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Original article

Forage Yield and Quality Attributes of Berseem Clover Genotypes under Mediterranean Climate

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Abstract

It is quite significant to develop annual and high-yield forage legumes to be pure-sown or intercropped with graminae species in winter cropping systems of Mediterranean region. In this sense, berseem clover is considered as a significant species for Mediterranean region. Therefore, this study was conducted to determine yield and herbage quality attributes of berseem clover (*Trifolium alexandrinum* L.) genotypes in Çukurova region with dominant Mediterranean climate. Experiments were conducted over the experimental fields of Eastern Mediterranean Agricultural Research Institute (Doğankent/Adana) in winter intermediate cropping system (November-April) of 2010-11 and 2011-12 growing seasons. Experiments were carried out in randomized blocks design with 3 replications. In the first and second year of the experiments, green herbage (GH) yields varied respectively between 53.3-79.7 and between 22.7-32.3 t/ha; dry matter (DM) yields varied respectively between 7.26-12.02 and 5.16-7.42 t/ha; crude protein (CP) ratios varied respectively between 11.37-15.39 and between 15.22-18.88%; digestible dry matter (DDM) ratios varied respectively between 61.10-68.08 and between 56.84-67.70%, acid detergent fiber (ADF) ratios varied respectively between 27.22-41.45 and between 23.07-31.41%; relative feed values (RFV) varied respectively between 99.3-155.0 and between 146.5-190.5. In both years, genotypes 3, 8, 15, 16, 17 and18 had greater DM yields than the standard cultivar and the other genotypes. These genotypes were also prominent w-for herbage quality attributes. Genotype 18 was registered under the name of "DERYA" in 2015 and the other promising genotypes are still being used in on-going breeding studies.

Keywords: Berseem Clover (*Trifolium alexandrinum* L.), Genotype, Yield, Forage Quality.

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INTRODUCTION

Berseem clover is quite common in Egypt, Turkey, India, Pakistan, Italy and Tunisia with mild winters. Selections made for resistance to harsh winters have facilitated adaption of berseem species to Mid-West of the USA with quite harsh winters (Knight, 1985).

Berseem clover cultivars are gathered under three groups based on number of harvests in a single year (single cut, intermediate and multiple cut) (Putnam et al., 1999; Ross et al., 2003). Single cut cultivars are mostly recommended for winter cropping systems (Rethwish and Graves, 2000).

Berseem clover (*Trifolium alexandrinum* L.) is an annual species of Mediterranean climate zone. It is a cool-season forage legume well adapted to semi-arid conditions. Although it has several species ranging from single cut to several cuts, the greatest herbage yields are obtained from the 1st and 2nd cuts. It is a forage legume with a feed value equal to alfalfa (Tansı et al., 1989; Iannucci et al., 1996; Çelen et al., 1991; Ranjbar, 2008). In Mediterranean ecologies, single cut is recommended for winter intermediate cropping systems as not to delay the sowing of subsequent species (Yücel et al., 2016).

It was reported in previous studies that leaf/stem ratio was a significant parameter, the cultivars with a greater leaf/stem ratio had greater quality and more preferred by the producers (Iannucci et al., 1996), stem ratio in total fresh and dry herbage was around 60% and leaf ratio was about 40% (Ranjbar, 2008), stems contained high nitrogen (CP) as well as high cellulose, lignin and pectin-like hard-to-digest substances (Pal et al., 2004).

Although berseem clover is pure-sown in Mediterranean coastal conditions, herbage and seed harvests are mechanized since the plants do not exhibit lodging as much as vetch. Among the trefoils, berseem has the greatest seed yield, thus it is easy to widespread. It was reported that berseem clover could either be pure-sown (Yücel et al., 2016) or reliably be intercropped with graminae species (Italian ryegrass and triticale) in Çukurova region and similar ecologies with intermediate winter cropping systems and could be an alternative forage crop (Yücel et al., 2018 a, b).

This study was conducted to determine herbage yield and quality of different berseem clover (*Trifolium alexandrinum* L.) genotypes and assess the genotypes as potential breeding materials of further breeding studies.

Materials and Methods

Material: Besides 18 berseem clover (*Trifolium alexandrinum* L.) genotypes, which have long been cultivated in Çukurova region (20-25 years), obtained through single plant selection and already brought to cultivar yield level, standard Alex cultivar were used as the plant material of the experiments.

Soil and Climate Characteristics of the Experimental Site

Soil samples were taken from the experimental site to determine soil characteristics. Total salt content was 0.026%, pH was 7.72, average lime content was 20%, organic matter content was 2%, sand content was 27.8%, clay content was 31.2% and silt content was 41% (Soil Lab. Results of Ç.Ü. Agricultural Faculty Soil Science Department).

Climate parameters for the experimental years (2010-11 and 2011-12 – November-April) are presented in Figure 1. Total precipitation during the vegetation period was 603.0 mm in the first year and 776.0 mm in the second year. Average temperature was 12.9 °C in the first year and 11.5 °C in the second year.

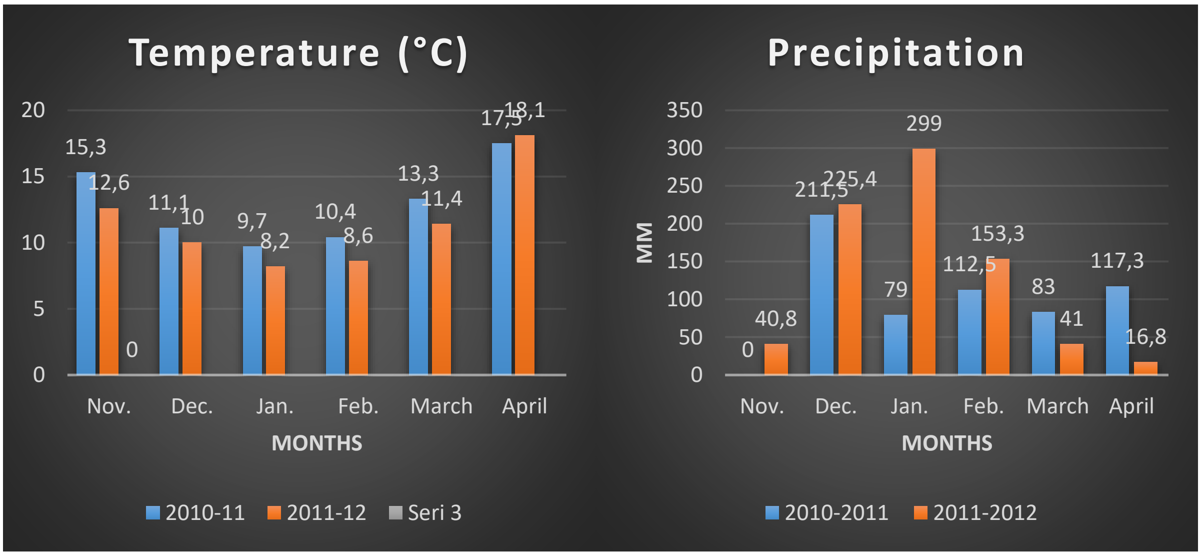

Figure 1. Climate parameters of the experimental years (2010-11 and 2011-12)

Figure 2. Image of berseem clover from the field experiments

Method: Field experiments were conducted over the experimental fields of Eastern Mediterranean Agricultural Research Institute during the winter intermediate cropping system of the years 2010-11 and 2011-12 (between November – April).

For cultivar yield trials, sowing was performed in the third week of November of both years. Sowings were performed at 25 cm row spacing. Each plot had 4 rows and plot length was 5 m. Sowing rate was 25 kg/ha. Before sowing, 30 kg/ha N and 80 kg/ha P were applied to plots. Dressing fertilization and irrigation was not performed throughout the growing season. Harvest was performed at 50-100% flowering period of each genotype (around 3rd and 4th week of April). Inner two rows were manually harvested to get green herbage yield per hectare. About 500 g green herbage samples of each plot were dried at 65 ^{o}C for two days until a constant weight and dry herbage ratio (%) was determined. Dry herbage ratios were then multiplied by green herbage yields to get dry matter yields. About 100-150 g dry herbage samples were ground in a mill and used later in quality analyses.

Herbage quality analyses were performed comparatively in NIRS (*Near Infrared Reflectance Spectroscop*y) analysis device (Shenk and Westerhaus, 1994) at the FOS XDS C0904FE-Hay and Fresh Forage calibration. Digestible dry matter (DDM) ratio, dry matter intake (DMI) and relative feed value (RFV) were calculated by using the equations provided by Jaranyama and Garcia (2004). These equations are: DDM=88.9-(0.779x%ADF); DMI=120/%NDF; RFV=(%DDM x %DMI)/1.29.

Experimental results were subjected to analysis of variance for each year separately with the aid of JUMP statistical software in accordance with randomized blocks design. Significant means were compared with the aid of Tukey’s multiple range test 5% significance level.

Results

With regard to number of days to flowering, genotypes, years, year x genotype interactions were found to be significant in both years. Number days to flowering varied between 133.3-146.7 days in the first year and between 141.3-152.7 days in the second year. Alex cultivar was identified as a late cultivar and number of days to flowering of the other genotypes were close to each other. Mean number of days to flowering was greater in the second year (144.5 days) than in the first year (139.7 days).

For plant height, only the years were found to be significant. Plant heights varied between 108.2-129.3 cm in the first year and between 75.1-86.4 cm in the second year. Alex cultivar had greater plant heights than the other genotypes in the first year and genotypes 5 and 8 and Alex cultivar had greater plant heights than the other genotypes in the second year (Table 1, 2). Mean plant height was lower in the second year (81.2 cm) than in the first year (122.9 cm).

With regard to green herbage yields, genotypes, years and year x genotype interactions were found to be significant in both years. Green herbage yields varied between 53.3-79.7 t/ha in the first year and between 22.7-32.3 t/ha in the second year. In the first year, genotype 8 had the greatest green herbage yield and Alex cultivar had the least green herbage yield. In the second year, genotype 20 had the greatest and genotype 6 had the least green herbage yield. Mean green herbage yield of the second year (27.4 t/ha) was lower than the mean green herbage yield of the first year (71.4 t/ha) (Table 1, 2).

Considering the dry matter yields, genotypes, years and year x genotype interactions were found to be significant in both years. Dry matter yields varied between 7.26-12.02 t/ha in the first year and between 5.16-7.42 t/ha in the second year (Table 1, 2). In the first year, genotype 8 had the greatest and Alex cultivar had the least dry matter yield. In the second year, genotype 20 had the greatest and genotype 6 had the least dry matter yield. Mean dry matter yield of the second year (6.33 t/ha) was lower than the mean dry matter yield of the first year (10.85 t/ha) (Table 1, 2).

With regard to crude protein ratios, genotypes and years were found to be significant only in the first year. Crude protein ratios varied between 11.37-15.39% in the first year and between 15.22-18.88% in the second year. In the first year, Alex had the greatest and genotypes 2 and 14 had the least crude protein ratios. In the second year, genotypes 1, 2, 3, 9 and 17 had crude protein ratios over 18% and genotypes 4, 13, 14, 15 and 16 had lower crude protein ratios (around 15%). Mean crude protein ratio of the first year (13.10%) was lower than the mean crude protein ratio of the second year (16.89%).

Considering the digestible dry matter (DDM) ratios, genotypes, years and genotype x year interactions were found to be significant in both years. DDM ratios varied between 61.10-68.08% in the first year and between 56.84-67.70% in the second year. In both years, genotype 2 had the least and Alex cultivar had the greatest DDM ratios. Mean DDM ratio of the first year (61.30%) was lower than the mean DDM ratio of the second year (67.10%).

With regard to neutral detergent fiber (NDF) ratios, genotypes, years and year x genotype interactions were found to be significant in both years. NDF ratios varied between 40.66-53.51% in the first year with the greatest value in genotype 2 and the least value in Alex cultivar. NDF ratios varied between 35.34-43.65% in the second year with the greatest value in genotype 1 and the least value in genotype 9. Mean NDF ratio of the first year (49.38%) was greater than the mean NDF ratio of the first year (38.19%) (Table 1 and 2).

Table 1. The averages of some examined characteristics of berseem clover genotypes in 2011

Genotypes	DF* (day)	PH (cm)	FFY (t/ ha)	DMY (t /ha)	CPR (%)	DDMR (%)	NDF (%)	ADF (%)	DMI (%)	RFV
1	136.3+	125.3	65.7	9.35	13.90	66.67	48.58	34.01	2.47	119.6
2	138.3	128.1	74.3	11.36	11.70	61.10	53.51	41.15	2.25	99.3 k
3	140.3	129.3	76.3	11.35	13.82	64.62	49.16	35.60	2.44	115.8
4	139.3	121.2	68.3	10.15	13.15	64.02	48.85	35.31	2.46	117.1
5	140.3	118.5	70.0	9.95	14.18	64.57	48.96	35.07	2.45	117.2
6	141.3	128.6	75.7	11.42	14.17	64.44	47.29	33.11	2.54	124.2
7	139.7	127.6	72.0	11.44	11.98	64.72	50.81	35.31	2.36	112.4
8	140.7	124.6	79.7	12.02	12.40	64.22	51.60	37.84	2.33	107.3
9	138.0	124.9	65.3	10.02	13.24	65.61	49.74	36.29	2.41	113.4
10	133.3	124.3	68.0	10.87	12.19	65.24	50.70	36.47	2.37	111.2
11	140.3	113.3	67.7	10.41	13.62	63.71	48.06	33.55	2.50	121.6
12	139.3	126.5	71.0	11.70	13.51	63.93	49.07	35.36	2.45	116.4
13	140.0	122.3	75.3	11.55	12.41	62.36	50.27	36.47	2.39	112.1
14	139.7	124.1	72.0	11.01	11.37	62.42	53.16	38.58	2.26	103.0
15	139.0	123.6	75.7	10.77	12.30	62.69	50.12	36.56	2.39	112.2
16	139.3	125.1	75.3	11.75	13.36	63.01	49.26	35.06	2.44	116.5
17	139.7	123.9	74.3	11.78	12.57	64.18	49.64	34.94	2.42	115.6
18	136.3	120.3	77.0	12.39	13.70	65.89	47.35	33.19	2.54	123.9
19 (Alex)	146.7	108.2	53.3	7.26	15.39	68.08	40.66	27.22	2.95	155.0
20 (Pop)	140.0	118.7	71.3	10.42	13.06	62.61	50.78	37.21	2.36	109.8
Mean	139.7	122.9	71.4	10.85	13.10 B[1]	61.30	49.38	35.42	2.44	116.2
CV (%)	0.74	5.42	5.89	7.56	8.09	1.84	3.47	4.08	3.40	4.78
F	**	NS	**	**	**	**	**	**	**	**

+) The means indicated with the same letters in the same column are not significantly different according to the Tukey's test at P≤0.05 **) Significant at P≤0.01

*) DF: Days to 50% flowering, FFY: fresh forage yield, DMY: dry matter yield, CPR: crude protein ratio, DDMR: digestible dry matter ratio, NDF: neutral deterjant fiber, ADF: acid deterjant fiber (ADF), DMI: dry matter intake, RFV: relative feed value

Table 2. The averages of some examined characteristics of berseem clover genotypes in 2012

Genotipler	DF* (day)	PH (cm)	FFY (t/ha)	DMY (t /ha)	CPR (%)	DDMR (%)	NDF (%)	ADF (%)	DMI (%)	RFV
1	143.3+	82.0	27.6	6.75	18.88	62.40	43.65	23.07	3.47	190.5
2	142.6	80.5	26.9	5.83	18.10	56.84	35.34	30.21	3.40	172.1
3	145.7	84.5	28.5	6.87	18.27	61.17	36.93	26.74	3.25	171.8
4	144.6	79.4	24.9	6.07	15.53	61.39	38.82	28.56	3.10	160.1
5	145.3	75.1	27.0	5.40	16.43	61.58	37.94	27.41	3.16	165.7
6	146.3	76.4	22.7	5.16	16.35	62.96	37.77	29.49	3.19	163.0
7	145.3	81.5	28.1	6.54	16.92	61.40	38.60	26.77	3.11	164.0
8	141.3	77.9	24.7	6.05	16.84	59.42	36.36	25.51	3.30	176.7
9	143.3	82.8	24.9	5.72	18.53	60.63	35.05	23.52	3.43	187.6
10	143.3	81.3	26.0	6.17	17.37	60.49	36.43	24.27	3.30	178.8
11	145.3	82.1	29.3	6.10	16.15	62.77	41.08	31.13	2.92	146.5
12	144.0	81.9	28.3	6.26	17.44	61.35	40.86	28.75	2.94	152.0
13	145.3	83.3	28.0	6.38	15.22	60.49	40.42	31.67	2.97	147.9
14	144.7	82.0	28.5	6.54	15.59	58.84	40.12	29.42	2.99	153.1
15	143.3	81.3	29.9	7.34	15.88	60.42	40.64	30.74	2.95	148.7
16	144.7	81.1	28.4	7.00	15.48	61.59	40.44	31.41	2.97	148.2
17	144.7	84.8	28.8	6.63	18.52	61.68	38.94	28.53	3.09	159.9
18	141.3	81.8	29.9	6.83	17.33	63.04	36.07	25.89	3.33	177.5
19 (Alex)	152.7	77.3	28.6	5.57	16.46	67.70	36.67	26.23	3.27	173.8
20 (Pop)	143.0	86.4	32.3	7.42	16.56	59.02	40.61	30.30	2.96	150.0
Mean	144.5	81.2	27.4	6.33	16.89	67.10	38.19	27.98	3.16	164.4
CV (%)	0.85	5.64	11.6	11.32	9.81	1.58	3.91	4.88	3.80	5.09
F	**	NS	*	*	NS	**	**	**	**	**
Y x C int.	*	NS	**	**	NS	**	**	**	**	**
Years	**	**	**	**	**	**	**	**	**	**

+) The means indicated with the same letters in the same column are not significantly different according to the Tukey's test at P≤0.05 **) Significant at P≤0.01

For acid detergent fiber (ADF) ratios, genotypes, years and genotype x year interactions were found to be significant in both years. ADF ratios varied between 27.22-41.15% in the first year and between 23.07-31.47% in the second year. In the first year, genotype 1 had the greatest and Alex cultivar had the least ADF ratios. In the second year, genotype 13 had the greatest and genotype 1 had the least ADF ratios. Mean ADF ratio of the first year (35.42%) was greater than the mean ADF ratio of the second year (27.98%) (Table 1 and 2).

With regard to dry matter intake (DMI), genotypes, years and genotype x year interactions were found to be significant in both years. DMI values varied between 2.25-2.95% in the first year with the least value in genotype 2 and the greatest value in Alex cultivar. DMI values varied between 2.92-3.47% in the second year with the lowest value in genotype 11 and the greatest value in genotype 1. Mean DMI value of the first year (2.44%) was lower than the mean DMI value of the second year (3.16%) (Table 1 and 2).

With regard to relative feed values (RFV), genotypes, years and genotype x year interactions were found to be significant in both years. Relative feed values varied between 99.3-155.0 in the first year and between 146.5-187.6 in the second year. In the first year, genotype 2 had the lowest and Alex cultivar had the greatest RFV. In the second year, genotype 11 had the lowest and genotype 1 had the greatest RFV. Mean RFV of the first year (116.2) was lower than the mean RFV of the second year (164.4) (Table 1 and 2).

Discussion

Years were found to be significant for all investigated parameters. With regard to vegetative characteristics, mean green herbage, dry matter yield and plant heights were greater, number of days to flowering was lower in the first year than in the second year. With regard to herbage quality parameters, CP, DDM, NDF, ADF, DMI and RFV of the second year were greater than the values of the first year. Lower NDF and ADF values are desired for feed quality of the herbage.

Number of days to flowering of the first year was lower than the second year, thus the second year was considered as later. Such differences were mainly attributed to greater precipitations and lower temperatures of the second year, such attributes were thought to influence number of days to flowering (Figure 1).

Mean plant heights of the second year were lower than the mean values of the first year. Greater precipitations of the second year and heavy and clay texture of the experimental soils slowed down plant growth and development. Together with recessing plant growth and development due to long and intense precipitations, weed populations also generated some problems and negatively influenced plant growth and development, thus ultimately yielded shorter plants. Similar findings were also reported by

Annicchiarico and Pecetti (2010) and Pecetti et al. (2012). In previous studies, plants heights were reported as between 64.8-99.9 cm (Anlarsal et al., 1996; Çelen, 1998).

Mean green herbage yields of the second year were lower than the mean values of the first year. Lower plants heights of the second year also resulted in lower green herbage yields. Significant positive correlations were reported between plant height and green herbage yields of trefoils (Yücel, 2005; Tucak et al., 2013). Present mean yields of the genotypes were greater than the values reported in previous studies (generally between 13.0 - 49.3 t/ha) (Tansı et al., 1989; Anlarsal et al., 1996; Çelen and Soya, 1997; Soya et al., 2003; Ranjbar, 2007; Yucel et al., 2016).

As it was in green herbage yield, dry matter (DM) yields of the second year were lower than the DM yields of the first year (Table 1, 2). Lower plant heights and green herbage yields of the second year also resulted in lower dry herbage yields. Significant positive correlations were reported between green herbage yield and dry matter yield of the trefoils (Yücel, 2005; Tucak et al., 2013). There was about 60% difference in yield of the years. Such differences in yields were attributed to climate and environmental conditions of the experimental years. Similar findings were also reported by Pecetti et al. (2012). Present findings on dry matter yields were similar with the values of earlier studies (between 3.4-15.2 t/ha) (Anlarsal et al., 1996; Soya, 1981; Soya et al., 2003; Çelen et al., 1991; Ranjbar, 2007; Yucel et al., 2016).

Crude protein (CP) ratios of the first year were lower than the CP values of the second year. In the first year, plants developed more because of more available climate conditions, thus leaf/stem ratios changed and decreasing leaf ratios resulted in lower CP values. Thusly, Abd El-Naby et al. (2015) reported significant positive correlations between crude protein ratio and leaf/stem ratio. As it was already known, CP ratio of legume leaves are greater than the stems. De Santis et al. (2004) indicated that plant heights increased with the progress of ripening and leaf/stem ratios decreased in this period. Tucak et al. (2013) also reported significant negative correlations of CP ratio with plant height, green herbage and dry matter yields. Crude protein ratios were reported as between 15.30-17.68% under Çukurova conditions (Yucel et al., 2016) and between 16.06-18.91% under Egyptian conditions (Abd El-Nabyet al., 2016).

The lower DDM ratios of the first year than the second year were because of greater plant heights, thus greater concentration of indigestible substances of the first year. Feed intake and digestion of the ruminants are related to composition of cell-wall substances. Previous researchers indicted that feed digestibility mostly varied with the plant sections, harvest periods, plant species, type and density of cell wall substances (Chesson et al., 1986; Buxton and Hornstein, 1986). Brink and Fairbrother (1994) reported negative correlations between cell wall components and digestibility of trefoils. DDM ratios were reported as between 56.9-62.2 under Çukurova conditions (Yucel et al., 2016) and between 63.03-67.86% under Egyptian conditions (Abd El-Naby et al., 2016).

NDF values of the first year were greater than the second year (Table 1 and 2). Greater plant heights of the first year decreased leaf ratios, thus increased indigestible cell wall substances and NDF ratios. Negative correlations of NDF were reported with plant height, green herbage and dry matter yields (Goeringand Van Soest, 1970; De Santis et al., 2017; Tucak et al., 2013). NDF values were reported as between 32.00-39.12% under Egyptian conditions (Abd El-Nabyet al., 2016) and between 47.34-53.31% under Çukurova conditions (Yucel et al., 2016).

As it was in NDF values, ADF values of the first year were also greater than the second year (Table 1 and 2). There are significant positive correlations between NDF and ADF. Similar findings were also reported by De Santis et al. (2017). ADF ratios were reported as between 23.09-26.12% under Egyptian conditions (Abd El-Nabyet al., 2016) and between 36.15-40.99% under Çukurova conditions (Yucel et al., 2016).

The DMI values of the first year were lower than the second year (Table 1 and 2). Higher DMI values are desired in quality feeds. Since DMI values are calculated with the aid of NDF value, greater NDF values of the first year resulted in lower DMI values. There is a inverse relationship between DMI and NDF values. DMI values were reported as between 3.11-3.75% under Egyptian conditions (Abd El-Naby et al., 2016) and between 2.27-2.52% under Çukurova conditions (Yucel et al., 2016).

Relative feed values (RFV) of the first year were lower than the second year (Table 1 and 2). RFV of alfalfa is calculated based on 100% flowering period. Since RFV is calculated with the aid of DDM ratio and DMI, lower DDM and DMI values also resulted in lower RFV. Such Such decreases in DDM, DMI and RFV were mainly attributed to greater NDF and ADF ratios (Wilson et al., 1991), greater lignification and lower leaf/stem ratios (Hides et al., 1983) with the progress of ripening. Abd El-Nabyet al. (2016) reported positive correlations between RFV and CP and negative correlations between RFV and NDF-ADF ratios. Hay Marketing Task Force of the American Forage and Grassland Council specified the standards for RFV as a criteria for hay quality (Rohweder et al., 1987). According to these criteria, the hays with RFV values of between 103-124 is graded as good, RFV values of between 125-151 is graded as Premium and RFV of >151 is graded as Prime class. Relative feed values were reported as between 149.9-197.3 under Egyptian conditions (El-Nabyet al., 2016) and between 80.5-102.2 under Çukurova conditions (Yucel et al., 2016).

Conclusion

Berseem clover are commonly either pure-sown or intercropped with graminae species in intermediate winter cropping systems of mild ecologies with dominant Mediterranean climate. Thus, berseem clover cultivars should be developed and sustained in sustainable ecosystems. In this study, it was quite remarkable that the genotypes 3, 8, 15, 16 and 18 had DM yields of greater than 9 t /ha and RFD of greater than 130. As the average of two years, genotype 18 had a DM yield of 9.61 t/ha and

RFV of 150.7 and these values were greater than the values of the other genotypes. Thus, this genotype was registered under the name of "DERYA" in 2015 and included into national cultivar list as the first local cultivar. The other promising genotypes are still being used in on-going breeding studies.

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