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Geographical Variation in Nutrient Composition of *Lotus tenuis* (Waldst.&Kit.) Populations from Seeds Collected from Different Locations*

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Abstract: To investigate the effect of seed origin on the nutritive value of narrowleaf birdsfoot trefoil (NBT, *Lotus tenuis* Waldst.&Kit.), seeds of 86 NBT populations were collected from plants spontaneously occurring in natural pastures and rangelands located at different geographical gradients of the Black Sea Region, Turkey. Some nutrient contents of these populations regrown under the same conditions were determined. Minimum and maximum values with regard to the crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), Ca, P, Mg, K and condensed tannin contents, relative feed value, Ca P^{-1} and K $(Ca+Mg)^{-1}$ ratios were found as 176.83 and 238.87, 201.00 and 351.61, 307.19 and 435.48, 15.01 and 21.60, 2.18 and 3.77, 3.03 and 4.02, 8.43 and 16.69, 20.00 and 31.50 g kg^{-1} dry matter, 189.70 and 263.41, 4.73 and 9.57, 0.35 and 0.88, respectively. The effects of seed genotype from different altitudes (Ca P^{-1}), latitudes (digestible dry matter and ADF) and longitudes (CP and Mg) on some nutrient contents and feed values were found significant ($P<0.05$). The correlations between altitude and NDF, P and Mg or between latitude and P and Mg contents of populations were significant. There were no specific trends in chemical composition and the nutritive value of tested NBT populations due to geographical variation. Thus, the results suggested that all studied geographical populations can be selected to obtain the nutritionally superior forage.

Keywords: Forage, narrowleaf birdsfoot trefoil, seed origin, chemical composition, feed value

1. Introduction

The livestock raised at high altitudes depend mostly on the feed resources in natural pasture and rangeland habitats. Therefore, livestock productivity are depend on quality of these areas include grass species as well as legume species (Ocak et al., 2006; Sun et al., 2014). In order to improve the quality of the herbage consumed by grazing animals, it is necessary to obtain information about its chemical composition and feed value, which thereafter could be related to its capacity to satisfy the requirements of the grazing animals (Dewhurst et al., 2009).

Legumes growing in pastures increase both the productivity and nutritional value of forages (Sleugh et al., 2000). There are three perennial legumes of the *Lotus* genus (Fabaceae) that are important as forage in some part of the world: birdsfoot trefoil (*L. corniculatus* L.), narrowleaf birdsfoot trefoil (NBT, *L. tenuis* Waldst.&Kit.), and greater lotus (*L. uliginosus* Schkuhr) (Acuña and Cuevas, 1999; Papadopoulos and Kelman, 1999). Different species of *Lotus* are currently used to improve pastures and hay quality where other forage legume species are not suitable (Papadopulous and Kelman, 1999). The NBT is a herbaceous perennial forage species of Mediterranean origin, is a diploid species with a taproot that grows in soils with a wide range of pH and water and nutrient availability, and also that is adapted to low fertility, waterlogged, and saline soils (Dear et al., 2003). It has been reported that this plant tolerates flooding, drought, and salinity (Striker et al., 2005; Acuña et al., 2010; Teakle et al., 2010). These characteristics give it added value when comparing it with other legumes such as

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alfalfa, white clover or birdsfoot trefoil. In fact, varieties of NBT are more tolerant to waterlogging or flooding, alkaline and salt conditions than any commercial varieties of birdsfoot trefoil (Clua et al., 2009). The forage plant breeding should be focussed on improving the economic sustainability of pastoral agriculture through feeding animals i.e. yields, quality and for truly sustainable livestock farming.

It seemed worthwhile to investigate the suitable fodder plants which provide high yield and reproductive performance, reduce the health problem, and develop grazing tolerant cultivars. Although there are studies investigated the effects of seed origin or collection site, i.e., latitude and longitude as well as altitude (Dragomir et al., 2011; Mountousis et al., 2011; Singh and Todaria, 2012; Uzun and Dönmez, 2015; Uzun et al., 2015) on the nutritional status of some perennial forages, the effects of these factors on the NBT populations were not investigated. Accordingly, the nutrient composition, including condensed tannins and the nutritive value of NBT populations grown from seeds collected from pastures and rangelands located at different altitudes, latitudes and longitudes requires further study. Therefore, the present study was designed to compare genetic potentials in a single environment in terms of the nutrient composition and the nutritive value of NBT populations regrown from seed collected from different altitudes, latitudes and longitudes of the Black Sea Region, Turkey.

2. Materials and Methods

This study was conducted at Ondokuz Mayis University, Samsun, Turkey for assessing effect of seed origin on the nutrient compositions and the nutritive value of NBT populations. *L. tenuis* seeds were obtained from naturalized populations of the Black Sea Region, Turkey. Therefore, the seeds of NBT populations were collected from different altitudes (1 to 1510 m a.s.l.), latitudes (40°06' to 42°03'N) and longitudes (31°33' to 37°32' E) ranges (Table 1) from July to September. The seeds were collected from mature pods of approximately 50 mature plants spontaneously occurring in natural pasture and rangelands, grazed by livestock throughout year, of studied region (Uzun et al., 2015). The seeds from each altitude, latitude and longitude were sown in nursery beds and then grown under the same climatic conditions in the experimental area at the Black Sea Agricultural Research Institute (situated at 41°17' N latitude, 36°21' E longitude and 4 m a.s.l. altitude). The local climate is mild and humid, with a mean annual temperature of 14.4 °C ranging from 3.1 °C in winter to 16.7 °C in summer and with a mean annual rainfall of 675.1 mm during the study period (Anonymous, 2015).

Initially, from each of 86 NBT populations representing all the seedlings, approximately 2 kg of tissue sample was freshly harvested and bulk sample was mixed thoroughly. Then, only 0.5 kg was finally dried at 60 °C for 72 h to determine the dry matter (DM) content and were grinded using mill having a sieve of 1 mm. The crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium (Ca), magnesium (Mg) and potassium (K) contents of these samples from NBT populations were determined by using near-infrared reflectance spectroscopy (NIRS). The near-infrared spectra were collected with a monochromator (FOSS NIR Systems 6500, Silver Spring, MD, USA), by scanning the 400-2500 nm spectral range. All spectra and reference data were recorded and managed with the WINISI version 1.6 software (Infrasoft International, Port Matilda, PA, USA). The calibration set was analyzed for CP concentration (Anonymous, 2000a). The condensed tannin (CT) contents were determined as described by Ramírez-Restrepo et al. (2006).

Table 1. Altitudes and positions of collection sites of *Lotus tenuis*

Provenance	Altitude (m a.s.l.)	Latitude (N)	Longitude (E)
Amasya	198-995	40°17'16.0"-40°52'53.1"	35°05'52.8"-36°28'54.0"
Bartın	13-312	41°06'36.5"-41°43'32.9"	32°19'31.2"-32°38'27.1"
Bolu	628-769	40°43'55.9"-41°02'47.5"	31°33'56.9"-32°04'49.9"
Çankırı	870-870	40°54'41.3"-40°54'41.3"	33°37'18.1"-33°37'18.1"
Çorum	415-501	40°58'42.5"-40°59'32.1"	34°38'36.8"-34°55'44.6"
Karabük	305-940	40°56'08.4"-41°23'12.9"	32°28'36.0"-32°43'27.2"
Kastamonu	19-1510	40°58'07.1"-41°53'36.0"	33°00'08.0"-35°52'53.4"
Ordu	4-27	41°01'18.2"-41°09'35.0"	37°12'19.0"-37°32'34.2"
Samsun	245-950	40°52'55.3"-41°07'13.5"	35°22'45.0"-35°26'43.7"
Sinop	5-18	41°58'05.0"-42°03'06.0"	34°25'20.3"-35°02'03.0"
Tokat	220-1165	40°06'36.0"-40°40'50.0"	36°18'16.0"-37°20'59.0"
Zonguldak	1-103	41°13'53.5"-41°34'07.6"	31°58'29.0"-32°09'43.2"

Each nutrient was analyzed with three replicated samples of altitudinal, latitudinal and longitudinal NBT populations.

As described by Moore and Undersander (2002) digestible DM (DDM) and DM intake (DMI) was calculated from percentage ADF and NDF values using the following equations, respectively; DDM (%) = 88.9 – (0.779 × % ADF) and DMI (% of body weight) = 120 / % NDF. Metabolizable energy (ME) was estimated using the following equation; ME (MJ/kg DM) = 0.17% DDM–2.0. Relative feed value (RFV) was estimated from DDM and DMI using the following equation; RFV = (DDM×DMI)/1.29. The tetany ratio is calculated on an equivalent weight basis using a so-called tetany ratio [K/(Ca+Mg)].

All the statistical analyses were performed using SPSS software package, version 17.0 (SPSS Inc., Chicago, USA). The NBT populations were studied by multifactor analysis of variance, including the effects of altitudes, latitudes, longitudes, and their interactions. Because the interaction effects of these factors on any of the studied parameters were not significant, these were not shown in the tables and elsewhere. Pearson's correlation analysis was performed to assess the association between different experimental parameters in each of factors. When the F-test was significant, differences were compared using Tukey test at a significance level of $P< 0.05$. Results are presented as means and a pooled standard error of mean.

3. Results and Discussion

In the first part of the present study, the morphologic, agronomic and phonologic traits of 86 NBT populations and the relationships between these traits and geographical features of the natural habitats were determined (Uzun et al., 2016). The results from this study indicated that NBT populations were present in all ranges of altitude, although there was a decrease in present frequency of NBT with increasing altitude. Indeed, 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. Similar suggestions reported by Vignolio et al. (2009) for the NBT populations naturalized in the Flooding Pampa grasslands.

The correlation coefficients between altitude and NDF, phosphorus (P) and Mg contents or between latitude and P and Mg contents of NBT populations were significant (Table 2). There were obvious the effects of seed genotypes from different altitudes (Ca P^{-1}, $P<0.05$), latitudes (DDM and ADF, $P<0.05$) and longitudes (CP and Mg, $P< 0.05$) on DDM, ADF, CP, Mg and Ca P^{-1} values (Table 3 and Table 4). These results can be related to the apparent high adaptability of the NBT that its spontaneous presence in this zone is very notorious (Acuña et al., 2012). Variations in quality of NBT populations also indicates their inherent ability to obtain nutrients from the soil and convert them to plant tissue with a favourable leaf to stem ratio in terms of CP, ADF, NDF, ME and RFV as well as mineral contents and ratios between minerals (Vignolio et al., 2009; Dragomir et al., 2011). Therefore, seeds selected from the NBT populations that have high nutrient composition can use to increase the plant population of pasture or rangelands. The nutritive value and mineral contents of the NBT populations were within the normal range and also were found

Table 2. Descriptive statistic and correlation coefficients between geography and the nutritive value, mineral and condense tannin contents of *Lotus tenuis* from different geographical sites

	Descriptive statistic				Correlation coefficient		
	Mean	SD	Minimum	Maximum	Altitude	Latitude	Longitude
DDM, %	69.50	1.96	61.51	73.24	-0.201	-0.201	-0.189
DMI, % of body weight	3.48	0.27	2.82	3.96	-0.205	-0.162	0.158
RFV	232.09	17.38	189.70	263.41	-0.206	-0.164	-0.159
ME, MJ kg^{-1} dry matter (DM)	9.82	0.33	8.46	10.45	-0.200	-0.200	-0.189
CP, g kg^{-1} DM	212.01	12.50	176.83	238.87	-0.194	-0.187	-0.209
ADF, g kg^{-1} DM	248.97	25.11	201.00	351.61	0.201	0.201	0.189
NDF, g kg^{-1} DM	351.67	28.33	307.19	435.48	0.227*	0.190	0.183
Ca, g kg^{-1} DM	18.56	1.24	15.01	21.60	-0.010	-0.029	-0.590
P, g kg^{-1} DM	2.98	0.33	2.18	3.77	-0.260*	-0.228*	-0.196
Ca P^{-1}	6.49	1.09	4.73	9.57	0.189	0.156	0.123
Mg, g kg^{-1} DM	3.50	0.20	3.03	4.02	0.225*	0.232*	0.188
K, g kg^{-1} DM	11.89	1.81	8.43	16.69	-0.117	0.074	-0.500
K $(Ca+Mg)^{-1}$	0.56	0.12	0.35	0.88	-0.048	-0.004	0.220
CT, g kg^{-1} DM	24.10	2.73	20.00	31.51	-0.138	-0.107	-0.106

Values are means of 86 populations with three replicates. DDM: Digestible dry matter, DMI: Dry matter intake, RFV: Relative feed value, ME: Metabolizable energy, CP: Crude protein, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, Ca: Calcium, P: Phosphorus, Mg: Magnesium, K: Potassium, CT: Condensed tannin, *: Significant at $P< 0.05$

to be comparable with other legumes (Dynes et al., 2003; Acuña et al., 2012). Moreover, they were within values recommended by the Anonymous (2000b, 2001, 2007) and confirmed other scientific suggestions (Moniello et al., 2005). All have excellent feed quality for ruminants; 12-25% crude protein content and dry matter digestibility of 50-73% (Cook et al., 2005). Thus, our findings indicated that the maintenance requirements of grazing livestock may meet until late autumn without additional protein and energy sources.

Protein, necessary for muscle development, growth and milk production is an important nutrient in animal production and is generally least expensive if supplied by forages. The CP contents of the NBT populations were in the same range as reported by Dynes et al. (2003) and Acuña et al. (2012). As reported by Dragomir et al. (2011), the nutritional value, calculated through the determination of some quality indices (CP, ADF and NDF), proved that there are not important differences between the studied geographical sites.

Table 3. Altitudinal, latitudinal and longitudinal variations in nutritive value of *Lotus tenuis* from different geographical sites

	n	DDM	DMI	RFV	ME	CP	ADF	NDF
Altitude								
<400	39	69.82	3.50	233.73	9.87	215.11	244.97	348.35
401-800	29	69.46	3.50	233.74	9.81	209.69	249.54	349.71
>801	18	68.90	3.38	225.87	9.71	209.06	256.75	361.99
	P-value	0.257	0.233	0.235	0.257	0.110	0.257	0.218
Latitude								
40°06'-40°58'	44	69.86a	3.51	234.17	9.88	213.88	244.40b	347.45
41°59'-42°03'	42	69.13b	3.44	229.91	9.75	210.06	253.77a	356.08
	P-value	0.043	0.263	0.259	0.054	0.157	0.048	0.159
Longitude								
31°33'-32°43'	27	70.00	3.50	233.58	9.90	218.00a	242.66	348.04
33°00'-33°59'	9	69.14	3.47	231.87	9.75	214.81ab	253.64	352.40
34°01'-34°55'	8	70.22	3.58	238.75	9.94	210.18ab	239.87	339.90
35°02'-35°65'	17	69.01	3.53	235.91	9.73	205.97b	255.28	346.93
36°11'-36°55'	19	69.36	3.39	226.76	9.79	213.64ab	250.88	361.06
37°12'-37°32'	6	68.75	3.33	222.91	9.69	210.31ab	258.66	366.25
	P-value	0.405	0.349	0.345	0.414	0.013	0.405	0.312
	SEM	0.211	0.029	1.874	0.036	1.348	2.708	3.055

SEM: Standard error of the mean, DDM: Digestible dry matter (%), DMI: Dry matter intake (% of body weight), RFV: Relative feed value (g kg^{-1} DM), ME: Metabolizable energy (MJ kg^{-1} DM), CP: Crude protein (g kg^{-1} DM), ADF: Acid detergent fiber (g kg^{-1} DM), NDF: Neutral detergent fiber (g kg^{-1} DM), a, b: Different superscripts within the same column indicate significant differences (P< 0.05)

Table 4. Altitudinal, latitudinal and longitudinal variations in mineral and condense tannin contents of *Lotus tenuis* from different geographical sites

	n	Ca	P	Ca/P	Mg	K	K/(Ca+Mg)	CT
Altitude								
<400	39	18.72	3.02	6.45ab	3.47	11.84	0.54	24.34
401-800	29	18.28	3.02	6.28b	3.50	12.25	0.58	24.26
>801	18	18.66	2.83	6.94a	3.57	11.43	0.54	23.30
	P-value	0.327	0.052	0.024	0.177	0.321	0.298	0.380
Latitude								
40°06'-40°58'	44	18.67	3.02	6.44	3.46	11.89	0.55	24.30
41°59'-42°03'	42	18.45	2.94	6.55	3.54	11.90	0.56	23.88
	P-value	0.414	0.283	0.634	0.053	0.969	0.503	0.481
Longitude								
31°33'-32°43'	27	18.60	3.06	6.31	3.46b	12.03	0.55	24.48
33°00'-33°59'	9	19.44	2.84	7.14	3.56ab	10.97	0.49	24.20
34°01'-34°55'	8	18.04	3.08	6.10	3.38b	12.43	0.59	23.82
35°02'-35°65'	17	18.27	3.00	6.34	3.55ab	12.17	0.58	23.65
36°11'-36°55'	19	18.47	2.95	6.57	3.50b	11.95	0.57	24.32
37°12'-37°32'	6	18.88	2.76	7.10	3.66a	10.99	0.50	24.16
	P-value	0.200	0.239	0.206	0.049	0.416	0.245	0.870
	SEM	0.134	0.352	0.118	0.021	0.196	0.012	0.294

SEM: Standard error of the mean, Ca: Calcium (g kg^{-1} DM), P: Phosphorus (g kg^{-1} DM), Mg: Magnesium (g kg^{-1} DM), K: Potassium (g kg^{-1} DM), CT: Condensed tannin (g kg^{-1} DM), a, b: Different superscripts within the same column indicate significant differences (P< 0.05)

In the present study, the correlation between CP and altitude does not support that N concentration of leaf increased from the tropics to mid-latitudes and then remained stable or decreased at high latitudes (Reich and Oleksyn, 2004) and that nitrogen (N) concentration in plants increased with the latitudinal (Han et al., 2012) and the altitudinal gradient (Singh and Todaria, 2012). In contrast to our results, Mountousis et al. (2011) reported that NDF, ADF and gross energy contents of forages were affected by the altitudinal zone as well as by the season.

The DMI and ME are a positive indicator of forage quality and an important component that makes up the diet of animals (Arzani et al., 2005). Waghorn and Clark (2004) noted that the ME content of forages provides a guide to nutritive value but ignores other components that affect intake, digestion and the products of digestion that are absorbed and metabolized to provide substrates for maintenance and production. Although herbage ME content was lower in the lowlands owing to faster maturation of plants as a result of higher air temperatures (Mountousis et al., 2011), this effect was not reflected on that of NBT populations from different altitudes, latitudes and longitudes. An increase in DDM generally leads to improve in performance by increasing DMI of ruminant animals (Ashikaga et al., 2009). In the present study, there were significant effects of the studied factors on DDM and ADF (Table 3). The minerals and the correct ratios of minerals (e.g. Ca P^{-1}, Ca Mg^{-1}, K/(Ca+Mg) and K Mg^{-1} ratios) in the forage as well as CP and ME contents and RFV of fodder feeds may affect animal growth and/or performance. Based on our findings and previous reports (Striker et al., 2005; Acuña et al., 2010, 2012; Teakle et al., 2010) on advantage of NBT, populations can select for multiplication of quality forage production.

Because legumes, including NBT have higher concentrations of Ca and Mg than grasses, avoiding legume decrease in rangelands may be useful to prevent the tetany risk (Aydın and Uzun, 2008; Uzun, 2010). Risk of tetany, causing yield decrease and death in cattle and sheep, increases by feeding forage with a ratio of K $(Ca+Mg)^{-1} \geq 2.2$ or the greater risk of grass tetany occurs at K:Mg ratio greater than 10:1. Indeed, K $(Ca+Mg)^{-1}$ and K:Mg ratios in all NBT populations were very lower (Table 4) than these values, resulting in tetany risk. Deficiency of P, an essential nutrient for all animals is the most widespread of all the mineral deficiencies affecting livestock. Besides, P must be balanced in the animal diet with adequate Ca and vitamin D for growth, reproduction, gestation, and lactation. The Ca:P ratio of forage is often discussed when examining forage quality and animal performance. An acceptable Ca:P ratio is between 1:1 and 7:1, as long as there is enough P to meet the nutritional requirements of livestock (Anonymous, 2000b, 2001, 2007). Therefore, the Ca:P ratios of all NBT populations in the present study were within the desirable Ca:P ratio range. Therefore, results on mineral concentrations and ratios between minerals of NBT populations suggest that NBT is suitable not only to prevent incidence or risky of milk fever and tetany but also to increase Ca, P, Mg and K utilization of the ruminant animals (Kume et al., 2001).

Perennial forage legume species, such as birdsfoot trefoil and NBT, contain condensed tannins, with severe consequences on the nutritional value of forage legumes and the health of animals eating such forage. The CT contents of NBT populations suggested that the level of secondary metabolites does not change with change in the altitude, latitude and longitude of plant origin. The CT levels of NBT populations were within normal ranges (up to 35 g kg^{-1} DM) which have reduced rumen degradable nitrogen and increase N-use efficiency (Acuña et al., 2012), increasing the protein absorption of the small intestine (Terrill et al., 1992; Acuña et al., 2012). Thus, grazing of pasture with NBT led to increased growth rate, fattening and reproductive performances as well as milk and meat quality (Speijers et al., 2004).

4. Conclusions

This study provided that nutritional information on NBT from different altitudes, latitudes and longitudes, which can be used to obtain average nutritional values in pasture management work. This study indicated that tested populations can be selected for development of new cultivars or the multi-trait indices may be a more effective tool to select quality genotypes. There were no specific trends in the chemical composition and the nutritive value of tested NBT populations when grown under the same conditions. Therefore, we recommend that the seeds of different altitudinal, latitudinal and longitudinal NBT populations could be selected for multiplication of *Lotus* for obtaining the nutritionally superior forage.

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