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Effect of altitude on morphological and nutritive characteristics of orchard grass (*Dactylis glomerata* L.) collected from natural flora of Ganos Mountain in Thrace region, Turkey

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Abstract
The aim of this research was to determine some morphological characters and nutritive values of orchard grass collected from four different elevations of natural flora. At each elevation, 70 plant samples were randomly collected at full-bloom stage. Altitudes did affect the plant height, number of tiller per plant, panicle axis length, number of spiklet per panicle, number of flower per spiklet and dry matter biomass per plant of orchard grass. The lowest plant height (75.0 cm), number of tiller per plant (9.1), panicle axis length (9.2 cm), number of flower per spiklet (3.5), number of spiklet per panicle (116.7) and dry matter biomass (55.6 g plant^{-1}) values were found at 845-890 m a.s.l. The acid detergent fiber, neutral detergent fiber, potassium, phosphorus, calcium and magnesium contents decreased with increasing elevation. The highest acid detergent fiber (36.49%), neutral detergent fiber (65.17%), potassium (1.23%), phosphorus (0.36%) and calcium (0.59%) contents were obtained at the low altitude site (5-24 m a.s.l.). The magnesium concentration of samples decreased from 501 m (0.13%) to 890 m (0.12%) altitudes.

Keywords*:* Altitude, Morphological characters, Nutritive traits, Orchard grass, Thrace region

Introduction
Altitude, slope and direction are known as topographic factors. Different effects of altitude on plants can be seen from sea level to high land areas. With an increase in altitude above mean sea level, there is progressive fall in temperature and as we go higher up with a decrease in temperature there is greater activity of the wind. A decrease in soil temperature reduces the absorption of water and nutrients by the crops (Singh, 2011). The northern aspect of high lands usually has higher levels of rainfall, thus facilitating loss of nutrients through surface runoff, a parameter also greater on steep slope sites (Chase *et al*., 2000; Ates, 2011). Besides, altitude and slope aspect plays an important part in defining properties of the floristic composition and soil. Ates and Tekeli (2011) reported that the morphological and chemical characters in clovers (*Trifolium* sp.) and sweet pea (*Lathyrus odoratus* L.) were changed depending on slope aspect and elevation. They emphasized that the variability in these traits were related to different photoperiod, precipitation, light intensity, temperature, moisture and soil characteristics.

Dactylis L. comprises a monospecific genus in the *Poeae* tribe of the *Poaceae* family, with the main species *D. glomerata* L. referred to as orchard grass or cocksfoot (Bushman *et al*., 2011). It is an herbaceous perennial forage grass, naturally distributed in the Anatolia, northern Africa, western and central Europe, and temperate lowlands and highlands of tropical Asia and exhibits remarkable local adaptation and ecotype differentiation (Sahuquillo and Lumaret, 1999; Xie *et al*., 2010; Copani *et al*., 2012). Orchard grass is characterized by high yield, good forage quality, stable productivity, rapid regrowth, highly palatable to all ruminant and non-ruminants, drought and cold tolerance. It is an aggressive grass, which often smothers other species in mixtures, is suited for cutting and grazing, especially when grown as a sole crop. It grows well on the soils that are not acidic and not waterlogged (Anonymous, 2017). Orchard grass performs best in areas that receive 18 inches annual precipitation or on irrigated sites but will establish and persist in areas that receive as little as 16 inches of annual precipitation. Plants are 50-120 cm tall. The leaves are 2-8 mm wide, and 20-30 cm long, v-shaped near the base tapering to a narrow tip with a prominent mid-nerve on the lower surface. The sheath is closed, auricles absent and the ligule is membranous and often split, merging with the throat margins. The inflorescence is a panicle, 5-20 cm long with spikelets laterally compressed, relatively small and 2-5 flowered in dense 1-sided clusters (Bush *et al*., 2012).

The aim of this research was to determine some morphological characters and nutritive values of orchard grass collected from four different elevations of natural flora.

Materials and Methods

Climatological parameters: This study was carried out in 2014-15 on the Ganos mountain of Thrace, Turkey located at 40.0 °N, 27.0 °E, with a typical subtropical climate (Fig 1). The prevailing potential natural vegetation in the studied area is *Pinus* sp., oak (*Quercus* sp.), linden (*Tilia* sp.), beech (*Fagus* sp.) and chestnut (*Castanea* sp.) forests. Altitudes were determined by GPS. The experimental mountain had an annual overall temperature of 11.2 °C at 5 m altitude. The air temperature decreases 0.49 °C per 100 m a.s.l. towards the mountain summit. The main soil type was designated as well-drained sandy clay mountain red yellow soil developed on sandy rock, which was classified as a xeralf. The altitude ranged from 5 to 905 m and annual precipitation was from 602 (5 to 251 m a.s.l.) to 1274 mm (501 to 905 m a.s.l.). The soils where the study was conducted was poor in organic matter (15-24 m: 1.21%, 248-251 m: 1.33%, 501-554 m: 1.88% and 845-890 m: 2.13%), low to moderate in P content (15-24 m: 58.2 kg ha^{-1}, 248-251 m: 48.1 kg ha^{-1}, 501-554 m: 39.7 kg ha^{-1} and 845-890 m: 38.4 kg ha^{-1}), but rich in K content (15-24 m: 501.8 kg ha^{-1}, 248-251 m: 421.7 kg ha^{-1}, 501-554 m: 189.4 kg ha^{-1} and 845-890 m: 95.6 kg ha^{-1}), and with pH ranging from 6.7 to 7.2.

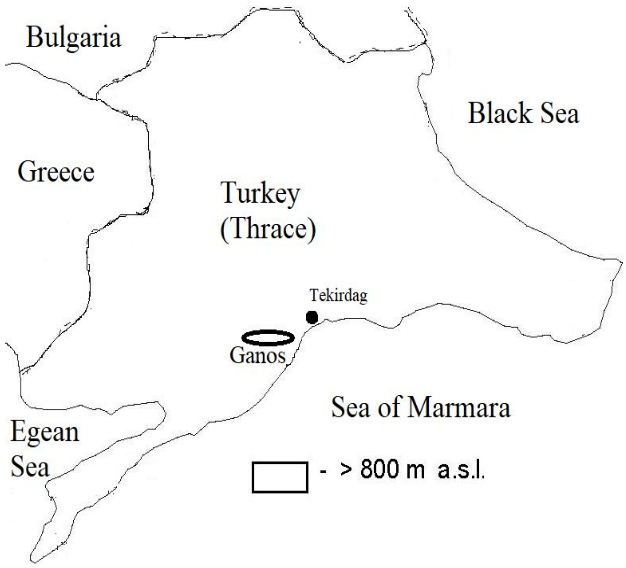

Fig 1. The Ganos Mountain in Thrace Map, Turkey

Sample collection, morphological traits and nutritional analyses: Four natural populations were sampled from different altitudes (5-25 m a.s.l., 248-251 m a.s.l., 501-554 m a.s.l. and 845-890 m a.s.l.) on south aspect of the mountain. At each elevation, 70 plant samples (Ayan *et al*., 2006) were randomly collected at full-bloom stage (March to July). These were chosen not damaged by biotic and abiotic factors (Pederson *et al*., 1999; Ates, 2011). The plant height (cm), the number of tillers per plant, panicle axis length (cm), the number of spiklet per panicle and the number of flower of spiklet were determined, then all collected samples were sterilized in 2% sodium hypochlorite solution for 30 minutes and washed in distilled water three times (Baji *et al*., 2002; Prakash *et al*. 2008; Ates, 2016). Dry matter (DM) biomass (g $plant^{-1}$) by each individual plant was clipped to a 5 cm above-ground level (Belesky, 2005), oven-dried at 55°C for 48 h and weighed (Ates and Tekeli, 2007; Ates, 2015). Dried plants were ground to small (1 mm) pieces and used for the analyses. The individuals of each altitude were mixed and three samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest *et al*., 1991) and N using the Kjeldahl procedure (AOAC, 2007). Crude protein content (%) was then calculated by multiplying the N content by 6.25. The samples were wet-fired with nitric-perchloric acid, and phosphorus (P) content was determined spectrophotometrically. Potassium (K), calcium (Ca) and magnesium (Mg) contents were determined using an atomic absorption spectrophotometer. Then, tetany ratios (K/Ca+Mg) were calculated (Cherney *et al*., 2002; Aydin and Uzun, 2008).

Statistical analyses: The results were compared using the TARIST statistical computer package program. There were no significant differences at P<0.05, 0.01 in the year x altitude interactions when comparing between the years of study neither between the altitudes. For this reason, the means were compared. The same program used for comparison of the means.

Results and Discussion

Morphological characteristics and dry matter biomass: The effects of the years on the plant height, number of spiklet per panicle and tetany ratio were all significant (P<0.01). The changes of some morphological characters and dry matter biomass depended on different altitudes (Table 1). Altitudes did affect the plant height, number of tillers per plant, panicle axis length, number of spiklets per panicle, number of flowers per spiklet and dry matter biomass per plant of orchard grass (P<0.01).

Table 1. Changes of some morphological characters and dry matter biomass depends on different altitudes in orchard grass

Altitudes (A)	*Plant height (cm)*			*Number of tiller per plant*			*Panicle axis length (cm)*		
	Years			Years			Years		
	2014	2015	Average	2014	2015	Average	2014	2015	Average
5-24 m	113.7a	120.1a	116.9a	14.3	14.8	14.6a	14.2	14.0	14.1a
248-251 m	99.3b	100.5b	99.9b	13.2	12.6	12.9bc	13.7	12.9	13.3b
501-554 m	90.7c	96.7c	93.7c	12.5	11.0	11.8c	12.4	11.3	11.9c
845-890 m	74.3d	75.8d	75.0d	8.7	9.4	9.1d	8.5	9.8	9.2d
Average	94.5b	98.2a	96.3	12.1	11.9	12.1	12.2	12.0	12.1
LSD (*P*=0.01)	Y: 1.331	A: 3.227		Y: NS	A: 1.078		Y: NS	A: 0.789	

Altitudes (A)	*Number of spiklet per panicle*			*Number of flower per spiklet*			*Dry matter biomass (g plant^{-1})*		
	Years			Years			Years		
	2014	2015	Average	2014	2015	Average	2014	2015	Average
5-24 m	165.1a	170.7a	167.9a	5.1	5.7	5.4a	100.8	105.8	103.3a
248-251 m	155.0b	160.3b	157.7b	4.7	4.5	4.6b	95.1	93.5	94.3bc
501-554 m	120.7c	126.8c	123.8c	4.5	3.9	4.2bc	90.7	92.4	91.6c
845-890 m	115.3d	118.1d	116.7d	3.8	3.2	3.5c	60.5	50.6	55.6d
Average	139.0b	143.9a	141.5	4.5	4.3	4.4	86.7	85.5	86.1
LSD (*P*=0.01)	Y: 1.422	A: 5.123		Y: NS	A: 0.423		Y: NS	A: 2.761	

Letters that differ within columns and row indicate values that are significantly different at $P < 0.01$; NS: $P > 0.01$

The plant samples showed decreasing trend in these traits with increasing altitude. The lowest plant height (75.0 cm), number of tillers per plant (9.1), panicle axis length (9.2 cm), number of flowers per spiklet (3.5), number of spiklets per panicle (116.7) and dry matter biomass (55.6 g plant^{-1}) values were found at 845-890 m a.s.l. Besides, maximum plant height (98.2 cm) and number of spiklets per panicle (143.9) were determined in second year (P<0.01). Topography is the principal controlling factor of plants growth in natural flora. Aspect, altitude and slope are three basic topographic factors that control the distribution and botanical composition of vegetation types in the areas. Temperature and precipitation caused by these topographic factors are considered as main limiting factors for growth, morphological traits and nutrient contents of plants in the vegetation (Ates, 2011; Ates, 2017). With increasing altitude, low temperature associated with low rates of soil biological activity were repeatedly considered as the main limiting factor for plant growth and nutrient supply (Körner, 1989; Macek *et al*., 2012). The high-altitude plants do not suffer water stress in the summer season; nevertheless, these plants have to face the unfavorable winter conditions with low temperatures accompanied by high irradiance (Kofidis *et al*., 2007) in subtropical ecological conditions and similar regions. Many different plant species live in the low-altitude have been determined to have lower root/stem biomass ratios compared to high- altitude plant species (Graves and Taylor, 1986; Kofidis *et al*., 2007). Tosun (1992), who studied the morphological, phenological and cytological characters on orchard grass (*Dactylis glomerata* sp. *hispanica* (Roth) Nyman) naturally growing in Erzurum Province, emphasized that the number of tiller per plant in orchard grass depends on plant age, plant growth and environmental factors. Morecroft and Woodward (1996) stated that the mass of inflorescences was greater in plants at the lower altitude. Intraspecific variations of leaf properties such as number of leaves, leaf size and leaf mass per shoot in *Sieversia pentapetala* L., along an altitudinal gradient in the central Japan was investigated by Taguchi and Wada (2001). They reported that the number of compound leaves per shoot and leaf size significantly decreased, resulting in reduction of leaf mass per shoot with altitude. The plant height, number of tillers per plant, panicle axis length, number of spiklets per panicle and number of flowers per spiklet ranged from 56.9-200.0 cm, 6.3-15.6, 8.5-15.0 cm, 94.3-175.4 and 2-5, respectively in orchard grass (Ayan *et al*. 2006; Hatipoglu and Kokten, 2009). According to Ates (2009), the different altitudes had significant effect on the plant height, number of stem per plant, number of leaves per main stem, leaflet length, leaflet width and leaf/stem ratio of some clover species. Roukas *et al*. (2017) emphasized that generally, forage production was negatively correlated with altitude. The present results were similar to those reported by these researchers.

Table 2. Changes of some nutritive values (in % DM basis) depends on different altitudes in orchard grass

Altitudes (A)	***Crude protein***			***ADF***			***NDF***		
	Years			**Years**			**Years**		
	2014	**2015**	**Average**	**2014**	**2015**	**Average**	**2014**	**2015**	**Average**
5-24 m	14.13	14.38	14.26c	36.23	36.74	36.49a	65.12	65.22	65.17a
248-251 m	14.73	14.53	14.63b	35.13	36.00	35.57ab	62.74	61.13	61.94b
501-554 m	15.23	15.45	15.34ab	34.72	34.24	34.48b	60.14	59.77	59.98b
845-890 m	16.00	15.97	15.99a	34.00	33.98	33.99b	59.88	58.00	58.94b
Average	15.02	15.08	15.05	35.02	35.24	35.13	61.97	61.03	61.52
LSD (*P*=0.01)	Y: NS	A: 0.650		Y: NS	A: 0.921		Y: NS	A: 3.222	

Altitudes (A)	**K**			**P**			**Ca**		
	Years			**Years**			**Years**		
	2014	**2015**	**Average**	**2014**	**2015**	**Average**	**2014**	**2015**	**Average**
5-24 m	1.22	1.25	1.23a	0.38	0.35	0.36a	0.60	0.58	0.59a
248-251 m	1.18	1.20	1.19b	0.30	0.32	0.31b	0.54	0.52	0.53b
501-554 m	1.15	1.12	1.13c	0.27	0.25	0.26c	0.50	0.47	0.48c
845-890 m	1.05	1.11	1.08d	0.22	0.22	0.22c	0.45	0.39	0.42d
Average	1.15	1.17	1.16	0.29	0.28	0.28	0.52	0.49	0.44
LSD (*P*=0.01)	Y: NS	A: 0.037		Y: NS	A: 0.043		Y: NS	A: 0.045	

Altitudes (A)	***Mg***			***Tetany ratio (K/Ca+Mg)***		
	Years			**Years**		
	2014	**2015**	**Average**	**2014**	**2015**	**Average**
5-24 m	0.20	0.18	0.19a	1.52d	1.64c	1.58c
248-251 m	0.19	0.21	0.20a	1.61c	1.64c	1.63c
501-554 m	0.14	0.13	0.13b	1.79b	1.87b	1.83b
845-890 m	0.12	0.11	0.12b	1.84a	2.22a	2.03a
Average	0.16	0.16	0.16	1.69b	1.84a	1.77
LSD (*P*=0.01)	Y: NS	A: 0.057			Y:0.049	A: 0.093

Letters that differ within columns and row indicate values that are significantly different at P < 0.01; NS: P> 0.01

Nutritional composition: The orchard grass samples showed higher crude protein (15.99 %) and tetany (2.03) ratios with increasing altitude (P<0.01, Table 2). The minimum tetany ratio (1.69) was found in first year. The ADF, NDF, K, P, Ca and Mg contents decreased with increasing elevation (Table 2). The highest (P<0.01) ADF (36.49 %), NDF (65.17 %), K (1.23 %), P (0.36 %) and Ca (0.59 %) contents were obtained at the low altitude site (5-24 m a.s.l.). The Mg concentrations of samples decreased from 501 m (0.13 %) to 890 m (0.12 %) altitudes. Our results were similar to some earlier studies. Nikki *et al*. (1960) reported that the crude protein and crude fiber contents of orchard grass increased with increasing altitude. Köhler *et al*. (2006), Macek *et al*. (2012) documented a decrease in foliar N, P, K and Ca contents with increasing altitude in some plant species. Ates (2009) reported that the crude protein, crude cellulose, Ca and K_2O ratios in field clover (*T. campestre* Schreb.), zigzag clover (*T. medium* L.), alsike clover (*T. hybridum* L.), suckling clover (*T. dubium* Sibth.) and hare's-food clover (*T. arvense* L.) were varied depending on altitude and slope aspect, who stated that the crude protein content of these plants from foothills zone increased with increasing altitude. Macek *et al*. (2009) documented a decrease in foliar P content with increasing altitude. Mineral elements balance is very important to keep animal healthy. Lack of one mineral element content cannot be balanced the others. These elements should be in certain ratio. For example, Ca and P are closely related to animal health and metabolism. It is very important to keep a proper balance of Ca and P in relation to vitamin D (Tekeli and Ates, 2005). Skeleton is containing approximately 68-73 % of the Mg in the total Mg content of animal body. The content of P in the rumen is also important, with higher levels of P favoring magnesium absorption. Cows grazing P-deficient pastures might have low concentrations of P in the rumen, and Mg absorption might be further impaired (Ates, 2017). Kemp (1960), Bush *et al*. (1979), Allison (2003) and Tekeli and Ates (2005) suggested that 0.20-0.25% Mg in the dry matter of forage crops should be a fairly safe level to prevent the likelihood of grass tetany. The Ca content in the blood also plays a role in the development of grass tetany in some cows. If it decreases, the concentration of Mg in the cerebrospinal fluid falls more rapidly when Mg in the blood decreases, as absorption is insufficient. Total protein and fiber contents are inversely related to growth stages of the forage crops, nevertheless, protein and fiber contents of forage crops could be quite variable among species and their cultivars. Generally, forage grasses typically contain lower protein levels (8-22%) compared with forage legumes (12-26%). The biological importance of the cell wall is related to a structural function and is of variable and often low digestibility in ruminants. Also, the physical volume occupied by cell walls in the rumen affects feed intake and animal performance (Jung, 1997). Redfearn *et al*. (2008) emphasized the high-producing dairy cows need hay with at least 20% CP, less than 30% ADF, and less than 40% NDF. Forages with better CP, ADF, and NDF values are not necessarily better for milk production. Tuna *et al*. (2004), Yavuz and Karadag (2016) reported that the NDF and ADF contents ranged from 64.31 to 65.31 % and 37.69-39.90 % respectively, in orchard grass. Sahin *et al*. (2010) obtained values of crude protein between 10.40-13.18 % in wild orchard grass lines. Can and Ayan (2017) found a Ca content of 0.41 %, a K content of 2.10 %, a P content of 0.41 %, a Mg content of 0.13 % and a tetany ratio of 3.92 for orchard grass at full-bloom stage. The results were similar to those reported by these researchers.

Conclusion

It was concluded that all morphological and nutritive characteristics of orchard grass were affected by altitude. The crude protein content and tetany ratios increased with increasing altitude whereas other morphological and nutritive characters decreased with increasing altitude. The changes in these traits we observed with increasing altitude could be described by different abiotic constraints (temperature, moisture, light intensity and quality, etc.). The decreases in plant mineral contents with increasing altitude could be explained by lower nutrient uptake due to low temperature and poorly developed soil biota under hill conditions.

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