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Full Length Article

Using Leaf Production Efficiency as an Effective Criterion for Evaluation of Berseem Clover (*Trifolium alexandrinum*) Cultivars

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ABSTRACT

Ten berseem clover (*Trifolium alexandrinum* L.) cultivars were considered for their forage production and leaf:stem dry matter ratio under Mazandaran conditions in 2003 and 2004. Results showed that genotypes (G), years (Y), harvests (H) and their interactions were significant for most of examined traits including total fresh yield (TF), stem and leaf fresh yield (SF & LF), stem and leaf dry weight (SD & LD). Sacromonte was superior cultivar for production of TF, SF, LF, SD and LD; however, it was significantly poorer than Khadrawi in SD and EF_1, Laura in LD and Tabor in leaf dry matter efficiency (EF_2). Genotypes, Y × G and H × G interactions illustrated no significant difference for SD, stem dry matter efficiency (EF_1), LD and EF_2 and H × G × Y interaction showed no significant difference in SD% and EF_1. Year 2004 was more productive than 2003 for traits TF, SF, LF, LD and EF_2; however, for traits SD, EF_1 and LD% year 2003 showed better results. In all traits except LD% and EF_2, second harvest was significantly higher than the other three cuts. Totally, cultivar Sacromonte and harvest 2 were the most important cultivar and harvesting time for producing high quality forages and hays in the tested province.

Key Words: Berseem clover; Forage production; Stem dry matter efficiency; Leaf dry matter efficiency; Leaf-stem ratio

INTRODUCTION

Berseem clover (*Trifolium alexandrinum* L.) is an annual forage plant well adapted as a second crop in rotation with rice in Mazandaran Provinces, Iran (Ranjbar, 2007). Also, in some part of Iran it is cultivated in spring because of its spring type origins (Ranjbar, 2007). The quality of forage yield of this berseem clover like other leguminous forage species is closely correlated with more leaf production (Lloveras & Iglesias, 1998). So the cultivars with high leaf:stem ratio produce higher quality forages or hays and thus are preferred for cultivation by farmers (Iannucci *et al*., 1996). Although animals' stomach is able to digest stem fraction of forage as well as leaf fraction, digestion of stem fraction in animal stomach is much harder than leaf fraction and therefore, takes more time to pass through the digestion process compared with leaf forage (Tylor & Touy, 1976; Lloveras & Iglesias, 1998). Stem of berseem clover contains higher amount of fiber and materials like cellulose, lignin and pectin than leaf which make it difficult for digestion (Pal *et al*., 2004), also it contains high nitrogen (crude protein) concentration (Brink & Fairbrother, 1992). In contrast, leaf with low nitrogen (crude protein) concentration, has softer tissues containing photosynthetic assimilates which is including carbohydrates, vitamins, more digestible proteins and other macro and micro nutrients compared with stem (Brink & Fairbrother, 1992). Therefore, cultivars producing more leaves are preferred in a breeding program (Ranjbar, 2007).

The knowledge of growth patterns and productivity under different cutting regimes is of primary importance in berseem clover breeding programs (Iannucci *et al*., 1996). Iannucci (2001) found that the developmental stage at cut greatly influenced dry matter accumulation in all cultivars. Using other leguminous forage species (Pohlman & Simon, 199; Xia *et al.,* 1993) and in berseem clover (Iannucci, 2001) found that delay cutting from sixth internodes elongation to flowering can result in a subsequent depression in herbage yield.

The objective of this study was to identify the differences of some berseem clover genotypes in their forage dry matter with emphasize on the role of cultivars with high leaf production in increasing forage quality.

MATERIALS AND METHODS

In order to consider differences existed among berseem clover cultivars for forage quality, ten genotypes, selected from various sources based on their possibilities for recommendation as the most productive genotype, were

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examined using a split plot-in time design based on a randomized complete block design with four replications. The experiment was undertaken on a silt-loam soil with pH 7.5 in Mazandaran conditions in fall growing seasons during 2003 and 2004. The experimental field consisted of plots with six m^2 area (4 × 1.5) including five rows and 30 cm row intervals. Ten genotypes of berseem clover with different origins were Karmel, Ecotypes A and B, Actanone, Tabor, Sacromonte, Saidi, Khadrawi, Miskawi and Laura. Planting was done using 30 kg ha^{-1} seeds formally recommended by local Agricultural department for farmers, means that, totally 18 g $plot^{-1}$ seeds were used. After preparation of seed bed 50 kg ha^{-1} urea and 200 kg ha^{-1} ammonium phosphate fertilizers were manually added on the experimental area prior to planting. Harvesting was conducted using a hand sickle mower. Since berseem clover genotypes are differential in their post harvest re-growth, plants were cut from 5-8 cm above ground height. Each harvest were conducted from area of 2.7 m^2 (to measure yield of each plot precisely, one row from both sides and one meter from top and down of each plot were considered as guard area). Immediately after harvest of each plot, fresh forage yield was determined using a portable balance right in the corner of field. To determine total forage dry weight, a 500 g sample was selected from each plot. Another 500 g sample from each plot was chosen for separating leaves from stems. Both types of samples were dried at room temperature without direct exposure to sunshine until constant weight to have approximate dry weight. Some of quality characters were calculated using the following formulas:

Stem Fresh yield (SF%) = (SF/500×TF) × 100
Leaf Fresh Yield (LF%) = (LF/500×TF) × 100
Stem Dry Matter (SDM%) = (SDM/500) × 100
Leaf Dry Matter (LDM %) = (LDM/500) × 100
Stem Dry Matter Efficiency (EF_1) = SDM/TDM*100
Leaf Dry matter Efficiency (EF_2) = LDM/TDM*100
Leaf Stem Ratio (LSR)=LDM/SDM.

Where TF, SF, LF, SDM, LDM, EF_1 and EF_2 are abbreviated for total, stem and leaf fresh yield, stem and leaf dry matter and efficiencies of stem and leaf dry matter, respectively.

Statistical analysis on fresh and dry forage yield and their ratios was done using MSTATC statistical software. To analyze the variance characters of each year with four harvests were firstly analyzed and then a combined analysis with two years and four harvests was also done.

RESULTS AND DISCUSSION

Differences in years, genotypes, harvests and their two and three ways interactions are highly significant for total fresh yield (TF), stem fresh and dry yield (SF & SD), leaf fresh and dry yield (LF & LD) (Table I). Except years, harvests and their interactions, differences amongst other variations were highly significant for percentage of SD and EF_1. Percentage of LD and EF_2 were also highly significant for all variations (Table I).

There was a direct relationship in between leaf contribution of a forage plant from total forage yield and its forage quality, so that, in most of legumes, the superior variety will be determined by comparing their leaf and stem dry matters. Leaf has higher nutrition value than stem fraction and its digestible materials are obviously higher than in stem. Moreover, stem has more cellulose, lignin and pectin than leaf, which has more water, vitamins carbohydrates and proteins. Physiologically, leaves are more active than stems in transforming raw materials uptake from soil into photosynthetic assimilates (Pal *et al*., 2004). Like alfalfa by aging the berseem clover plants, their stem part may transform into woody and make more fiber in total produced forage (Bhargava & Raghupathi, 1993). Therefore, knowledge about the leaf contribution of each genotype in berseem clover is very important. Greater the leaf production and more the leaf quotas in total fresh and dry matter production, the better quality of forage is expected.

It seems that in year 2004 the environmental conditions were more suitable for fresh forage yield production of berseem clover genotypes than the first year, however, during 2003 the prepared materials were mostly transferred into stem fraction and less number of leaves were totally developed. Higher ratio of leaf: stem dry matter in second year implied that totally less water removal of leaves (1.9 t ha^{-1}) occurred in comparison with first year. In the first year, percentage of leaf dry matter produced from leaf fresh yield (20.0%) was significantly higher than that of the second year (14.3%) (Table II). To explain this phenomenon focused can be directed on two subjects. Water removal from leaves is much faster than stem and so in drier year with lighter leaf production, more water removal of leaves will occur than the wetter year. Another reason is related to lesser number of leaves during drier year, which can result in lesser leaf: stem ratio in comparison with wetter year.

The second harvest with 11.5, 7.5, 4.0, 1.2 and 0.8 t ha^{-1} for total, stem and leaf fresh yield and stem and leaf dry matter respectively was significantly higher than the other harvests (Table II). There were no significant differences among harvests 2, 3 and 4 in percentage of stem dry matter obtained from fresh stem (16.0, 16.2 & 16.0%, respectively), Moreover, there was no significant difference between harvest 1 and 2 in efficiency of stem dry matter (64.0 & 61.9%, respectively, Table II). Meanwhile, the fourth harvest produced the highest results for both of percentage and efficiency of leaf dry matter regarding to fresh leaf and/or total dry matter (21.0 & 46.2%, respectively) (Table II). Genotypes with 32.1, 19.9, 13.3 and 1.9 t ha^{-1} had produced more fresh forage yield, stem fresh yield, leaf fresh yield and leaf dry weight in second year of experiment respectively. However, they obtained better outcomes in first year of experiment for stem dry weight

Table I. Analysis of variance of fresh and dry weight of total, stem and leaf along with their percentages in 10 berseem clover genotypes

Source of variation	TF	SF	SD	SD%	EF_1	LF	LD	LD%	EF_2	LSR
Year (Y)	**	**	**	**	**	**	**	**	**	**
Genotype (G)	**	**	**	ns	ns	**	**	**	**	**
Y × G	**	**	**	ns	ns	**	**	ns	ns	ns
Harvest (H)	**	**	**	**	**	**	**	**	**	**
H × G	**	**	**	ns	ns	**	**	ns	ns	ns
H × Y	**	**	**	**	**	**	**	**	**	**
H ×G × Y	**	**	**	ns	ns	**	**	**	**	ns

Y = Year, G = Genotype, H = Harvest, TF = total fresh yield, SF = stem fresh yield, SD = stem dry matter, EF1 = efficiency of stem dry matter, LF = leaf fresh yield, LD = leaf dry matter, EF2 = efficiency of leaf dry matter

Table II. Differences amongst years and harvests for producing total, stem and leaf fresh yield and stem and leaf dry matter of berseem clover genotypes

Character	Means									
	TF	SF	LF	SD			LD			
	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	%	EF_1	t ha^{-1}	%	EF_2	LSR
Years										
2003	21.6b	14.1b	7.5b	2.8a	19.9a	67.3a	1.5b	20.0a	35.6b	0.5b
2004	32.1a	19.9a	13.3a	2.7a	13.6b	57.9b	1.9a	14.3b	41.4a	0.9a
LSD 5%	0.97	0.26	0.26	0.25	0.47	0.73	0.39	0.80	0.62	0.22
CV	5.22	2.65	2.44	0.22	2.47	4.35	0.64	2.31	2.36	1.45
Harvest										
1	6.7b	3.7b	2.4b	0.5b	14.0b	64.0a	0.3b	12.0d	35.5c	0.9a
2	11.5a	7.5a	4.0a	1.2a	16.0a	61.9a	0.8a	19.0b	38.2b	0.6c
3	5.8c	3.5b	1.9c	0.6b	16.2a	56.2b	0.3b	17.1c	31.7d	0.7b
4	3.2d	1.8c	1.3d	0.3c	16.0a	51.2c	0.3b	21.0a	46.2a	0.7b
LSD 5%	0.26	0.62	0.35	0.14	0.40	4.23	0.23	1.12	2.54	0.09
CV	2.22	1.16	0.11	0.09	1.31	3.84	0.04	2.26	3.14	1.15

TF = Total Fresh yield, SF = Stem Fresh yield, LF = Leaf Fresh yield, SD = Stem Dry matter, LD = Leaf Dry matter, EF_1 = Efficiency of stem dry matter, EF_2 = Efficiency of leaf dry matter

Table III. Means of fresh and dry weights of total, stem and leaf along with their ratios in 10 berseem clover genotypes

	Means									
	TF	SF	LF	SD			LD			
Gnotype	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	%	EF_1	t ha^{-1}	%	EF_2	LSR
Karmel	51.1e	31.4g	19.9d	5.3de	16.8b	61.1f	3.5bcd	17.5b	40.4b	0.7b
Ecotype B	55.9c	34.5e	21.4b	5.5cd	16.0d	61.1f	3.7abc	17.1c	40.4b	0.7b
Actanone	50.1f	31.9f	18.1f	5.1e	16.0d	63.3c	3.1de	17.4b	39.0c	0.8a
Tabor	49.7f	31.4g	18.3ef	4.8f	15.4e	61.8e	3.2cde	17.7b	41.4a	0.7b
Sacromonte	62.3a	39.5a	22.8a	6.4a	16.2d	63.0d	3.9a	17.3b	38.6d	0.8a
Saidi	53.4d	34.8d	18.5e	5.6c	16.2d	63.7b	3.1de	9.8e	32.5f	0.6c
Khadrawi	49.3f	31.1h	18.4e	5.3de	17.2a	64.2a	3.0e	16.2d	35.9e	0.6c
Miskawi	57.8b	37.2b	20.6c	6.0b	16.1d	63.1cd	3.6abc	17.6b	38.3d	0.6c
Ecotype A	57.7b	36.4c	21.6b	5.5cd	15.0f	60.3g	3.7ab	17.3b	41.1ab	0.8a
Laura	49.9f	32.2f	17.7g	5.3de	16.5c	61.7e	3.3bcde	18.8a	38.9cd	0.8a
LSD (5%)	0.97	0.26	0.26	0.25	0.25	0.23	0.39	0.45	0.37	0.03
C.V.	4.25	1.11	1.11	1.09	1.09	1.07	1.68	2.03	1.64	0.55

TF = Total Fresh yield, SF = Stem Fresh yield, LF = Leaf Fresh yield, SD = Stem Dry matter, LD = Leaf Dry matter, EF_1 = Efficiency of stem dry matter, EF_2 = Efficiency of leaf dry matter

(2.8 t ha^{-1}), SD (19.9%) and EF_1 (67.3%) (Table II). Although better results were achieved for production and the efficiency of leaf dry weight in the second year, the percentage of transformed fresh yield into dry weight in the first year were greater than that of in the second year (Table II). This indicated that for producing higher tonnage of fresh yield and total dry weight the environmental conditions in the second year of experiment was much better than that of in the first year; however, the first year was more suitable than the second year for transforming leaf fresh yield into dry matter. In other words, less amount of water has been removed from leaf fraction in the second year. Also, the second year demonstrated significantly higher leaf – stem dry matter ratio (LSR) than the first year of experiment (Table II). Superiority of the second year showed that the climatic conditions in this year helps berseem clover cultivars to produce more leaves with less water removal and/or less photosynthetic assimilates have been transferred into stem fraction of plant compared with the first year. First harvest showed the best for producing high LSR, which is significantly greater than other harvests (Table II). As the first harvest in both years of experiment has been conformed

in early spring, it implies that the cool or moderate weather conditions keep nutritive materials in leaves and do not allow them to be transferred into stem fraction of plant. Overall, it has been proven that the more LSR is a very good criterion to define high quality of forage. In current study first cut with LSR of 0.9 produced much higher quality forage than the other three harvests with LSR of 0.7, 0.7 and 0.6, respectively (Table II).

Genotypes are different for total, stem and leaf fresh forage yield, so that, cultivar Sacromonte with 62.3, 39.5 and 22.8 t ha^{-1} produced the highest amount of fresh yield for all three mentioned characters, respectively. Also, it produced the most stem (6.4 t ha^{-1}) and leaf (3.9 t ha^{-1}) dry weight in comparison with other genotypes (Table III). The ratio of transforming stem fresh dry weight (percentage of stem dry weight from stem fresh yield, e.g., SD of cultivar Sacromonte was not high enough but cultivar Khadrawi with 17.2% showed the highest result for producing dry weight from stem fraction. Khadrawi was the most efficient cultivar for stem dry weight (64.2%). This showed that its stem dry weight quotas regarding to total dry matter was greater than other genotypes (Table III). The highest amount of leaf dry matter has been produced by cultivar Sacromonte (3.9 t ha^{-1}), however, cultivar Laura as a poor genotype for total, stem and leaf fresh forage yield produced the highest percentage of dry weight from leaf fraction (18.8 t ha^{-1}) and Tabor as the second poorest fresh forage yield producer with efficiency 41.4% was the best genotype for leaf dry weight production (Table III).

Again genotypes showed significant differences for LSR, so that, Sacromonte, Actanone, Ecotype A and Laura with LSR of 0.8 illustrate higher LSR than the other genotypes (Table III). Sacromonte as one of the high forage yielding cultivar in present study is also one of the best cultivar for LSR means that it can be recommended to be replaced with Karmel cultivar in Mazandaran province.

These results are in agreement with Iannucci and Martiniello (2001) demonstrated that growth characteristics in genotypes were severely affected by harvests and that their response varied between cultivars. Although a number of research workers mostly in Italia, USA and Egypt have been published on berseem clover, still knowledge about this forage legume especially in Iran is severely restricted (Martiniello *et al*., 1996; Peterson *et al*., 1999; Iannucci & Martiniello, 2001; Akbar *et al*., 2006). Cultivar Karmel has been recommended for several years for growing in riceland of Mazandaran province as a winter forage crop. However, according to the results of the present work, it seems that little considerations have been done for introducing this cultivar. The current results proved that there are several cultivars and genotypes with higher amount of forage fresh and dry yield and also with higher hay quality than this previously recommended cultivar. Iannucci *et al*. (1996) have mentioned importance of knowledge about growth patterns and productivity under different cutting regimes in a breeding program for berseem clover. To add some extra information about genotypes behaviors in Mazandaran conditions the results of current study may play beneficial roles. These results are in accordance with achievement about effect of developmental stage of genotypes at cut on dry matter accumulation (Iannucci, 2001). Also, delay in cuttings can influence herbage yield and quality (Pohlman & Simon, 1993; Xia *et al*., 1993; Iannucci, 2001).

According to Iannucci (2001) more leaf dry weight production makes quality value a cultivar higher than the other genotypes, so that breeders can provide programs for incorporating genes controlling these quality aspects into a high yielding well adapted cultivar. In conditions of current experiment, if cultivar Sacromonte can be adapted as a high yielding cultivar, then Khadrawi and Laura can be used for improving its forage quality. Also, studies on forage compositions of genotypes are necessary to find the right weakness and/or ability of cultivars for including into an improvement program, precisely. Shrestha *et al*. (1998) stated that forage nutritive value was variable across years and locations. Berseem clover consistently had crude protein similar to alfalfa as an index forage crop in both years. Lloveras and Iglesias (1998) pointed that the nutritive value of the stem fraction decreased with plant maturity. Indeed, delay in each harvest will promote stem water removal and reducing its digestibility. Sanderson and Wedin (1989) reported different digestibility concentrations over several phonological stages of plant. In current work harvests have been conformed at full bloom conditions. According to the results of Lloveras and Iglesias (1998), it may reduces nutritive value of stem fraction. Also, due to different leaf:stem ratio differences in forage quality between the early and late-maturing cultivars varied between years (Ross *et al.*, 2004). Berseem clover regrowth in second cut had higher nutritional quality but its protein contents declined with increasing days of regrowth (Ross *et al.*, 2004). Also Ross *et al.* (2004) stated that the second cut produced higher forage yield which are in accordance with current work's achievements.

CONCLUSION

Sacromonte as an Italian cultivar of berseem clover showed better agronomic and quality characters compared with other genotypes. With high difference between Sacromonte and Karmel (as the recommended cultivar for Mazandaran region), it seems their replacement is economically necessary and can effectively help in high forage production per unit area. The second year climatic conditions of the experiment were more suitable than the first year conditions for forage production. The first harvest was very important for high quality forage production. Also, differences in dry matter due to the different harvests resulted in significant changes in SD yield, SD percentage, EF_1, LD yield, LD percentage, EF_2 and LSR. Finally, breeding or molecular methods can be recommended for transferring some important quality properties of some

cultivars like Khadrawi, Laura and Tabor into cultivar Sacromonte.

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