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Performance of four blue melilot (*Melilotus caeruleus* (L.) Desr.) lines grown at two locations in the Thrace region of Turkey

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Abstract
The aim of this research was to determine the performance of forage yield and quality in four blue melilot (*Melilotus caeruleus* (L.) Desr.) lines grown in the Thrace region of Turkey. Four blue melilot lines (BG-1, BG-2, BG-3 and BG-4) and one population as control were used in the experiment. The lines were evaluated with mass selection in the Department of Field Crops, Faculty of Agriculture, Namik Kemal University, Turkey. Blue melilot population seeds were collected at mature stage from grasslands (43.0 °N, 26.0 °E) of the Belovets village in Razgrad, the north-east of Bulgaria. Some forage quality traits, yield and their components of candidate variety BG-3 was determined to be higher than other lines. Besides, genetic improvement using phenotypic selection there will also be required selection under multiple locations.

Keywords*:* Blue melilot, Forage quality, Forage yield, Mass selection

Introduction

Almost 1400-1500 species of *Fabaceae* family can be used as forage for livestock, although only about 55-65 species have been developed and widely used as cultivated forage crops. Forage legumes agriculture is highly dependent on a reliable source of protein as the primary feed base for animal production. Besides, they furnish essential energy, minerals, vitamins, and fibers. The benefits are reduction or elimination of nitrogen (N) fertilizer, extended grazing season, weed control, erosion control, and crop rotation.

Forage legumes are divided into annuals, biennials, and perennials, and each of these categories is further divided into warm and cool season forage legumes. The *Melilotus* L. genus in forage legumes shows considerable variations in botanical and agricultural characters. The primary center of origin of blue melilot (*M. caeruleus* (L.) Desr.) is the Turkey, near east and the central Mediterranean region (Dangi *et al*., 2004), Caucasus on the border between Asia and Europe, the mountains of central, eastern and south eastern Europe (Katzer, 2014). It is an annual, winter or spring annual legume normally growing 20 to 100 cm tall and can survive at -8 °C. Blue melilot is adapted to a wide range of soil types, but it is best-suited in low-lying areas, with well-drained, chernozem, vertisol and airy textured soils of pH from 6 to 8. It has been successfully grown in areas that receive 450 to 1200 mm annual rainfall (Ates, 2012). The leaves are trifoliate, alternate, with stipules adnate to the leaf-stalk, and heads or dense spikes of small blue, purple or white flowers; the small, one seeded roundish pods are enclosed in the calyx. The seeds are usually dark goldenrod or ochre in colour. Germination of seeds may be limited by a hardseededness of the embryo. The hardseededness ratio of these are softened during the summer, autumn and winter under uncontrolled storage conditions at subtropical regions by a combination of high and fluctuating temperatures and humidity. Besides, blue melilot is known as the Blue fenugreek, but it is genetically and morphologically different from *Trigonella* species. Based on genetic similarity indices, higher diversity is observed in blue melilot as compared (Dangi *et al*., 2004) to fenugreek (*Trigonella foenum-graceum* L.).

It is used as forage, pasture, silage, soil improvement, aromatic (alpha keto acids), medical [á-ketoisocaproic acid ($C_6H_{10}O_3$), pyruvic acid ($C_3H_4O_3$), á-ketoisovaleric acid ($C_5H_8O_3$) and á-ketoglutaric acid ($C_5H_6O_5$)] and culinary plants (Ates, 2011). Nevertheless; in Balkan countries and Georgia, the dried plants and seeds of blue melilot are widely utilized as a spice, usually sold as a playe greenish-brown powder consisting of leaves, pods and seeds. The aim of this work was to determine the some forage quality traits, forage yield and its components in four blue melilot lines in the Thrace region of Turkey.

Materials and Methods

A field experiment was conducted during 2011-2013 (October-June) at two locations (Tekirdag and Kirklareli) in Thrace, Turkey, one on xeralf soil (phosphorus content of 58.7 kg ha^{-1}, potassium content of 451.2 kg ha^{-1}, organic matter of 0.95 % and pH 6.9) at the Field Crops Department experimental area of Namik Kemal University, Tekirdag (41.0 °N, 27.5 °E) at 6 m above sea level with a total precipitation of 585 mm on average and an annual overall temperature of 14.02 °C. The other was on ustalf soil (phosphorus content of 55.7 kg ha^{-1}, potassium content of 501.4 kg ha^{-1}, organic matter of 0.88 % and pH 6.1) at the Oruclu village, Kirklareli (41.25 °N, 27.05 °E) at 100 m above sea level with a total precipitation of 561.3 mm on average and an annual overall temperature of 13.2 °C. Four blue melilot lines (BG-1, BG-2, BG-3 and BG-4) and one population as control were planted in Randomized Block Design with four replications. Blue melilot population seeds were collected during 2005-2006 at mature stage from grasslands (43.0 °N, 26.0 °E) of the Belovets village in Razgrad, the north-east of Bulgaria. The lines were evaluated with mass selection.

At each location, a basal fertilizer containing N and P (50 kg ha^{-1}) was incorporated into the soil at the time of land preparation. At both locations, each genotype was sown in plots of 20 rows, with a spacing of 25 cm and 5 m in length. The green fodder yield (t ha^{-1}) was determined in 3 m^2 at the full-bloom at 3 cm height from ground level and calculated per hectare. One cut was made in each year. Approximately 500 g herbage samples were dried at 55 °C for 48 h and stored for a day at room temperature for calculating dry matter (Ates and Tekeli, 2007).

The plant height (cm), main stem diameter (mm), number of leaves per main stem, leaf length (cm), leaflet length (cm) and width (cm), number of heads per plant were determined on twenty plants, which were randomly chosen from all plots at full-bloom stage. The main stem diameter was measured between the second and third node. The leaf length, leaflet length and width were measured on the leaf at the third node of the plants. Measurements of width and length of leaflet were concluded on the middle leaflet. Samples were hand-separated into leaf (including leaf sheath and inflorescence) and stem components to calculate leaf/stem ratio. The number of seeds per head was counted on ten plants, which were randomly chosen from all plots at mature stage. The seed yield (kg ha^{-1}) was determined in 2 m^2 at mature state at 2 cm height form ground level. All dried fodder samples were ground to small (< 2mm) pieces and used for the analyses. The crude protein (CP) content was found by the micro-Kjeldahl method (AOAC, 2007). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined following Romero *et al*. (2000). All samples were analyzed in duplicate. The results were statistically analyzed by using MSTAT-C procedures and mean separations were made based on Fisher's Least Significant Difference (LSD). There were no significant differences between years at each location.

Results and Discussion

The results for the some morphological characteristics and quality parameters, green fodder, dry matter and seed yields are given in Tables 1 to 3. The effect of location on main stem diameter, leaf length, leaflet length, leaflet width, leaf/stem ratio, number of heads per plant, number of seeds per head, green fodder yield, dry matter yield, crude protein, NDF and ADF ratios were found to be not significant ($P>0.05$, 0.01). A significant line and line x location interaction were found for leaf length, leaflet length, leaflet width, leaf/stem ratio, number of seeds per head, green fodder yield and dry matter yield measurements. The maximum leaf length (8.88-9.00 cm), leaflet length (4.23-4.47 cm), leaflet width (2.17-2.26 cm), leaf/stem ratio (0.88-0.92), number of seeds per head (45.21-45.41), green fodder yield (11.22-11.78 t ha^{-1}) and dry matter yield (2.98-3.11 t ha^{-1}) were determined for BG-3 blue melilot line at both the location. Plant height and number of leaves per main stem were influenced significantly by genotype and environmental factors such as line, location and interaction effect of line x location (Table 1). Higher plant height (94.68-108.71 cm) and leaves/main stem (23.07-28.79) were observed for BG-3 blue melilot line grown at Tekirdag compared to those grown at Kirklareli. Plant height, main stem diameter, leaf length, leaflet length, leaflet width, number of leaves per main stem or plant, leaf/stem ratio, number of heads per plant and number of seeds per head are important characters used to estimate forage yield, seed yield and forage quality. Forage quality, seed yield, green fodder and dry matter yields are known to be a complex properties governed by polygenes and therefore are influenced more by environmental factors. This type of interaction effect indicates that improvement in forage quality, forage and seed yields using selection of forage crops grown in different environments over a short term will be difficult and that genotypic improvement using phenotypic selection will require selection under multiple locations. Acikgoz (2001) stated that white and yellow

melilots grew to a height of 120-200 cm, whereas Silbir (2009) and Tekeli and Ates (2011) reported this value to be only 120-150 cm. Hay yields upto 7-8 t ha^{-1} are achievable in white melilot (*M. alba* Medik.) (Frame, 2002). Meyer (2005) determined that forage yields of yellow melilot (*M. officinalis* (L.) Pall.) at mid-bud, 10% bloom and late-bloom stages ranging from 7.18 t ha^{-1}, 7.43 t ha^{-1} and 5.20 t ha^{-1}, respectively. Badrzadeh and Ghafarzadeh-namazi (2009) found a plant height of 25-60 cm, a leaflet length of 2-5 cm and a leaflet width of 1-2 cm for blue melilot. Ates (2011) studied the forage yield and its components in blue melilot, who measured that plant height, number of leaves per main stem, leaf length, leaflet length, leaflet width, leaf/stem ratio, green fodder and dry matter yields at full-bloom stage ranging from 100.43 cm, 23.80, 4.97 mm, 8.49 cm, 3.78 cm, 1.85 cm, 0.70, 9.97 t ha^{-1} and 2.62 t ha^{-1}, respectively. Besides, that the blue melilot can be sown and cut at ¼ bloom, ½ bloom and full-bloom stages in the subtropical conditions. Dzyubenko and Dzyubenko (2014) reported that leaf length, leaflet length and plant height ranging from 2-5 cm, 1-2 cm and 30-60 cm, respectively.

In addition to a significant genotypic effect, location x line effect was significant for number of heads per plant and main stem diameter (Table 1 & 2). Higher main stem diameter (5.63 mm) and number of heads per plant (24.40) were measured for BG-3 line grown at Tekirdag compared to at Kirklareli. Seed yield of blue melilot was influenced significantly by genotype and environmental factors such as location, line and interaction effect of location x line (Table 3). The maximum seed yield (600.78 kg ha^{-1}) was found for same blue melilot line at Tekirdag. The effects of genotype, environmental factors and interactions on CP ratios were found to be not significant (Table 3). The CP ratios were ranged from 17.89 to 18.52 %. Basu *et al*. (2009) found that mean seed yield varied from 127 to 169 kg ha^{-1} in two blue melilot accessions under irrigated conditions at two locations of Canada. Canbolat and Karaman (2009) obtained CP ratios to be only 15.33 % to 15.78 % for white and yellow melilots, whereas, Ates (2011) emphasized that the CP and CF ratios, main stem diameter ranged from 17.35-19.38 %, 18.90-21.03 % and 4.92 to 5.05 mm, respectively in blue melilot at different growth stages. The growth pattern of a forage legume and grass is affected by the genetic makeup of the crop and the environmental conditions to which it is exposed. The genetic makeup is determined by the species, cultivar and line selected. Growth will vary within a season and among seasons, depending on the weather complex with in the different climatic

Table 1. Some morphological characters of blue melilot lines (mean of two years) at Kirklareli and Tekirdag locations

	Plant height (cm)			Main stem diameter (mm)			Leaves/main stem			Leaf length (cm)			Leaflet length (cm)		
Lines	**Kirklareli**	**Tekirdag**	**Mean¥**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**
BG-1	90.81^{d}	94.80^{cd}	92.81^{c}	4.78^{e}	4.80^{de}	4.79^{b}	21.82^{d}	22.05^{d}	21.94^{b}	7.91^{c}	7.62^{c}	7.77^{c}	3.62^{b}	3.46^{b}	3.54^{b}
BG-2	95.42^{c}	96.22^{c}	95.82^{b}	5.11^{c}	4.98^{d}	5.05^{b}	23.37^{c}	24.14^{c}	23.76^{b}	8.10^{b}	7.92^{c}	8.01^{b}	3.71^{b}	3.88^{ab}	3.80^{b}
BG-3	103.27^{b}	108.71^{a}	105.99^{a}	5.22^{b}	5.63^{a}	5.43^{a}	25.62^{b}	28.79^{a}	27.20^{a}	8.88^{a}	9.00^{a}	8.94^{a}	4.23^{a}	4.47^{a}	4.35^{a}
BG-4	92.25^{d}	93.54^{d}	92.90^{b}	5.04^{c}	5.10^{c}	5.07^{b}	20.81^{d}	21.13^{d}	20.97^{c}	7.74^{c}	7.50^{cd}	7.62^{c}	3.61^{b}	3.47^{b}	3.54^{b}
Popul ation	75.57^{f}	80.14^{e}	77.86^{c}	3.77^{e}	3.89^{d}	3.83^{c}	19.10^{e}	19.22^{de}	19.16^{c}	6.83^{d}	7.11^{d}	6.97^{d}	3.27^{c}	3.00^{c}	3.14^{c}
Means†	91.46^{b}	94.68^{a}	93.07	4.78	4.88	4.83	22.14^{b}	23.07^{a}	22.61	7.89	7.83	7.85	3.69	3.66	3.67
F-test	Location (L): 2.777**			L: NS			L: 0.887**			L: NS			L: NS		
(LSD)	Lines (Ls): 3.311**			Ls: 0.357**			Ls: 2.097**			Ls: 0.833**			Ls: 0.361**		
	LxLs: 4.200**			LxLs: 0.210**			LxLs: 3.102**			LxLs: 0.785**			LxLs: 0.583**		

**: p<0.01, ¥Line means and line x location interactions with different letter for the same column are significantly different (p<0.01); †Location means with different letter for the same row are significantly different (p<0.01); NS: p>0.01

Table 2. Green fodder yield and some morphological characters of blue melilot lines (mean of two years) at Kirklareli and Tekirdag locations

	Leaflet width (cm)			Leaf/stem ratio			Heads/plant			Seeds/head			Green fodder yield (t ha^{-1})		
Lines	**Kirklareli**	**Tekirdag**	**Mean$^{¥}$**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**
BG-1	1.89^{b}	1.89^{b}	1.89^{b}	0.75^{b}	0.71^{c}	0.73^{b}	21.20^{d}	20.10^{e}	20.65^{c}	32.45^{c}	32.00^{c}	32.23^{c}	9.97^{c}	10.05^{b}	10.01^{b}
BG-2	1.78^{b}	1.88^{b}	1.83^{b}	0.72^{c}	0.77^{b}	0.75^{b}	22.00^{c}	21.78^{cd}	21.89^{b}	34.12^{b}	31.78^{d}	32.95^{c}	10.13^{b}	10.55^{b}	10.34^{b}
BG-3	2.17^{a}	2.26^{a}	2.22^{a}	0.88^{ab}	0.92^{a}	0.90^{a}	23.12^{b}	24.40^{a}	23.76^{a}	45.21^{a}	45.41^{a}	45.31^{a}	11.22^{a}	11.78^{a}	11.50^{a}
BG-4	1.77^{b}	1.74^{bc}	1.76^{bc}	0.71^{c}	0.78^{b}	0.75^{b}	21.00^{d}	21.74^{cd}	21.37^{b}	34.78^{b}	35.11^{b}	34.95^{b}	8.78^{d}	8.95^{d}	8.87^{bc}
Population	1.56^{c}	1.44^{d}	1.50^{c}	0.68^{d}	0.61^{e}	0.65^{c}	16.72^{f}	16.45^{f}	16.59^{d}	15.70^{e}	16.72^{e}	16.21^{d}	7.78^{e}	8.13^{e}	7.96^{c}
Means†	1.83	1.84	1.84	0.75	0.76	0.76	20.81	20.89	20.85	32.45	32.20	32.33	9.56	9.89	9.73
F-test (LSD)	Location (L): NS Lines (Ls): 0.297** LxLs: 0.267**			L: NS Ls: 0.077** LxLs: 0.039**			L: NS Ls: 0.531** LxLs: 1.123**			L: NS Ls: 2.097** LxLs: 2.317**			L: NS Ls: 1.158** LxLs: 0.567**		

**: p<0.01, $^{¥}$Line means and line x location interactions with different letter for the same column are significantly different (p<0.01); †Location means with different letter for the same row are significantly different (p<0.01); NS: p>0.01

Table 3. Some forage quality parameters, dry matter and seed yield of blue melilot lines (mean of two years) at Kirklareli and Tekirdag locations

	Dry matter yield (t ha^{-1})			Seed yield (kg ha^{-1})			Crude protein (%)***			NDF (%)***			ADF (%)***		
Lines	**Kirklareli**	**Tekirdag**	**Mean$^{¥}$**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**	**Kirklareli**	**Tekirdag**	**Mean**
BG-1	2.44^{d}	2.65^{b}	2.55^{b}	411.11^{c}	421.72^{c}	416.42^{c}	18.23	18.21	18.22	45.22^{a}	45.11^{a}	45.17^{a}	29.78^{b}	30.12^{b}	29.95^{a}
BG-2	2.73^{b}	2.76^{b}	2.75^{b}	415.12^{c}	412.78^{c}	413.95^{c}	18.11	17.98	18.05	44.89^{a}	44.78^{a}	44.84^{a}	31.11^{a}	30.21^{b}	30.66^{a}
BG-3	2.98^{a}	3.11^{a}	3.05^{a}	565.73^{a}	600.78^{a}	583.26^{a}	18.00	18.52	18.26	40.14^{b}	40.00^{b}	40.07^{b}	28.78^{c}	27.84^{c}	28.31^{b}
BG-4	2.31^{e}	2.55^{c}	2.43^{c}	501.42^{b}	499.25^{b}	500.34^{b}	17.89	18.22	18.06	45.16^{a}	44.82^{a}	44.99^{a}	29.84^{b}	29.94^{b}	29.89^{a}
Population	2.12^{f}	2.24^{e}	2.18^{d}	310.00^{d}	325.45^{d}	317.73^{d}	18.14	18.21	18.18	44.77^{a}	45.14^{a}	44.96^{a}	30.44^{b}	30.75^{a}	30.60^{a}
Means†	2.52	2.66	2.59	440.68^{b}	452.00^{a}	446.34	18.07	18.23	18.15	44.04	43.97	44.01	29.99	29.77	29.88
F-test (LSD)	Location (L): NS Lines (Ls): 0.293** LxLs: 0.147**			L: 11.0.23** Ls: 47.777** LxLs: 46.389**			L: NS Ls: NS LxLs: NS			L: NS Ls: 4.557** LxLs: 3.778**			L: NS Ls: 1.555** LxLs: 1.046**		

**: p<0.01, $^{¥}$Line means and line x location interactions with different letter for the same column are significantly different (p<0.01); †Location means with different letter for the same row are significantly different (p<0.01); NS: p>0.01; ***In dry matter

location. Pasture and forage species are often grown extensively in environments that are similar but that are considerably away from their natural center of origin (Nelson and Volenec, 1995).

The means of the ADF and NDF ratios from the genotype and interaction of location x line are significantly different by a LSD test at the P=0.01 level of probability. The lowest NDF (40.00-40.14 %) and ADF (27.84-28.78 %) ratios were determined in BG-3 blue melilot line at all locations. The protein and fibre contents of forage crops can be quite variable among species and their genotypes. However, a forage quality property of forage legumes and grasses varies with different ecological conditions of locations, soil traits and growth stages. Generally, forage legumes typically contain higher protein levels (12-26%) compared with grasses (8-22%). With forages, however, leaves and stems quality begin to decline early in the growth cycle due to deposition and lignification of NDF especially in stems (Moore *et al*., 2007; Ates, 2011). NDF varies from roughly 10% in corn grain, which is nearly 90% digestible, to approximately 80% in straws and tropical grasses, which generally ranged from 20 to 50% in digestibility. ADF ranges from approximately 3% in corn grain to 40% in mature forages and 50% in straws. ADF values are slightly higher than are those for crude fibre (CF) because all the lignin and some ash are included in the former (Fisher *et al*., 1995). Alford *et al*. (2003) investigated intercropping irrigated corn with annual legumes for fall forage in the high plains and obtained lower value for CP (13.5 %), higher values for ADF (45.8 %) and NDF (56.1 %). Yisehak (2008) emphasized that the CP, ADF and NDF ratios ranged from 22.5, 33.1 and 37.2 %, respectively in white melilot. The results were similar to those reported by this researcher.

Conclusion

It is concluded that the some forage quality traits, yield and their components of candidate variety BG-3 blue melilot line was determined to be higher than other lines. Besides, genetic improvement using phenotypic selection there will also require selection under multiple locations.

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