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Full Length Article

Leaf Size Variation in Natural Wild Cherry (*Prunus avium*) Populations in Turkey

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Abstract

Variation in leaf size in relation to population location was investigated in 25 natural wild cherry (*Prunus avium* L.) populations in Turkey. A total of 25 populations were identified across Turkey. In each population 20 trees were selected and 20 fully expanded leafs were collected from each tree. Petiole length (P) and lamina length (L) and width (W) were measured. All, but one, of the populations are in northern Anatolia, at elevations from 59 to 1900 m. Mean annual temperature and precipitation range from 4.1 to 13.9°C and from 609 to 1051 mm, respectively. Climate types at each location range from humid to semi-arid. On population basis, large and significant amount of variation in L (9.75 – 13.62 cm), W (5.34 – 7.39 cm) and P (2.48 – 3.39 cm) were observed. Greater L and W values were observed in populations at lower elevations with higher mean annual temperatures. Similarly, increased precipitation was associated with larger leafs, but leaf size was significantly smaller at locations with higher precipitation between April to August. The populations can be grouped as inland and coastal based on the results of the hierarchical cluster analysis. More than 40% of the total variation in leaf size was explained by differences among populations and trees within populations. Until through genetic studies are conducted, variation in moderately heritable leaf traits can be used to discern *P. avium* populations for conservation purposes in Turkey. © 2018 Friends Science Publishers

Keywords: *Prunus avium*; Wild cherry; Population differentiation; Gene conservation; Geographic variation

Introduction

Natural distribution of wild cherry (*Prunus avium* L.) is in temperate forest regions of Europe, Anatolia and proximal regions of the North African Maghreb, and western Asia (Welk *et al*., 2016). In Turkey it is found in the Black Sea region as individual trees or small groups mixed with other forest tree species such as *Castanea sativa*, *Acer* spp. *Carpinus betulus*, *Abies* sp., *Fagus orientalis*, *Fraxinus* spp., *Quercus* spp. and *Picea orientalis* (Esen *et al*., 2012).

In recent years there is a growing interest in the species due to fast growth, valuable timber and environmental services it provides in the ecosystems where *P. avium* is present. *P. avium* is a shade-intolerant species with individuals reaching up to 120 cm in diameter and 35 m in height. Its height and diameter growth is rapid, volume yield is high and stands can be managed with 65–75 years rotations. On good sites it can reach up to 9.1 m^3 ha^{-1} mean annual increment (Pryor, 1988). Wood of *P. avium* is decorative and has favorable mechanical properties for wood working resulting in high demand (Savill *et al*., 2009). These factors resulted in increased *P. avium* cultivation (Russell, 2003) and initiation of several breeding programs across Europe. In the wild, *P. avium* is a pioneer species and provide food for wildlife contributing establishment and continuity of biodiversity (Hernandez, 2008; Grunewald *et al*., 2010). Natural stands of *P. avium* are primary source of genetic variation and rootstock development in breeding sweet cherry for fruit production (Wolf *et al*., 2000; Ercisli, 2004). Finally, different parts of the plant can be used for medicinal purposes (Kim *et al*., 2005; González-Gómez *et al*., 2009; Ferretti *et al*., 2010).

Determined by the genes (Kessler and Sinha, 2004) and the environmental factors (Wright *et al*., 2005), leaf shape and structure in forest tree species display tremendous amount of variation at both between and within species levels (Nicotra *et al*., 2011; Guet *et al*., 2015). Thus, investigation of leaf size variation in relation to habitat is a useful tool in identifying population structures of wide ranging species, especially for those where there is little information is available (Gratani *et al*., 2003; Bayramzadeh *et al*., 2012).

While there are many studies on cultivated forms of *P. avium*, studies dealing with variation in natural *P. avium* populations in Turkey are very limited both in number and range. Thus, the goals of this study were 1) to document the variation in leaf size among the natural *P. avium* populations, and 2) to investigate relationship between leaf size and geography and climate at population locations in Turkey.

Materials and Methods

Experimental Details

In the summer of 2015 a total of 25 natural *P. avium* populations in Turkey were visited (Table 1 and Fig. 1). In each population 20 trees were randomly selected. The select trees were at least 100 m apart but within a 300 m elevation band. Twenty fully expanded and exposed leaves were randomly collected from south facing branches in mid-crown of each tree. The leaves were placed in separate plastic bags for each tree, transferred to the laboratory and kept in a refrigerator at 4°C until the measurements were conducted. Leaf measurements were completed within three days of collection for each population.

Geography of Populations and Climate Data

Approximate center coordinates (in decimal degrees) and elevation (in meters) of each population was recorded using a Garmin® GPS receiver (Olathe, KS, USA). Climate data at population locations were extracted from WorldClim (Hijmans *et al.*, 2005). WorldClim is a set of global interpolated climate layers with a spatial resolution of one square kilometer and provides data on 67 different variables including monthly mean, minimum and maximum temperatures and precipitation for given geographic coordinates based on weather data from the years 1950 – 2000. Data extraction was based on approximate center coordinates of each population. Thus, a total of 70 geography and climate variables were considered.

Measurements on Sample Leaves

Petiole length (P) and lamina length (L) and width (W) were measured (in cm to the closest mm) with a digital caliper. P is the distance from petiole's point of attachment to the stem to the lamina base. L was measured from the lamina base to the apex and W across the lamina at its widest point perpendicular to the midrib (Fig. 2). Additional three variables were created as the ratios (L/W, L/P and W/P) and all of the six variables will be called 'leaf variables' hereafter.

An analysis of variance (ANOVA) was conducted on the leaf variables according to the following statistical model;

$$Y_{ijk} = \mu + p_i + t_{j(i)} + e_{ijk} \quad \text{[Eq. 1]}$$

where Y_{ijk} is the k^{th} leaf from the j^{th} tree in the i^{th} population, μ is the overall mean, p_i is the random effect of the i^{th} population, $t_{j(i)}$ is the random effect of j^{th} tree in the i^{th} population, and e_{ijk} is the random error. PROC GLM procedure of SAS/STAT® software (SAS, 1999) was used to conduct ANOVAs. Variance components were estimated from the expected mean squares and their proportions in total variance were calculated.

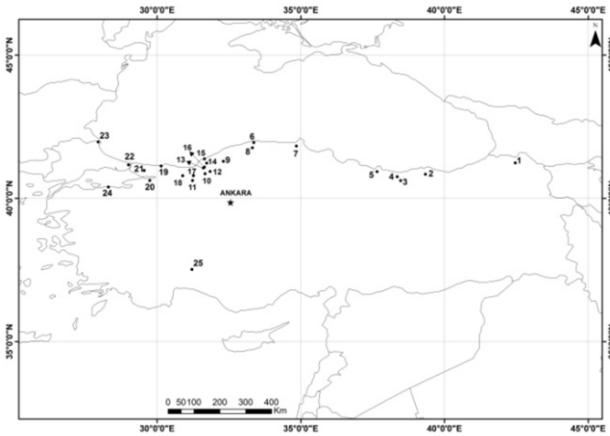

Fig. 1: Population locations across Turkey. See table 1 for population information

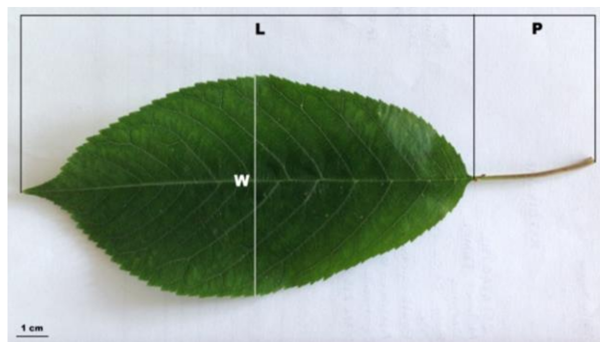

Fig. 2: Measurement of lamina length (L), lamina width (W) and petiole length (P) on a *Prunus avium* leaf

Relationship between Leaf Variables and Climate

Climate type at each population location was determined using Thornthwaite (1948) Climate Classification (TCC; Eq. 2) method. This method is based on precipitation, temperature and potential evapotranspiration (Thornthwaite and Mather, 1957).

TCC index is calculated as:

$$I_m = \frac{100s - 60d}{n}, \quad \text{[Eq. 2]}$$

Where I_m is the index value, s is the annual water surplus (mm), d is the annual water deficit (mm), and n is the annual potential evapotranspiration.

In order to investigate the relationships between geography and climate at population locations and the leaf variables, first a simple correlation analysis was conducted between the leaf variables and 70 geography and climate variables. After examining all the correlations, simple regression analyses were conducted between the leaf variables and geo-climatic variables. In addition, a hierarchical cluster analysis was performed and a dissimilarity matrix was computed using Ward (1963) method.

Table 1: Location, elevation, mean annual temperature (MAT) and mean annual precipitation (MAP) of the natural *P. avium* populations included in this study

Population No.	Population Name	Forest Management[1]	Latitude (N)	Longitude (E)	Elevation (m)	MAT (°C)	MAP (mm)
1	Veliköy	Artvin-Şavşat-Veliköy	41.238048	42.466309	1900	4.1	705
2	Düzköy	Trabzon-Trabzon-Düzköy	40.840889	39.339694	1129	9.8	622
3	Kümbet	Giresun-Dereli-Kümbet	40.618276	38.477146	1364	8.3	609
4	Kemerköprü	Giresun-Giresun-Kemerköprü	40.754308	38.354558	1184	9.2	670
5	Fatsa	Giresun-Ünye-Fatsa	40.933353	37.654874	950	9.7	732
6	Doğanyurt	Kastamonu-İnebolu-Doğanyurt	41.945785	33.365948	567	11.0	785
7	Dıranas[2]	Kastamonu-Sinop-Erfelek	41.825464	34.848940	676	11.1	679
8	Çamlıbük[2]	Kastamonu-Azdavay-Çamlıbük	41.762338	33.316392	1180	7.7	762
9	Tefen	Zonguldak-Devrek-Tefen	41.292890	32.306848	560	11.7	695
10	Yedigöller	Bolu-Bolu-Kale	40.861044	31.670558	1279	8.3	733
11	Abant	Bolu-Bolu-Abant	40.621022	31.234583	1556	6.9	751
12	Elemen	Zonguldak-Dirgine-Kozdere	40.937555	31.847664	1200	8.8	742
13	Bendere	Zonguldak-Kdz. Ereğli-Bendere	41.060263	31.613458	1127	8.9	745
14	Çaylıoğlu[2]	Zonguldak-Zonguldak-Çaylıoğlu	41.227080	31.726431	300	13.4	957
15	Ereğli	Zonguldak-Kdz. Ereğli-Ereğli	41.377426	31.647808	250	13.1	1051
16	Alaplı[2]	Zonguldak-Kdz. Ereğli-Alaplı	41.091701	31.645816	550	11.0	671
17	Düzce	Bolu-Düzce-Düzce	40.793153	31.265231	288	12.8	765
18	Melen	Bolu-Düzce-Melen	40.788537	30.890832	422	12.1	766
19	Kefken	Sakarya-İzmit-Kefken	41.126759	30.142403	59	13.8	858
20	Gölcük	Sakarya-Gölcük-Gölcük	40.622819	29.746048	747	11.0	785
21	Mollafenari	Sakarya-Gebze-Gebze	40.972346	29.552005	257	12.9	829
22	Araştırma	İstanbul-Bahçeköy-Araştırma	41.168350	29.006756	69	13.4	890
23	Macara	İstanbul-Demirköy-Macara	41.967806	27.949375	195	12.5	621
24	Bursa	Bursa-Mustafakemalpaşa-Karacabey	40.394440	28.304440	130	13.9	689
25	Isparta	Isparta-Sütçüler-Tota	37.521110	31.211940	880	12.9	653
Mean(Standard Deviation)					753 (512.86)	10.7 (2.50)	751 (104.78)

[1] Regional Forest Directorate – Forest Management District – Forest Management Unit
[2] Gene conservation forest

Results

Geography of Population Locations

The studied natural *P. avium* populations are in the Lakes Region (25-Isparta), the Marmara Region (populations 19 – 24, Kefken, Gölcük, Mollafenari, Araştırma, Macara and Bursa) and all across the Black Sea Region (rest of the populations) of Turkey (Fig. 1). The elevation ranged from 59 to 1900 m at population locations. Mean annual temperature and precipitation ranged from 4.1 to 13.9°C and from 609 to 1051 mm, respectively (Table 1). Climate types at population locations are Humid (two locations), Semi-humid (eight locations) or Semi-humid – Semi arid (15 locations; Table 2).

Variation in Leaf Variables

Mean lamina length (L) ranged from 9.75 cm (8-Çamlıbük) to 13.62 cm (22-Araştırma) (mean = 11.48 cm), width (W) from 5.34 cm (1-Veliköy) to 7.39 cm (22-Araştırma) (mean = 5.91 cm), and petiole length (P) from 2.48 cm (1-Veliköy) to 3.39 cm (11-Abant) (mean = 2.96 cm). On average, L was twice the W (mean = 1.97, range: 1.81 (24-Bursa) – 2.17 (9-Tefen), but about four times the P (mean = 4.12, range: 3.48 (12-Elemen and 25-Isparta) – 4.96 (15-Ereğli). Mean W was about twice the P (mean = 2.13, range: 1.59 (12-Elemen) – 2.45 (15-Ereğli) (Table 3).

Table 2: Climate types at population locations based on Thornthwaite (Thornthwaite, 1948) Climate Classification (index value = I_m)

Population name	I_m	Climate type
Veliköy	24.83	Humid
Düzköy	-10.96	Semi-humid – Semi-arid
Kümbet	-2.63	Semi-humid – Semi-arid
Kemerköprü	-3.43	Semi-humid – Semi-arid
Fatsa	-4.06	Semi-humid – Semi-arid
Doğanyurt	-3.69	Semi-humid – Semi-arid
Dıranas	-12.67	Semi-humid – Semi-arid
Çamlıbük	14.38	Semi-humid
Tefen	-11.57	Semi-humid – Semi-arid
Yedigöller	11.21	Semi-humid
Abant	23.72	Humid
Elemen	7.36	Semi-humid
Bendere	5.96	Semi-humid
Çaylıoğlu	4.23	Semi-humid
Ereğli	12.87	Semi-humid
Alaplı	-12.50	Semi-humid – Semi-arid
Düzce	-9.24	Semi-humid – Semi-arid
Melen	-7.55	Semi-humid – Semi-arid
Kefken	-4.47	Semi-humid – Semi-arid
Gölcük	-0.70	Semi-humid – Semi-arid
Mollafenari	-2.72	Semi-humid – Semi-arid
Araştırma	2.33	Semi-humid
Macara	2.35	Semi-humid
Bursa	-15.25	Semi-humid – Semi-arid
Isparta	-12.92	Semi-humid – Semi-arid

Effect of Climate and Geography on Leaf Size

Of the climatic and geographic variables investigated, mean annual temperature and elevation are significantly ($P < 0.05$) associated with L and W. Both L and W increase with increased mean annual temperature (Fig. 3), but decrease as population elevations increase (Fig. 4). Petiole length (P) exhibits similar patterns of relationship with these environmental variables, but the relationships are not significant (Fig. 3, 4 and 5). Leaf size increased, albeit slightly and insignificantly ($P > 0.05$) with increased mean annual precipitation (Fig. 5), increased precipitation from April to August is significantly ($P < 0.05$) associated with smaller L and W (Fig. 6). No significant relationship between climatic and geographic variables and L/W, L/S and W/S was detected.

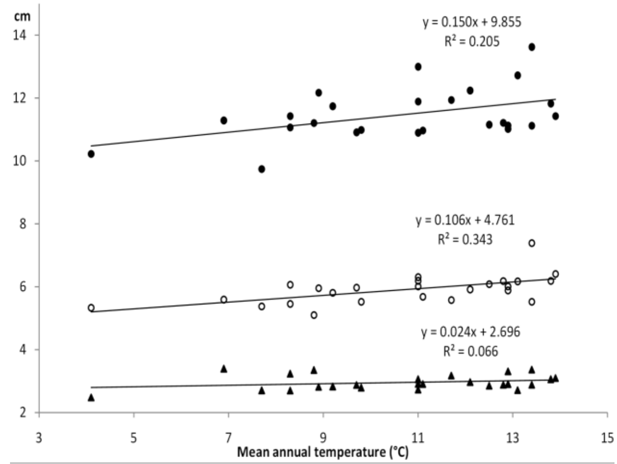

Fig. 3: Relationship between mean annual temperature and leaf length (●), leaf width (○) and petiole length (▲). The relationship is significant for leaf length and leaf width at P = 0.05

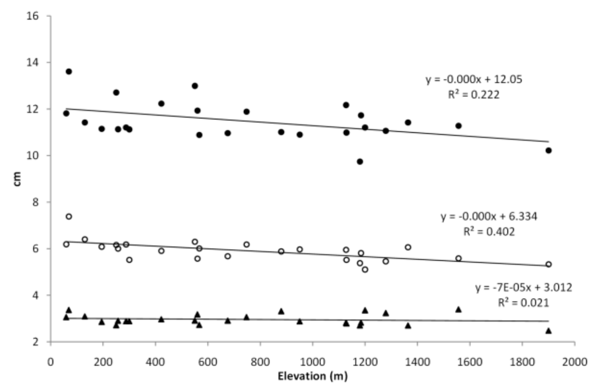

Fig. 4: The relationship between population elevation and leaf length (●), leaf width (○) and petiole length (▲). The relationship is significant for leaf length and leaf width at P = 0.05

Population Relatedness based on the Leaf Variables

Results of the ANOVA indicated that both populations and trees within populations were significantly different ($P < 0.01$) for all leaf variables. On average, while more than half (58%) of the total variation is due to leaf-to-leaf variation, significant amount of variation is explained by populations (9.7%) and trees within populations (32.3%; Table 4).

Examination of the diagram (Fig. 7) resulting from the hierarchical cluster analysis indicates two distinct groups. One group includes 11-Abant, 10-Yedigöller, 25-Isparta, 12-Elemen and 9-Tefen while all other populations are in the second group.

Discussion

Altitudinal range of studied populations is from sea level to almost timberline, indicating the ability of *P. avium* to inhabit diverse ecosystems in Turkey. Ballian *et al.* (2012) report wide elevation range (155 to 1226 m) in species distribution in Bosnia. The range in Bosnia is narrower only probably because mean elevation of this country (500 m) is lower than that of Turkey (1132 m). While there is tremendous amount of variation in elevation, climate types at population locations are mostly humid.

Mean annual temperature is the major driving environmental factor in determining leaf size and shape (Royer *et al.*, 2005; Peppe *et al.*, 2011). In this study, larger leafs were sampled from locations with higher mean annual temperatures and lower elevations. There are other leaf characteristics such as leaf specific area, nitrogen content and teething which are also related to temperature and precipitation (Read *et al.*, 2014), but they were not measured in this study.

Investigating leaf size variation is a relatively quick method in identifying population structure of less studied forest tree species and can be useful in designing more detailed population genetics studies. Bayramzadeh *et al.* (2012) were able to discern three major *Fagus orientalis* groups in northern Iran based on leaf morphology. Similarly, Shiran *et al.* (2011) used leaf form along with molecular markers to investigate population structure of *Quercus brantii*. Natural *P. avium* populations can also be grouped based on leaf morphology, but the variation between populations is less than the variation among individual trees within populations. This suggests that there is a significant amount of out-breeding and gene flow limiting population differentiation (Hamrick *et al.*, 1992). High rates of gene flow were also obtained using microsatellite DNA markers in natural *P. avium* populations in Croatia (Crmaric *et al.*, 2011), Greece (Ganopoulos *et al.*, 2011) and Italy (De Rogatis *et al.*, 2013).

In addition to the genetic structure of the populations and environmental factors seem to be at play in determining leaf size in natural populations of *P. avium* in Turkey. The findings showed that increase in temperature and precipitation coupled with lower elevation is associated with larger leaf size.

Table 3: Means and standard deviations (in parentheses) of leaf variables for each population

	Lamina		Petiole length (P, cm)	L/W	L/P	W/P
Pop. No.	Length (L, cm)	Width (W, cm)				
1	10.23 (1.64)	5.34 (0.95)	2.48 (0.46)	1.94 (0.28)	4.27 (1.11)	2.22 (0.56)
2	10.99 (2.03)	5.52 (1.07)	2.79 (0.51)	2.02 (0.35)	4.08 (1.13)	2.04 (0.51)
3	11.43 (2.47)	6.07 (1.17)	2.70 (0.65)	1.90 (0.34)	4.42 (1.24)	2.35 (0.65)
4	11.73 (1.97)	5.81 (0.93)	2.83 (0.68)	2.04 (0.30)	4.41 (1.44)	2.18 (0.68)
5	10.91 (2.14)	5.98 (1.10)	2.88 (0.72)	1.84 (0.25)	3.97 (1.15)	2.18 (0.61)
6	10.89 (2.84)	6.01 (1.44)	2.73 (0.77)	1.82 (0.32)	4.16 (1.21)	2.32 (0.70)
7	10.97 (2.40)	5.68 (0.96)	2.91 (0.76)	1.93 (0.31)	3.97 (1.24)	2.07 (0.62)
8	9.75 (2.03)	5.38 (1.07)	2.70 (0.64)	1.83 (0.28)	3.81 (1.24)	2.10 (0.65)
9	11.94 (1.79)	5.58 (0.97)	3.18 (0.76)	2.17 (0.32)	3.95 (1.05)	1.85 (0.52)
10	11.07 (2.35)	5.46 (0.97)	3.24 (0.69)	2.05 (0.41)	3.58 (1.11)	1.77 (0.51)
11	11.28 (2.13)	5.59 (0.91)	3.39 (0.77)	2.04 (0.34)	3.50 (1.08)	1.73 (0.49)
12	11.21 (1.89)	5.10 (0.82)	3.35 (0.76)	2.22 (0.33)	3.48 (0.85)	1.59 (0.42)
13	12.17 (1.75)	5.96 (0.81)	2.82 (0.65)	2.06 (0.25)	4.57 (1.30)	2.24 (0.65)
14	11.12 (1.94)	5.52 (1.09)	2.89 (0.81)	2.05 (0.33)	4.15 (1.36)	2.08 (0.76)
15	12.72 (2.43)	6.17 (1.21)	2.71 (0.69)	2.10 (0.41)	4.96 (1.50)	2.45 (0.89)
16	12.99 (2.93)	6.30 (1.15)	2.91 (0.92)	2.08 (0.38)	4.86 (1.79)	2.38 (0.93)
17	11.21 (1.73)	6.18 (1.09)	2.89 (0.84)	1.85 (0.36)	4.19 (1.33)	2.31 (0.74)
18	12.24 (2.29)	5.91 (1.13)	2.97 (0.76)	2.10 (0.36)	4.40 (1.46)	2.14 (0.77)
19	11.82 (1.81)	6.19 (0.89)	3.06 (0.74)	1.93 (0.27)	4.09 (1.15)	2.15 (0.64)
20	11.89 (2.23)	6.19 (1.07)	3.06 (0.73)	1.94 (0.29)	4.16 (1.51)	2.17 (0.78)
21	11.13 (2.27)	6.01 (1.23)	2.91 (0.67)	1.87 (0.30)	3.99 (1.13)	2.16 (0.59)
22	13.62 (2.83)	7.39 (1.40)	3.37 (0.85)	1.85 (0.23)	4.28 (1.36)	2.32 (0.68)
23	11.15 (2.36)	6.08 (1.19)	2.85 (0.61)	1.84 (0.22)	4.04 (1.10)	2.20 (0.53)
24	11.43 (2.47)	6.41 (1.32)	3.10 (0.88)	1.81 (0.34)	4.00 (1.47)	2.22 (0.72)
25	11.01 (1.88)	5.88 (1.04)	3.31 (0.78)	1.90 (0.30)	3.48 (0.99)	1.87 (0.58)
Overall mean	11.48 (2.35)	5.91 (1.18)	2.96 (0.77)	1.97 (0.34)	4.12 (1.32)	2.13 (0.70)

Table 4: Mean squares (and variance as proportion of total variance) for each source of variation for all leaf variables and expected mean squares. Populations and trees within populations are significantly different for all leaf variables (*p*< 0.01)

Source of variance	DF	Leaf variables						
		Lamina length (L)	Lamina width (W)	Petiole length (P)	L/W	L/S	W/S	Expected mean squares
Populations	24	278.07 (0.11)	83.41 (0.13)	22.84 (0.08)	5.61 (0.11)	57.80 (0.07)	18.33 (0.08)	$\sigma_e^2 + 20\sigma_{j(i)}^2 + 400\sigma_i^2$
Trees	475	36.31 (0.30)	10.16 (0.34)	4.48 (0.35)	0.75 (0.29)	11.33 (0.29)	3.86 (0.37)	$\sigma_e^2 + 20\sigma_{j(i)}^2$
Error	9999	3.33 (0.59)	0.74 (0.53)	0.34 (0.57)	0.07(0.60)	1.12 (0.64)	0.26 (0.55)	σ_e^2

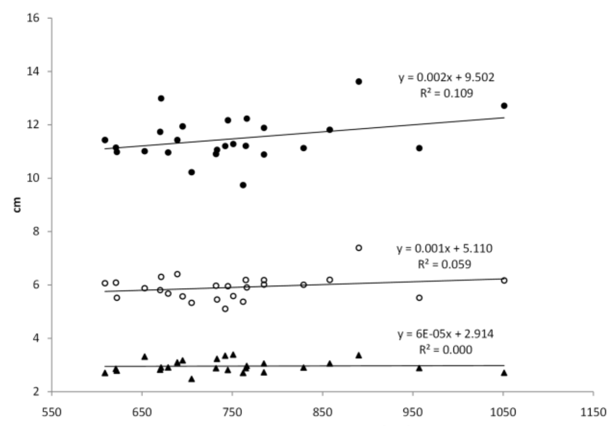

Fig. 5: The relationship between mean annual precipitation and leaf length (●), leaf width (○) and petiole length (▲). The relationships are not significant P = 0.05

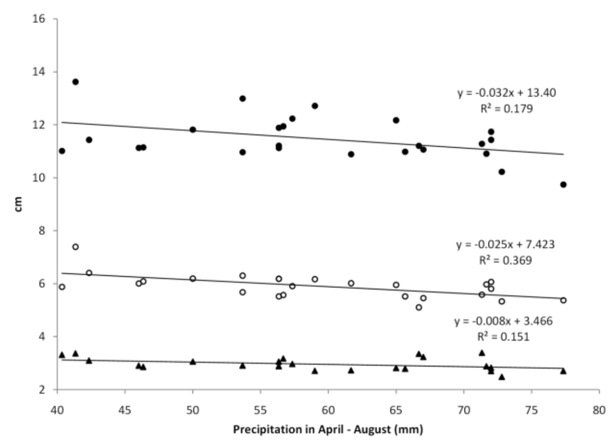

Fig. 6: The relationship between mean monthly precipitation from April to August and leaf length (●), leaf width (○) and petiole length (▲). The relationship is significant for leaf length and leaf width at P = 0.05

However, influence of precipitation on leaf size seems closely related to its distribution during the vegetation season because increased precipitation between April and August is associated with smaller leafs. A potential explanation for this may be the greater effect light availability on leaf size than that of water.

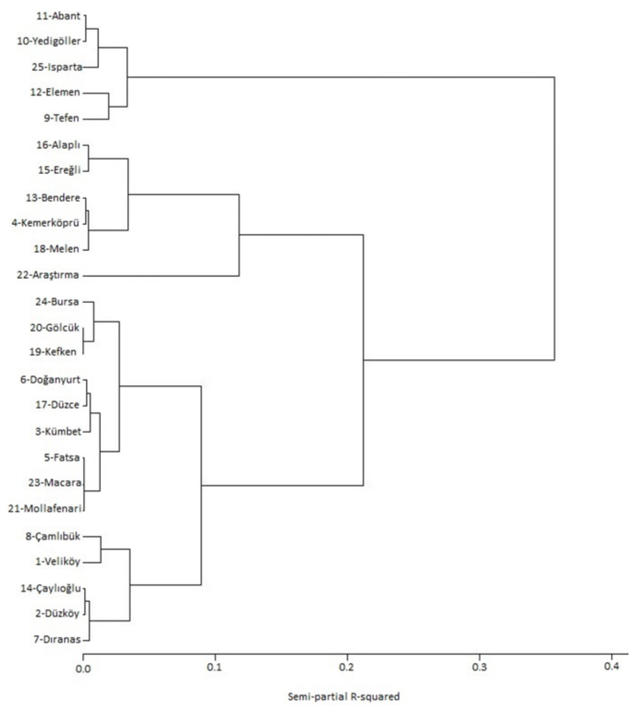

Fig. 7: Cluster of populations based on Ward (1963) method

Xu *et al.* (2009) reported that while leaf size increases with increased water and light availability, the influence of light is more significant than that of water on the size of *Q. acutissima* leafs. Reduction in light intensity due to rain bringing clouds during April through August may be responsible for smaller *P. avium* leaf sizes in locations with higher April-August precipitation.

The first step in any genetic conservation effort is usually documenting present genetic variation in conserved population(s). While greatly influenced by the environmental factors, leaf size is under moderate genetic control in *P. avium* (Santi *et al.*, 1998). The results from this study indicate that more than 40% of variation on leaf traits is explained by differences between populations and trees within populations. Until through genetic studies are conducted, variation in moderately heritable leaf traits can be used to discern populations for conservation purposes. Based on leaf traits the populations can be divided into two major groups (Fig. 7). Five populations (population numbers 9–12 and 25) in the first group are clearly located in more inland than the other populations (Fig. 1). Currently a total of four natural populations of *P. avium* are protected as gene conservation stands in Turkey (Table 1). All of these populations, however, are located in coastal regions and does not include any of the inland populations. Therefore, more inland populations and populations at the extremes should be included in the conservation program. The program can be refined once more detailed genetic studies are done.

Conclusion

P. avium is found from sea level to almost timberline in Turkey. While the elevational distribution is very diverse, the climate types at population locations display humid characteristics. This fact needs to be taken into account when using *P. avium* seed sources for afforestation purposes. The leaf size is highly variable and this variation is associated with elevation, temperature and precipitation at population locations. Lower elevations and higher temperatures result in larger leafs. Distribution pattern of precipitation throughout the year is more important than its annual amount. Higher precipitation from April to August is associated with smaller leafs, supporting the hypothesis that the light availability is more important than that of water in leaf expansion. About 10% of the total variation in leaf size is explained by significant differences between populations, allowing grouping of the populations based on leaf size variables. This information can be used for gene conservation purposes until through genetic description of the populations are conducted. Population variation is mild probably because out-crossing nature of mating in this species and high amount of gene flow between populations.

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