting that the location or the enviroment of the plant would have an impact on its morphologicial characteristics. Muhammad *et al*. (2016), examined the comparative ecophysiology of seeds germination in two pairs of realted pines of Mediterranean and Himalayan, concluded that, the differences observed in the germination behaviour of the pines may be attributed to varying ecophysiological strategies evolved in response to the particular geographical distribution of a species. The result of study on Jarusalem artichocke revealed that reproductive and tuberization development varied based on the rain (Paungbut *et al.*, 2015).

Cyclamen genus is represented by 12 species in Turkey and 6 of them are endemic to Anatolia (*C. pseudibericum* Hildebr., *C. trochopteranthum* O. Schwarz, *C. parviflorum* Pobed., *C. cilicium* Boiss.e.Heldr., *C. cilicium* var. *intaminatum* Meikle Grey-Wilson, and *C. mirabile* Hildebr.) (Davis, 1978; Güner *et al*., 2000).

Cyclamen species are long-lived, perennial herbs with a tuberous rootstock and lack vegetative reproduction. The long-petiolate leaves arise directly from the tip of the floral trunk that emerges from the upperside of the tuber. The hermaphroditic flowers are solitary and pendent at the tip of an erect leafless pedicel emerging from the floral trunk. They do not produce nectar (Grey-Wilson, 1988). The corolla is divided into five reflexed lobes and the five sepals are fused at the base. The anthers are positioned inside the corolla and attached to the base of the flower by a very short filament. Anther dehiscence is introrse and pollen is mainly liberated through an apical pore. The superior ovary contains numerous ovules. After fertilization the corolla falls and the erect pedicel coils from the apex downwards bringing the capsule to the substrate surface where the brownish seeds are liberated and dispersed by ants (Gündoğan, 2003).

There have been a number of researches undertaken to identify the types of cyclamen. Most of these studies classify the type of cyclamen depending on the similarities and differences of both genotype and morphological traits. *C. alpinum*, one of the species have been studied, there were no significant differentiation between locations of the species and quantitative morphological traits e.g. tuber diameter, height and weight, leaf and flower numbers per plant, lamina length and width (Şahin & Bürün, 2010). Ecological characteristics and habitat differences of two congeneric cyclamen species (*Cyclamen balearicum* and *C. repandum*) were quantified in a survey of 19 ecological variables linked to climate, topography, soil and vegetation at 125 sites (Debussche & Thompson, 2003). Debussche *et al.* (2004), examined variation in the phenology and morphology of 17 species of the genus *Cyclamen* cultivated in uniform garden conditions, have shaped with climatic factors and phylogenetic constraints and quantify the extent to which traits differ among subgenera and thus represent conserved traits within evolutionary lineages. However, the same cyclamen genotypes should show similar morphological features if cultivation conditions were the same. Given that the cultivation condition of cyclamen was the same, the genotypes of the same cyclamen species should show different morphological traits regarding their location of plant sampled. The reason of these differences could be weather conditions, genetic drift, etc. Therefore, the following three hypothesises in the nul form are developed and tested in this study: Ho1. Morphological traits of *C. mirabile* from two different locations are not different, Ho2. Morphological traits of *C. alpinum* from two different locations are not different, Ho3. Morphological traits of *C. hederifolium* from two different locations are not different.

The aim of this research was to analyse the differences of morphological traits of the same cyclamen species of which tubers were sampled from two different locations and grown in the same controlled environment (greenhouse conditions).

Material and Methods

Three cyclamen species (*C. alpinum*, *C. hederifolium* and *C. mirable;* figures 1, 2 and 3 respectively) were analysed in this study and locations and numbers of tubers were sampled from each species presented in table 1.

Cyclamen plants have been collected from natural environments in autumn 2010 and in spring 2011, were cultivated at the same controlled environment (greenhouse condition), controlling the factors likely to effect the growth of cyclamen, (types of soil, temperature, light, etc.) in Adana. Their propagation and cultivation processes such as prunning roots and planting into pots containing (1:1:1) peat/perlit/sand (v:v:v), placed on to the bench in the greenhouse, using net for shading when necessary, watering manually 300ml/week and fertilizing with 0, 2% solution of N, P, K (%20-% 20 - % 20 + trace elements, respectively) were carried out.

Cyclamen species were characterized morphologically one year later in regenerated plants. In these plants, total of 12 quantitative morphological traits (petal length, petal width, flower number/plant, pedicel length, basal corolla ring diameter, pistil length, stamen length, leaf number/plant, petiole length, lamina length, lamina width, plant growth habit) of each cyclamen genotypes were measured during the flowering period according to Çürük *et al*. (2015).

Both parametric Anova and it's non parametric alternative Mann-Whitney U test were run to test the hypothesis, i.e. find out the comparison of morphological traits of cyclamen species depending on the locations of their tuber (Kalıpsız, 1981; Özdamar, 2002).

Fig. 1. *C. alpinum:* A), B) Plants in nature and in pots, C) Fruits of the species, D), E) Abaxial and adaxial sides of the leaves.

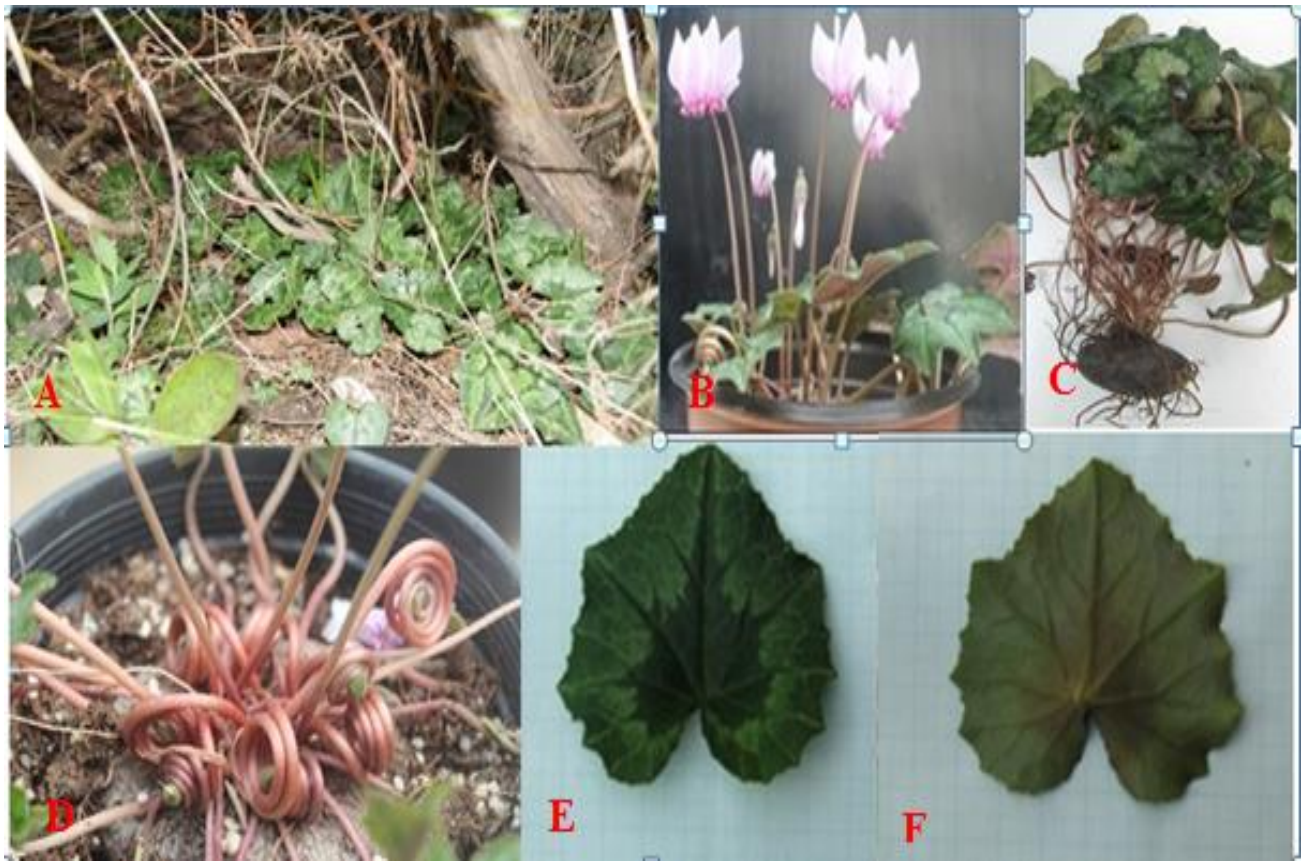

Fig. 2. *C. hederifolium*: A), B) Plants in nature and in pots, C) Intact plant, D) Fruits of the species, E), F) Abaxial and Adaxial sides of the leaves.

Fig. 3. *C. mirabile*: A), B) Plants in nature and in pots, C) Intact plant, D) Fruits of the species, E), F) Abaxial and adaxial sides of the leaves.

Table 1. List of the Cyclamen species locations and number of tubers sampled

Species	Number of sample	Collection site	Sampling date	Coordinates	Elevation (m)	Vegetation
Cyclamen hederifolium	34	Meze Köyü, Köşk / AYDIN Ilıdağ Köyü, Köşk / AYDIN	07.12.2010	37°54′24″N 28°03′32″E	289	*Pinus brutia*, *Quercus ilex* forest and evergreen shrubland
	45	Dağkaraağaç Köyü, Germencik / AYDIN	20.12.2010	37°56′45″N 28°02′37″E	541	
	40	Meryemana Yolu, Selçuk / İZMİR	22.12.2010	37°54′00″N 27°36′27″E	159	
	31		22.12.2010	37°55′24″N 27°20′53″E	197	
Cyclamen mirabile	36	Barla / ISPARTA	16.12.2010	38°01′00″N 30°47′00″E	1085	Woodland and shrubland dominated by *Platanus orientalis* and *Pinus brutia*
	20	Yeşilyurt Köyü, Karacasu / AYDIN	21.12.2010	37°40′22″N 28°40′57″E	575	
Cyclamen alpinum syn. *C. trochopteranthum*	39	Cankurtaran Mevkii / DENİZLİ	14.04.2011	37°41′37″N 29°12′30″E	1400	Woodland and shrubland dominated by *Pinus brutia*, *Liquidambar orientalis*, *Laurus nobilis* and *Ceratonia siliqua*
	20	Sakarca Mevkii, Kızılkaya Köyü, Köyceğiz / MUĞLA	15.04.2011	36°51′53″N 28°51′43″E	570	

Table 2. Differences in morphological characters of *C. mirable* depending on the locations.

Traits		Mean	Std. Deviation	Minimum	Maximum	Anova	
						F	Sig.
Petal length (mm)	Isparta	18,4672	2,03495	14,21	22,98	0,952	0,334
	Aydın	17,8865	2,30544	13,48	23,27		
	Total	18,2598	2,13311	13,48	23,27		
Petal width (mm)	Isparta	6,4336	,99069	5,03	9,08	10,586	0,002
	Aydın	7,2790	,81174	5,44	8,61		
	Total	6,7355	1,00959	5,03	9,08		
Flower number/plant	Isparta	8,81	6,637	1	28	14,514	0,000
	Aydın	3,05	1,504	1	6		
	Total	6,75	6,046	1	28		
Pedicel length (cm)	Isparta	11,15	2,840	6	20	8,193	0,006
	Aydın	9,06	2,180	3	13		
	Total	10,40	2,793	3	20		
Basal corolla ring diameter (mm)	Isparta	3,1256	0,58317	2,22	4,46	2,165	0,147
	Aydın	2,8860	0,58496	2,12	4,90		
	Total	3,0400	0,58995	2,12	4,90		
Pistil length (mm)	Isparta	5,5769	1,08566	3,85	8,03	5,643	0,021
	Aydın	4,9405	0,67222	4,17	6,82		
	Total	5,3496	1,00042	3,85	8,03		
Stamen length (mm)	Isparta	4,6731	0,55532	3,58	5,72	4,818	0,032
	Aydın	4,3395	0,52508	3,10	5,00		
	Total	4,5539	0,56347	3,10	5,72		
Leaf number/plant	Isparta	18,08	7,640	5	37	13,817	0,000
	Aydın	11,10	4,633	3	21		
	Total	15,59	7,480	3	37		
Petiole length(cm)	Isparta	8,044	2,5739	3,7	12,8	4,706	0,034
	Aydın	9,687	2,9551	5,5	17,0		
	Total	8,631	2,8041	3,7	17,0		
Lamina length (cm)	Isparta	3,081	0,5880	1,7	4,6	0,173	0,680
	Aydın	3,150	0,6134	2,2	4,3		
	Total	3,106	0,5926	1,7	4,6		
Lamina width (cm)	Isparta	2,861	0,5281	1,6	3,9	3,550	0,065
	Aydın	3,150	0,5862	2,1	4,3		
	Total	2,964	0,5618	1,6	4,3		
Plant growth habit	Isparta	1,0761	0,05288	0,98	1,20	24,242	0,000
	Aydın	0,9985	0,06268	0,89	1,13		
	Total	1,0484	0,06741	0,89	1,20		

Note: Bolded characters showed differences at the 0.05 significance level

Results

Descriptive statistics together with Anova and Mann-Whitney U test results were compared to identify whether there were significant differences in 12 morphological traits of three cyclamen species, *C. mirable*, *C.alpinum* and *C.hederifolium* are shown in tables 2, 3 and 4 respectively.

The test results tend to reject the H01 for 8 out of 12 morphological traits of *C. mirable* analysed in this study. The results showed that only petal length, basal corolla ring diameter, lamina length and lamina width of the *C. mirable* genotypes collected from Isparta were not significantly different from those traits of the same cyclamen species collected from Aydın, at the 0.05 significance level. Only average petal width and petiole length of *C. mirable* collected from Aydın were found to be significantly greater than those traits of *C. mirabile* collected from Isparta. On the other hand, it is rather interesting that the average number of flowers per plant, petiole length, pistil length, stamen length, leaf numbers per plant of the *C. mirable* collected from Isparta was found to be significantly greater than those traits of the same cyclamen sp. from Aydın. These results indicated that most of the morphological traits of *C. mirable* showed differences significantly (p<0.05) depending on the location of plants taken because of climatic factors, altitudes, temperatures, light intensity, facing north or south of the collecting site.

The statistical test results tend to reject the H02 only for 4 out of 12 morphological traits of *C. alpinum* analysed in this study, shows that pedicel length, petiole length, lamina length and lamina width of *C. alpinum* of which tubers were sampled from Muğla were significantly different (greater) than those traits of *C. alpinum* of which tubers were sampled from Denizli at 0.05 significance level (Table 3). It is rather interesting that average petiole length of *C. alpinum* from Muğla (19,19 cm) was found to be almost 3,11 times greater than those of *C. alpinum* from Denizli (5,97 cm). However, majority of the other morphological characters analysed in this study (8 out of 12) of *C. alpinum* of which plants were sampled from two different locations were not significantly different (H02 were not rejected 8 out of 12 morphological characters of *C. alpinum*). These results suggest that even though most of the morphological traits of *C. alpinum* of which plants were sampled from two different location were not significantly different, still some morphological traits, especially petiole length changes significantly depending on the location of plants were taken. Şahin & Bürün, (2010) mentioned that there were no significant differentiation between locations of the *C. alpinum* species and quantitative morphological traits e.g. tuber diameter, height and weight, leaf and flower numbers per plant, lamina length and width.

Anova test results as regards to morphological characters of *C. hederifolium* genotypes were sampled from Aydın and İzmir but cultivated at the same controlled environment showed that (Table 4) six out of 12 morphological characteristics Cyclamen from Aydın and İzmir were not the same (significantly different p<0.05). That is, H03 were rejected for six out of 12 morphological characters of *C. hederifolium*. The interesting results shown on table 4 is that however the mean score of petal length, number of flowers and leaves per plant of *C.hederifolium* from Aydın were greater than those of *C. hederifolium* from İzmir, pedicel length, stamen length and petiole length of *C. hederifolium* from İzmir were greater than those of *C. hederifolium* from Aydın. These results also indicated that considerable numbers of the morphological characters of *C. hederifolium* changed significantly depending on the location of plants taken.

Discussion

Most of the morphological traits of *C. mirable* showed differences significantly (p<0.05) depending on the location of plants taken because of climatic factors, altitudes, temperatures, light intensity, facing north or south of the collecting site. Because Isparta is located at the inland or continental part of the country and the altitude is higher than that of Aydın (Mammadow *et al.*, 2016), it has continental climate. Thefore the growth of plants, the sizes of petal and leaves, the length of pedicle and petiole decreased in Isparta. Temperature is also one of the effective factors for plant growth. Because the temperature in Aydın is higher than that of Isparta, plant morphology is affected positively (Olcay *et al*., 2013). The occurrence of high numbers of endemic species, mainly on islands and in mountain ranges in the Mediterranean region, affirms to the high levels of geographic differentiation that occurs in the flora. Many species have disjunct distributions such that geographically isolated populations could also exhibit high levels of differentiation (Debussche & Thompson, 2002; Debussche *et al*., 2004).

Debussche & Thompson, (2003) found that ecological characteristics and habitat differences of two congeneric plant species were quantified in a survey of 19 ecological variables linked to climate, topography, soil and vegetation at 125 sites. One of these species, *Cyclamen balearicum* (Primulaceae), is endemic to the Balearic Islands and southern France, the other, *C. repandum*, is more widely distributed across the Mediterranean basin. How climatic factors and phylogenetic constraints have shaped variation in the phenology and morphology of 17 species of the genus *Cyclamen* cultivated in uniform garden conditions examined. The researchers quantify the extent to which traits differ among subgenera and thus represent conserved traits within evolutionary lineages (Debussche *et al*., 2004).

As there is not many studies running for identifying the effect of location of tuber of cyclamen on their morphological characteristics, it is not possible to compare the result of this study with other studies. Furthermore, literature on research on possible factors influencing the morphological traits of cyclamen is also limited, it may not possible to identify the exact reasons of the differences among the morphological traits of cyclamens found in this study based on the previous study. It may, however, possible that the size of tuber taken from different location may be one of the factors causing such differences. One of the main limitations of

this study is that the size of tuber could not be controlled. Therefore, further research may be undertaken to identify if the location of tuber of cyclamen is an important factors affecting the morphological traits by controlling the tuber size. In Çürük *et al*. (2015)'s study, the PCA result according to morphological characterisation showed that different Cyclamen species originated from a similar geographical area or the same species originated from different geographical areas were placed into the different groups. Zhao *et al*. (2007) found that the lack of conformity between the genetic and geographical variation exists because of an exchange of genetic material, the introduction of new accessions, genetic drift and natural selection or human interference. Persson *et al*. (2006), also indicated that location influenced on some the morphological traits of rye genotypes. Altundağ *et al*. (2012) mentioned that plants were nearly always restricted to specific geographic and climatic zones, in areas with great topographical and climatic variation.

As in most geophytes (e.g. Dafni *et al*., 1981; Halevy, 1990), seasonal variation in temperature may play a significant role in the timing of flowering in the genus *Cyclamen*. The climatic variables related with extreme temperatures were more effective than average temperatures or rainfall to explain the morphological variability (L´azaro *et al*., 2001).

In conclusion, the results of this study (under the above mentioned limitation) indicated that location of the plant could be the most important factor effecting the morphological traits of cyclamen. According to the location of species, all three species (*C. mirabile, C hederifolium, C. alpinum*) showed morphological differentiation in two traits (pedicel length and petiole length). *C. mirabile* showed high levels of morphological differentiation from other species (in eight traits out of 12, 66, 7%), followed by *C. hederifolium* (in six traits out of 12, as 50%) and *C. alpinum* (in four traits out of 12, as 33, 3%). Therefore, location of plant grown in different temperature, altitudes, soil types and vegetation, relative humidity and light intensity should be considered in cyclamen cultivation.

Table 3. Differences in morphological characters of *C. alpinum* depending on the locations.

Traits		Mean	Std. Deviation	Minimum	Maximum	Anova	
						F	Sig.
	Denizli	**10,5854**	**1,56759**	**8,02**	**13,60**	**3,611**	**0,062**
Petal length	Muğla	11,4465	1,79689	8,90	14,58		
	Total	10,8773	1,68426	8,02	14,58		
	Denizli	8,9328	0,99307	6,88	10,85	1,324	0,255
Petal width	Muğla	9,2845	1,31596	6,78	11,81		
	Total	9,0520	1,11428	6,78	11,81		
	Denizli	3,64	2,641	1	14	0,224	0,638
Flower number/plant	Muğla	3,30	2,577	1	9		
	Total	3,53	2,602	1	14		
	Denizli	**7,367**	**2,0020**	**4,0**	**14,3**	**33,890**	**0,000**
Pedicel length	Muğla	11,313	3,1944	7,0	16,7		
	Total	8,705	3,0851	4,0	16,7		
	Denizli	3,3023	0,44566	2,48	4,85	0,494	0,485
Basal corolla ring diameter	Muğla	3,3795	0,28554	3,03	3,87		
	Total	3,3285	0,39773	2,48	4,85		
	Denizli	5,1123	0,67551	3,79	7,21	2,509	0,119
Pistil length	Muğla	4,8405	0,50511	4,03	6,12		
	Total	5,0202	0,63197	3,79	7,21		
	Denizli	4,8100	0,79256	3,30	7,21	0,007	0,934
Stamen length	Muğla	4,8260	0,44200	4,03	5,69		
	Total	4,8154	0,68964	3,30	7,21		
	Denizli	9,08	4,343	3	19	2,064	0,156
Leaf number/plant	Muğla	10,65	3,133	6	16		
	Total	9,61	4,017	3	19		
	Denizli	**5,97**	**2,145**	**3**	**12**	**206,607**	**0,000**
Petiole length	Muğla	19,19	4,933	9	26		
	Total	10,45	7,128	3	26		
	Denizli	**2,751**	**0,5246**	**1,8**	**4,7**	**7,359**	**0,009**
Lamina length	Muğla	3,195	0,7147	1,8	4,7		
	Total	2,902	0,6265	1,8	4,7		
	Denizli	**2,863**	**0,6653**	**1,6**	**4,5**	**4,259**	**0,044**
Lamina width	Muğla	3,252	0,7233	1,8	4,8		
	Total	2,995	0,7042	1,6	4,8		
	Denizli	0,9777	0,12698	0,77	1,33	0,045	0,833
Plant growth habit	Muğla	0,9840	0,05567	0,86	1,08		
	Total	0,9798	0,10765	0,77	1,33		

Table 4. Differences in morphological traits of *C. hederifolium* depending on the locations.

Traits		Mean	Std. Deviation	Minimum	Maximum	Anova	
						F	Sig.
	Aydın	**20,8550**	**3,31925**	**11,79**	**27,73**	**4,448**	**0,037**
Petal length	İzmir	19,3932	3,86678	7,61	24,86		
	Total	20,5529	3,47683	7,61	27,73		
	Aydın	9,0903	1,52033	5,02	12,92	0,014	0,908
Petal width	İzmir	9,1266	1,72660	4,43	13,12		
	Total	9,0980	1,56033	4,43	13,12		
	Aydın	**7,8319**	**5,60919**	**1,00**	**29,00**	**6,293**	**0,013**
Flower numbers/plant	İzmir	5,2500	2,94027	1,00	11,00		
	Total	7,2848	5,25912	1,00	29,00		
	Aydın	**10,9166**	**2,94927**	**3,00**	**17,00**	**13,069**	**0,000**
Pedicel length	İzmir	13,0334	2,90748	6,33	17,70		
	Total	11,3652	3,05664	3,00	17,70		
	Aydın	6,0439	0,88926	3,72	8,45	0,601	0,439
Basal corolla ring diameter	İzmir	5,9066	0,89093	4,61	8,25		
	Total	6,0148	0,88842	3,72	8,45		
	Aydın	7,29252	0,801666	4,030	9,770	1,462	0,229
Pistil length	İzmir	7,08562	1,050229	4,940	9,410		
	Total	7,24868	0,860646	4,030	9,770		
	Aydın	**5,1700**	**0,76293**	**2,15**	**7,53**	**5,609**	**0,019**
Stamen length	İzmir	5,5241	0,70244	4,45	7,13		
	Total	5,2450	0,76220	2,15	7,53		
	Aydın	**14,4622**	**7,19556**	**3,00**	**35,00**	**13,720**	**0,000**
Leaf numbers/plant	İzmir	9,5000	4,52235	4,00	21,00		
	Total	13,4106	7,00692	3,00	35,00		
	Aydın	**8,6824**	**3,04591**	**2,30**	**19,00**	**31,180**	**0,000**
Petiole length	İzmir	12,3084	3,97496	5,50	20,20		
	Total	9,4508	3,57412	2,30	20,20		
	Aydın	5,2815	1,45520	2,00	8,80	0,134	0,714
Lamina length	İzmir	5,1803	1,08624	3,40	7,40		
	Total	5,2601	1,38255	2,00	8,80		
	Aydın	4,7025	1,35456	1,60	8,00	0,000	0,985
Lamina width	İzmir	4,7072	0,97731	3,00	6,70		
	Total	4,7035	1,28093	1,60	8,00		
	Aydın	1,1382	0,15538	0,94	2,28	1,791	0,183
Plant growth habit	İzmir	1,1003	0,07377	0,90	1,25		
	Total	1,1302	0,14269	0,90	2,28		

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