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Morphological, Agronomical, Phenological and Stand Persistence Traits of Some Wild Narrowleaf Birdsfoot Trefoil (*Lotus tenuis* Waldst. & Kit.) Populations

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ABSTRACT

Existing information on the ecology, distribution and plant traits of *Lotus* taxa is scant for the natural flora of Turkey, which is one of the most important countries in the Mediterranean zone for genetic resources and genetic diversity of these species. Therefore, the aim of this study was to determine (i) morphologic, agronomic, phenologic, stand persistence traits and growth habits of some wild narrowleaf birdsfoot trefoil (NBT; *Lotus tenuis* Waldst. & Kit.), and (ii) the relationships between these traits and altitude, latitude or longitude of the natural habitats, the samples of which were obtained from diverse natural areas of Black Sea Region, Turkey. The altitudes of sampling sites ranged from 1 to 1510 m. A total of 86 wild NBT were sampled in the study. NBT was present in all ranges of altitude, although its frequency was the highest in altitudes between 1-300 m. On the other hand, considerable variations were determined for the investigated traits, except for growth habit in which all populations had decumbent growth habit. The results of the present study indicate that NBT better adapted to lowlands and studied region offers important genetic resources for further researches and also may serve as a valuable breeding material for new grazing-type and disease resistant NBT varieties.

Keywords: *Lotus tenuis*; Genetic resources; Variation; Growth habit; Disease resistance

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Bazı Yabani Dar Yapraklı Gazal Boynuzu (*Lotus tenuis* Waldst. & Kit.) Populasyonlarının Morfolojik, Agronomik, Fenolojik ve Tesis Ömrü Özellikleri

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ÖZET

Genetik kaynaklar bakımından Akdeniz İklim Kuşağı'nda yer alan en önemli ülkelerden biri olan Türkiye'nin, doğal florasında yer alan gazal boynuzu türlerinin; ekolojisi, dağılımı ve bitkisel özellikleri hakkındaki bilgi oldukça sınırlıdır. Bu nedenle bu çalışmada, Türkiye'nin Karadeniz Bölgesi doğal alanlarından örneklenen yabancı daryapraklı gazal boynuzu (DGB) populasyonlarının; morfolojik, agronomik, fenolojik ve tesis ömrü özellikleri ile gelişme formlarının belirlenmesi ve bu özellikler ile örnek alanlarının rakım, enlem ve boylam değerleri arasındaki ilişkilerin ortaya konulması amaçlanmıştır. Örnek alanlarının rakımları 1 ile 1510 m arasında değişmiştir. Çalışmada 86 adet yabancı DGB örneklenmiştir. DGB, çalışma yapılan her rakım derecesinde bulunmakla birlikte, bulunma sıklığı 1-300 m rakım aralığında daha fazla olmuştur. Diğer yandan gelişme formu hariç, incelenen tüm özelliklerin çok önemli varyasyonlar gösterdikleri belirlenmiştir. Bütün DGB populasyonları yatık gelişme formu göstermişlerdir. Çalışmadan elde edilen sonuçlar, DGB populasyonlarının düşük rakımlı alanlara daha iyi uyum sağladığını ve çalışılan bölgenin, başta otlakiye amaçlı ve hastalıklara dayanıklı yeni DGB çeşitlerinin geliştirilmesi olmak üzere yapılacak her türlü çalışmalar için zengin genetik materyal kaynağına sahip olduğunu göstermiştir.

Anahtar Kelimeler: Dar yapraklı gazal boynuzu; Genetik kaynak; Varyasyon; Gelişme formu; Hastalığa dayanım

1. Introduction

Turkey is one of the most important countries in the Mediterranean zone for plant genetic resources and genetic diversity. The natural environment of Turkey is very diverse, ranging from subtropical to the cold temperate zones. This ecological diversity has contributed to high genetic diversity and has allowed the successful introduction and cultivation of a great number of plant species. Davis (1970) reported 925 legume species in 60 genera from Turkey. The Black Sea Region in the north of Turkey is also one of the richest regions for plant genetic resources diversity. This region is show much variation and distribution for many legume species and thus it is an important region for investigation of birdsfoot trefoil genetic resources (Tan 2010).

The narrowleaf birdsfoot trefoil (*Lotus tenuis*) (NBT) is herbaceous perennial forage which is adapted to low fertility, water logged and saline soils (Dear et al 2003). It is increasingly used in pastures over the world because of their high adaptation capability and productivity in a wide range of soils and climatic conditions like other *Lotus* species (Blumenthal & McGraw 1999). In Turkey, the scientific information on the ecology, distribution and plant characteristics of *Lotus* taxa genetic resources, including NBT are scant (Uzun et al 2008). Efficient utilization of the genetic potential requires detailed knowledge about the genetic

resources, including genetic diversity studies, evaluation and classification (Beuselinck & Steiner 1992).

The objectives of this research were to determine morphologic, agronomic and phonologic, stand persistence traits and growth habits of some wild NBT populations sampled from the Black Sea Region of Turkey, and also to determine the relationships of these traits with geographic features of sampling sites.

2. Material and Methods

A total of 86 wild populations of NBT seeds were sampled from the natural areas of the Black Sea Region (31°33' - 37°32' E and 40°06' - 42°03' N), Turkey in summer periods of 2009 and 2010. The sampling area has an irregular topography with elevations from 1 to about 1510 m (Table 1 and Figure 1). The distance between two sampling sites was at least 8 km. All the chosen sites were checked for NBT plant and as it stumbled up on, this site was recorded as sampling site. The size of the each site was nearly 1 ha. Each population was represented by minimum 50 plants (Brown & Marshall 1995). The populations were sampled mainly from meadows or pastures that had not been cultivated or actively managed for pasture improvement. In each population, mature pods were randomly sampled on

a site with uniform growing conditions. Accession codes of NBT populations were created by traffic codes of province, the first two letters of county and sample order (Table 1).

The glasshouse and field studies of this study were carried out in the Black Sea Agricultural Research Institute, Samsun, between 2010 and 2012 years. The experimental site was located at 41°17' N, 36°21' E, elevation 4 m. The area has a mild and humid climate with a mean annual temperature of 14.4 °C and a mean annual precipitation of 675.1 mm (1974-2013 years).

Firstly, the seeds of each population were sown into plug trays containing peat and grown in glasshouse conditions under natural light in mid of September 2010. Forty-five seedlings from each population were randomly selected and transplanted into the field with three replications at the 8-10 cm length stage at spacing of 0.9 x 0.9 m in end of November 2010.

In this study, hay yield, seed yield, and root and crown-rot diseases severity data were obtained in 2011 and 2012 years. Other data were obtained at the first experimental year. The 15 plants from each population were harvested manually at the beginning of flowering stage for hay three times in 2011 and two times in 2012. When 65-70% of the pods turned brown in colour, the other 15 plants were harvested for seed yield. The ratios of disease of populations were determined at the end of the second growing season. Morphological and phenological data were obtained on the remaining 15 plants. The data were obtained and evaluated according to ICARDA (2001) and IBPGR (1991).

Firstly, the data were analyzed by means of descriptive statistics (mean±standard deviation). Secondly, the relationship between evaluated traits and geographical features of the collecting-site locations were investigated by using correlation analysis. Thirdly, for a better overview of diversity or similarity in populations, principal component analysis (PCA) and scatter plot analysis were performed with PASW/SPSS Statistics 18 programme on traits to determine relationships

among populations. In the PCA, the data were used to generate Eigen Values, the percentage of the variation accumulated by PCA and the load coefficient values which relate the values. Those PC with Eigen Values >1.0 were selected and those traits with load coefficient values >0.6 were considered highly relevant traits scored for that PC (O'Rourke et al 2005).

3. Results and Discussion

3.1. Distribution regarding to altitude

Distributions of populations based on altitude of sampling sites are given in Table 1. The ratio of gathered NBT populations were 41% in 0-300 m, 25% in 301-600 m, 20% in 601-900 m, 12% in 901-1200 m and 2% in 1201-1510 m altitude. These results indicate that there was a decrease in present frequency of NBT with increasing altitude. Although it has been reported that NBT may be adapted to all ranges of altitude (Davis 1970; Drobná 2010), based on findings of present study and some other studies (Steiner & Santos 2001; Drobná 2010), it can be said that NBT better adapted to lowlands.

3.2. Population description

In the study, it was observed that all of populations had spreading growth habit and there were no rhizome.

Based on the leaflet measurements which were performed at bloom stage the populations were variable for middle leaflet length and width. The middle leaflets size ranged between 0.90-1.76 cm and (length) and 0.28-0.67 cm (width). The populations differed in the number of stems per plant, ranged from 23.33 to 73.17. The length of main stem varied between 50.00 and 96.67 cm. The populations were also quite variable for stem height. The stem height of populations changed between 8.22 and 30.67 cm (Table 2). Plant heights of all populations were low due to the decumbent growth habit. As known, shorter forage species are considered more suitable for grazing than taller types (Ayala 2001). Indeed, collecting sites of the populations have been grazed throughout the year.

Table 1- Distribution of 86 wild narrowleaf birdsfoot trefoil populations based on altitude of sampling sites

Çizelge 1- Örneklenen 86 yabani dar yapraklı gazal boynuzunun bölge yükseltilerine göre dağılımı

No	Altitude (m)				
	0-300	301-600	601-900	901-1200	1201-1510
1	37CI01(37)*	05MI02(2)	05TA04(15)	05GU02(5)	37DE02(39)
2	67CA01(43)	05MI03(3)	05GU01(4)	78ES02(58)	37TO02(42)
3	67CA02(44)	05MI01(1)	05HA01(8)	55LA01(80)	
4	67CA03(45)	05GO01(6)	05HA02(9)	60AR01(22)	
5	67CA04(46)	05GO02(7)	60MI01(16)	60AR02(23)	
6	67DE01(47)	05ME01(10)	37AR01(35)	37MI02(33)	
7	67GO01(49)	05SU01(11)	37MI01(32)	37AG01(34)	
8	67GO02(50)	60MI02(17)	37TO01(41)	37IH01(36)	
9	67FI01(51)	60MI03(18)	78ES01(57)	37IN01(40)	
10	67SA01(52)	60MI04(19)	78SA02(56)	37DE01(38)	
11	74MI01(59)	60MI05(20)	14MN01(77)		
12	74MI02(60)	60MI06(21)	14MN02(78)		
13	74MI03(61)	60RE01(31)	14MN03(79)		
14	74IN01(63)	78MI02(54)	14MI01(76)		
15	52UN01(71)	78MI01(53)	55VE02(82)		
16	52UN02(72)	78SA01(55)	18IL01(85)		
17	52UN03(73)	74AM01(62)	18IL02(86)		
18	52UN04(74)	19OS01(66)			
19	52FA01(75)	19OS02(67)			
20	57MI01(83)	19OS03(68)			
21	57HE01(84)	19OS04(69)			
22	05TA01(12)	19OS05(70)			
23	05TA02(13)				
24	05TA03(14)				
25	60ER01(24)				
26	60ER02(25)				
27	60ER03(26)				
28	60NI01(27)				
29	60NI02(28)				
30	60NI03(29)				
31	60NI04(30)				
32	67DE02(48)				
33	74UL01(64)				
34	74UL02(65)				
35	55VE01(81)				

*, the values in the parenthesis described the codes of population's distribution in Figure 2

**Figure 1- Black Sea Region from which wild narrowleaf birdsfoot trefoil (*Lotus tenuis* Waldst. & Kit.) genetic resources were sampled**

Şekil 1- Yabani dar yapraklı gazal boynuzu genetik kaynaklarının toplandığı Karadeniz Bölgesi

The number of raceme per plant, number of flowers and pods per raceme varied from 604 to 2566, 3.73 to 6.80 and 2.17 to 4.93, respectively. The maximum number of seed per pod was 28.7 and the minimum 7.0. There were significant correlations between leaflet length ($r = -0.255^*$), stem number ($r = -0.278^{**}$), stem length ($r = -0.376^{**}$), stem height ($r = -0.213^*$), number of seed per pod ($r = -0.280^*$) and altitude. In addition to, the correlation between raceme number ($r = -0.227^*$) and latitude was statistically significant (Table 2).

As shown in Table 2, average DM yields were 146.40 and 121.30 g plant⁻¹ in the first and the second years, respectively. The populations 74MI03 and 19OS02 had highest mean DM yields per plant (223.60 and 257.80 g plant⁻¹, respectively), while the lowest mean DM yields per plant was 64.00 g plant⁻¹ in population 37DE01 at the first year and 42.26 g plant⁻¹ in 37MI02 at the second year, respectively. The low DM yield might be attributed to the less cutting number according to first year, because plants did not re-grow or die after second cutting in 2012.

The leaf to stem ratio varied between 0.54 and 1.02. Leaf to stem ratio is an important trait in determining herbage quality, diet selection and forage intake (Forbes & Coleman 1993). This trait has been used successfully to predict forage quality parameters in birdsfoot trefoil and alfalfa (Buxton et al 1985). Also, these authors reported that attempts to improve forage quality of alfalfa and birdsfoot trefoil should be directed toward the lower portion of the plant canopy and stem.

The populations were also quite variable for seed yield. Average seed yields were 28.10 and 24.25 g plant⁻¹ in 2011 and 2012 years, respectively. The reason of the differences of seed yields in first and second year might be attributed to differences of insect pollinator amount and activity in these years.

The highest seed yields per plant were 74.64 and 91.90 in population 19OS02, the lowest 3.33 and 2.39 g plant⁻¹ in population 37IH01 in 2011 and 2012, respectively. 1000 seed weigh ranged between 0.78 and 1.61 g.

There were significant correlations between DM yield ($r = -0.477^{**}$ and $r = -0.247^*$ in 2011 and 2012, respectively), leaf/stem ratio ($r = 0.224^*$), 1000 seed weight ($r = 0.479^{**}$) and altitude. Furthermore, the correlation between seed yield ($r = -0.232^*$) and latitude was statistically significant in the first year but no significant in the second year.

The first flower, full flower and end of flowering stage of populations were ranged between 239 and 266, 269 and 275, and 307 and 316 days, respectively. Mean number of days to first pod and mature pod were varied from 257 to 274 and from 279 to 296 days, respectively. Correlation between altitude and number of days to last flower was significant (0.237^*). There were significant correlations between number of days to first flower ($r = -0.395^{**}$), first pod ($r = -0.325^{**}$), full flower ($r = -0.380^{**}$), first mature pod ($r = -0.547^{**}$) and altitude. No significant relationship was found between evaluated traits and longitude (Table 2).

The results related to morphological traits were corroborated those obtained by Drobná (2010) who reported that the most variable were the quantitative traits related to stem and number of inflorescence per stem. Variability also exists for specific agronomic traits, including herbage yield and quality, flowering habit, insect resistance and reproductive compatibility (Santos et al 2001). The morphological traits of plants in terms of size and growth habit are influenced by genotype and environment or their interaction. Furthermore, morphological variations among populations are strongly associated with geographical features of the collecting-site locations (Drobná 2010). In the present study, considerable variations for the morphological traits of NBT populations in terms of size and reproductive growth were resulted in the genotype due to the populations grown in the same environment. The number of stem per plant ($r = 0.465^{**}$), stem length ($r = 0.628^{**}$) and stem height ($r = 0.628^{**}$) were the main contributor to DM yield (data not shown in table) because the general appearance of the plants ranged from small and weak to large plants with a high number of stems (Drobná 2010). The DM yields

Table 2- Descriptive statistics of morphologic, agronomic and phenologic traits and correlation coefficients (r) between traits and sampling-site altitude and latitude of narrowleaf birdsfoot trefoil populations (n= 86)

Çizelge 2- Yabani dar yapraklı gazal boynuzunun morfolojik, agronomik ve fenolojik özelliklerine ilişkin tanımlayıcı istatistikler ve bu özellikler ile yükselti ve enlem arasındaki korelasyon katsayıları (r), (n= 86)

Traits	Mean±SD	Min-Max	Among- population CV(%)	Correlation coefficient	
				Altitude	Latitude
Central leaflet length (cm)	1.27±0.17	0.90-1.76	13.44	-0.255*	-0.110
Central leaflet width (cm)	0.44±0.09	0.28-0.67	19.74	-0.107	-0.073
No. of stems per plant	45.44±9.70	23.33-73.17	21.34	-0.278**	0.155
Length of main stem (cm)	73.94±11.1	50.00-96.67	14.96	-0.376**	-0.021
Height of stem (cm)	17.23±4.76	8.22-30.67	27.62	-0.213*	-0.185
No. of raceme per plant	1544±439.3	604-2566	28.44	-0.189	-0.227*
No. of flowers per raceme	5.59±0.51	3.73-6.80	9.10	-0.131	0.165
No. of pods per raceme	3.32±0.48	2.17-4.93	14.46	-0.075	0.101
No. of seeds per pod	16.02±3.91	7.00-28.70	24.39	-0.280**	0.095
DM yield (g plant ⁻¹)	2011 146.40±40.2	64.00-257.80	27.48	-0.477**	0.085
	2012 121.30±44.83	42.26-234.89	37.00	-0.247*	-0.109
Leaf/stem ratio (%)	0.73±0.11	0.54-1.02	15.69	0.224*	0.010
Seed yield (g plant ⁻¹)	2011 28.10±14.2	3.33-74.64	50.48	-0.175	-0.232*
	2012 24.25±16.3	2.39-91.90	67.00	-0.057	-0.189
1000 seed weight (g)	1.11±0.17	0.78-1.61	15.48	0.479**	-0.127
Disease severity (%)	2012 42.38±19.5	0-92	46.02	0.180	-0.144
No. of days to first flower	255±4.86	239-266	1.91	0.119	-0.395**
No. of days to first pod	263±3.57	257-274	1.36	0.077	-0.325**
No. of days to full flower	271±1.48	269-275	0.55	-0.009	-0.380**
No. days to first mat. pod	286±4.10	279-296	1.43	0.113	-0.547**
No. of days to last flower	311±2.15	307-316	0.69	0.237*	-0.011

***P*<0.01; **P*<0.05

of 74MI03, 67FI01, and 60MI05 populations were higher compared to other populations based on stem number, stem length and height, which are the most variable morphological traits in the present study. Therefore, our results on variation in morphological traits also support the idea that variation in morphological traits are revealed by the existence of considerable variation in form and growth of plants such as stem length and shape, number of flowers in inflorescence etc. as mentioned by Drobná (2010).

As reported by Drobná (2010), morphological and agronomical variations were strongly associated with altitude of the collecting-site. The traits of stem, leaflet length, number of seed per pod, herbage yield, leaf to stem ratio and 1000 seed weight were influenced highly by altitude (Table 2). All morphological traits exhibited negative correlation with altitude in our study. Most of the populations from locations with lower altitude tended to produce plants with high DM yield, number of longer stems, seed yield and the number

of raceme per plant, inflorescence per plant and seed per pod etc. compared to populations from locations with higher altitude.

A major factor limiting more widespread use of *Lotus* taxa is the fact that achieving a high seed yield in these species is difficult (McGraw & Beuselinck 1983). In high variation in terms of seed yield of *Lotus* taxa, number of raceme per plant, number of pod per raceme, number of seed per pod and seed weight have been identified as major contributing traits (Garcia-Diaz & Steiner 2000; Vignolio et al 2010). Furthermore, Li & Hill (1989) and Qingfeng (1989) reported that the seed yield is primarily determined by the number of inflorescences produced per plant, in turn, inflorescence number is primarily depending on the number of shoots available at the time of flowering. In the present study, there are the significant positive relationships between seed yield and the number of raceme per plant ($r=0.423^{**}$) and also seed per pod ($r=0.281^{**}$) (data not shown). Therefore, it can be suggested that high number of raceme, number of seed per pod, 1000 seed weight and stem number should be used as selection criteria for improving seed production according to our results and reports by above researcher.

A high phenotypic variability among the populations in terms of studied phenological traits can be related to a high phenotypic plasticity and/or genetic variations among the individuals (Ferraro et al 2010). This causes the spreading of NBT in the heterogeneous environment. Indeed, genetic differentiation in phenotypic traits has been frequently demonstrated among populations of wide-ranging species (Quinn & Wetherington 2002). Flowering habit of NBT is indeterminate, so flowering is extended over a long period of time. Qingfeng (1989) reported that the continuous development of new shoots replacing older flowering shoots was the main cause of the long flowering period.

3.3. Stand persistence

There was no diseased plant in the first year, while the ratio of disease of populations varied from

17 to 92% in 84 populations in the end of second year. Disease was not observed in 37TO01 and 67CA01 populations. These two populations can be very important in disease resistance studies. *Lotus* taxa have poor stand persistence (Altier & Kinkel 2005). These authors reported stand losses of 68 to 90% within 2 year of establishment due to root and crown-rot diseases for birdsfoot trefoil. Persistence in humid regions is shortened by disease (Beuselinck et al 1984). The moisture was quite high our study area in vegetation period varied between 78.1 and 82.6%. The results of our study were similar to the study of these authors. Astley (1987) reported that germplasm collections can contain genes for resistance to pests, diseases, and abiotic stresses, and help to ensure that potentially useful genetic variation is preserved for future needs. Rhizomatous types of *Lotus* taxa offer an additional strategy to improve stand persistence (Beuselinck et al 2005). However, no a rhizomatous growth form within populations was observed.

3.4. Principal component and cluster analysis

To assess the relative significance of evaluated traits, PCA was carried out. PCA revealed that the first six PCs explained 68.47% of the total variation. The attributes that appeared to be the major sources of diversity within the evaluated populations were the number of days to first flower, first pod, full flower and first mature pod (PC1). Length of main stem, number of raceme per plant, dry matter and seed yields explained PC2. The 1000 seed weight, leaflet length and width, and number of stems per plant explained on the PC3, PC4, and PC6, respectively. Therefore, the above-mentioned traits primarily must be taken into consideration for selection. Based on the distance matrix for the distributions of populations on the first two axes, some populations (e.g. populations of 45, 49, 64 and 78 etc.) were closely similar to each other while some (e.g. populations of 32, 13, 61 and 76) were notably quite different from each other (Figure 2).

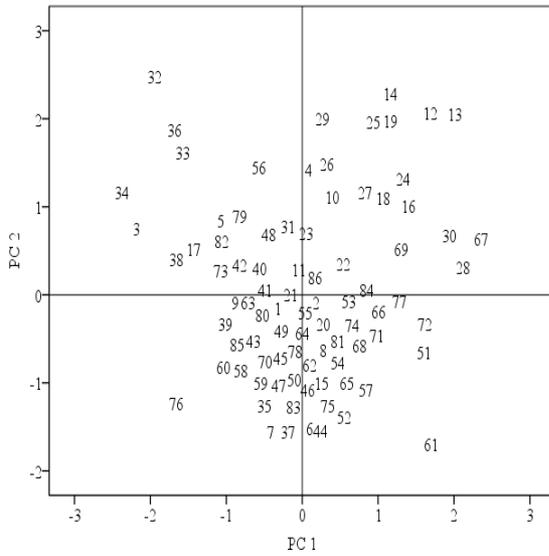


Figure 2- Distribution of populations on the PC1 (22.59) and PC2 (39.92) refer to the codes of Table 1

Şekil 2- Çizelge 1'deki kodlara göre PC1 (22.59) ve PC2 (39.92) popülasyon dağılımı

4. Conclusions

The results of the present study indicate that studied region may offer important NBT genetic resources for further researches and serve as a valuable breeding material. For example, new grazing-type and disease resistant NBT varieties may be improved from studied populations for improving pasture where alfalfa and other legumes cannot grow.

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