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Determination of Mineral Concentration and Cell-Wall Energy Content of Some Alfalfa Cultivars and Genotypes (*Medicago sativa* L)

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Increasing forage yields remains a top of most alfalfa (*Medicago sativa*) breeding programs besides yield other agronomic traits need to be considered in additive to yield, especially when trying to develop breeding material from non adapted materials. Four cultivars and seventeen genotypes were examined in a field experiment in 2003-2004. Crude protein (CP), dry matter (DM), P, K, Mg, Na, Ca, Fe, Cu, Mn and Zn as a mineral concentration, neutral detergent fiber (NDF), acid detergent lignin (ADL) and acid detergent fiber (ADF) as a cell-wall energy content were studied. A greater proportion of significant phenotypic variations were observed among genotypes. Considerable significant correlations in chemical concentrations between genotypes were measured. Amongst the characteristics examined in this experiment there are highly negative significant correlation observed between DM with P (r = -0.479**), Zn (r = -0.419**), NDF (r = -0.971**), ADL (r = -0.792**) and ADF (r = -0.819**). Highly significant positive relationships were determined between NDF and P (r = 0.416**), Zn (r = 0.415**). ADL measurement revealed significant correlation with P (r = 0.309*), Cu (r = 0.438**) and NDF (r = 0.754**). There are highly positive significant correlation observed between ADF with NDF (r = 0.814**) and ADL (r = 0.815**). This result suggest that Savas cultivar, Gülsinberk, Mahmudiye and Adigüzel genotypes should provide useful genetic material for enhancing mineral concentration in alfalfa forage. The variation could be exploited as an additional source of genetic variation in breeding programs for quality trials to achieve a higher genetic gain for breeding cycle.

Key Words: Correlation coefficient, *Medicago sativa*, Mineral concentration, Cell-wall energy content.

INTRODUCTION

Alfalfa is the most important forage crop in Turkey. As a perennial crop, alfalfa is the high yielding economical crop with high feature value. Genotypic variation in alfalfa forage has been documented and forage quality has been altered through selection. Juan *et al.*[1] found differences in crude protein, neutral detergent fiber and acid detergent fiber among multifoliate and trifoliate entries. Divergent selection for alfalfa lignin concentration resulted in higher leaf to stem ratio and lower NDF in low lignin than in high lignin selections[2]. The concentration of most nutrients is greatest in leaves with the greater concentration of K in stems. Differences exist among leaves according to their position on the plant. Concentrations of N and P are less in basal leaves than the top of stems. In contrast, basal leaves have higher concentrations at Ca and Mg. Stem concentrations of N, P, Ca and Mg decrease from the top to the bottom of the shoot. The concentration of K increases progressively to near the top of the plant then decreases slightly. The variation in environmental conditions will influence nutrient concentrations in forage, because of changes in rate of dry matter production, ion movement in soil, root activity and the uptake of nutrients by the plant[3,4].

Current selection procedures often include feeding value characters (digestibility and fiber contents) to improve the energy value of alfalfa forage. Genetic variation among cultivars for digestibility or fiber contents has been described by Lenssen *et al.*[5]. But the identification and development of high yielding, highly digestible cultivars are complicated by the negative relationship between digestibilities and forage yield[6]. A wide range of variation for digestibility could be found at the individual level, as for the others traits. Depending on the importance of within cultivar variation compared with among cultivar variation and on the genetic correlations when including this additional source of variation, breeding programs could be include the analysis on individual plant digestibility[7].

Energy and protein are the most valuable components of alfalfa. The crude protein content of alfalfa can be determined directly in a laboratory, but there is no direct chemical test to determine energy value. The energy value of alfalfa hay is closely related to its fiber content as the alfalfa plant matures, its fiber content increases and its energy value decreases.

Neutral detergent fibre (NDF) reflects the bulkiness of forage, there is a limit to the amount of NDF that will fit into an animal rumen. When that limits is reached, she will stop eating. There is no more room until a significant portion of the fiber in the rumen is digested and/or passes on to the lower gut[8].

Alfalfa breeders are currently showing considerable interest in using seeding year data to rapidly determine forage quality differences among alfalfa entries, but information about quality differences between the seeding year and production years is lacking[9,10].

Forage quality can be defined as the relative performance of animals when herbage is fed to livestock. It is the product of nutrient concentration, intake potential, digestibility and partitioning of metabolized products within the animal. In addition to the direct response of animals to forage quality, because of limitations associated with cost and time in using animals. However, forage quality often is estimated by *in vitro* or chemical means[11]. One of the most affected focuses on quality is the plant genus and species dependent on plant genotypes[12-14].

The objective of this study was to measure within cultivars variances for alfalfa traits related to main mineral concentration, the energy value and the potential nutritive capacity of these native alfalfa ecotypes.

EXPERIMENTAL

During the spring of year 2000, 218 alfalfa cultivars were established in a spaced plant nursery at the experimental fields of the Field Crops Department, Faculty of Agriculture, Atatürk University, Erzurum, Turkey (39°55'N lat. 41°16'E long and 1950 m above sea level). Among them, 21 cultivars were used for this study Adiguzel, Alaköy, Burcu (12), Çayirbasi, Dilburnu, Dönemeç, Ercis, Gülgören, Gülsinberk, Hidirkoy, Kasimoglu, Köprüler, Mahmudiye, Mollakasim, Otluca, Otluyazi were landraces[15]. Kayseri, Savas, L-1312, Ladak and Diableverde were cultivars chosen in the fall of year 2002 to represent a wide range of genetic variation and different areas of breeding. 20 Plants (*i.e.*, genotypes) per cultivar were grown in plastic pots (number 8) in green house and these single plants transplanted to the field on 15 May 2003 at Field Crop Department, Erzurum in a deep clay silt soil in a randomized complete block design (Table-1). The location is arid characterized by dry, cool temperate summers and 187 mm rainfall during April-August. 46.5 % of the annual average rainfall (Table-2). Weeds were controlled with hand weeding when necessary. Experiment used for three different purpose (i) for forage yield measurement (ii) for seed yield measurements (iii) for quality measurement made on mineral concentration, the energy value and the potential nutritive capacity. On ten randomly chosen plants were cut at early flowering period and forage was dried, weight ground to pass a 1 mm grid. On all samples with dry weight higher than 4 g near infrared spectra (NIRS) were collected (NIR systems 6500, NIR systems Inc. Silver Spring, MD) between 1100 and 2500 nm at every 2 nm Van Soest methods for NDF, ADF and ADL were used[16]. Total nitrogen was determined according to the Kjeldahl method and crude protein percentage was calculated using the factor 6.25

TABLE-1
SOIL PROPERTIES OF THE RESEARCH LOCATION CL (CENTILITRE)

Organic matter (%)	1.35	Sand (%)	33.48
$CaCO_3$(%)	8.20	Clay (%)	25.24
pH (in water)	8.32	Silt (%)	41.28
Saturation	54CL	P (ppm)	13.18

TABLE-2
CLIMATIC DATA OF THE RESEARCH LOCATION
LONG-TERM AVERAGE (LTA) (75 YEARS 1929-2004)

Months	Mean temperature (ºC)				Relative humidity (%)				Rainfall (mm)			
	2002	2003	2004	LTA	2002	2003	2004	LTA	2002	2003	2004	LTA
Jan	-16.1	-7.7	-9.0	-8.8	72.4	77.6	76.9	76	14.0	17.7	14.3	22.5
Feb	-3.4	-8.2	-8.7	-7.6	72.6	73.3	77.8	76	8.9	30.7	90.0	26.5
Mar	-1.0	-6.6	-1.7	-2.8	71.3	75.8	69.7	74	37.4	32.9	33.7	35.0
Apr	4.2	4.4	4.0	5.3	67.1	62.2	58.0	64	81.2	81.4	36.0	51.9
May	9.8	11.6	9.7	10.6	55.8	52.0	63.5	60	73.1	29.9	121.7	70.5
Jun	14.3	14.5	14.5	14.9	57.0	50.6	52.8	56	74.0	45.7	40.7	47.9
Jul	18.3	18.9	17.9	19.3	53.0	49.3	41.9	50	39.1	18.5	2.4	27.4
Aug	16.6	20.0	19.6	19.4	53.6	42.7	41.1	47	54.6	5.1	1.3	17.1
Sep	13.6	13.8	13.8	14.7	52.9	46.3	40.9	50	52.9	19.3	6.0	24.4
Oct	8.9	8.8	17.2	8.1	61.9	64.1	59.2	61	61.9	90.9	27.4	44.6
Nov	1.3	-0.7	-0.5	1.1	69.4	74.5	71.9	72	69.4	36.1	43.6	33.9
Dec	-12.0	-6.6	-14.1	-5.6	73.5	71.3	78.0	76	73.5	16.1	8.2	22.9

× N. Determination of Ca, Mg, Fe, Cu, Zn, Mn contents of the samples were carried out by atomic absorption spectrometry and that of Na and K by flame emission using a Perkin-Elmer 2380 atomic absorption spectrophotometer. P was determined by a colorimetric method[17]. Data obtained from the experiment were analyzed by using SPSS 11.0 statistical program.

RESULTS AND DISCUSSION

Generally there were considerable variations in chemical composition between genotypes (Table-3). The DM content in alfalfa ranged from 59.8-76.4 g kg^{-1} Savas cultivar and Gülsinberk genotype had significantly ($p < 0.001$) higher DM content than for the other genotypes. Otluyazi had the highest CP content (164.7 g kg^{-1}) in contrary Mahmudiye had the lowest CP content (93.4 g kg^{-1}). P, Mg, K, Na and Ca generally were similar to or higher than previously published reports, while concentrations of Fe, Cu, Mn and Zn were similar or to slightly below other reports[18-21]. Rominger *et al.*[22] reported that leaves of alfalfa contained higher concentrations of Ca, P, Mg and N than stems or roots, but stem contained more K. Differences in germplasms growth stage sampling may have biased differences in mineral content because concentration of P, Ca, Mg, Mn, Fe, Cu and Zn decline with advancing alfalfa maturity[23,24]. Besides energy protein relationship, marginal mineral content can also limit productivity. Thus, Ca and Mg contents in small grain forages affected performance and health in pregnant or lactating cows[25,26]. Mineral concentration among four alfalfa germplasm studied by Townsend *et al.*[18] had similar result with present study.

TABLE-3
SHOOT TISSUE CHEMICAL MINERAL AND CELL-WALL ENERGY CONCENTRATION FOR ALFALFA CULTIVAR AND ECOTYPES

Genotype	CP	DM	P	Mg	K	Na	Ca	Fe (10^{-1})	Cu (10^{-1})	Mn (10^{-3})	Zn (10^{-3})	Ca/P	K/Ca + Mg	NDF	ADL	ADF
Adigüzel	107.5efg	69.6bcd	1.36 h	2.6 de	26.05ab	8.80b.e	15.95c	1.39hji	3.7ab	2.14fg	282j	9.35f	0.66 a	38.99e .i	8.72abc	33.07 bc
Alaköy	116.6c.f	70.5bc	1.50ef	3.45a	27.75ab	9.15a.e	29.0abc	4.14a	3.4ab	3.47ab	8.08a	15.55a.e	0.41bcd	40.34d.g	7.82bc	33.03bc
Burcu 12	116.3c.f	70.0bc	1.43g	2.8b.e	25.15ab	9.20a.e	27.0abc	1.62fgh	2.1b	3.28a.d	6.09e	15.15a.f	0.41bcd	39.80e.h	8.51abc	31.91c
Çayirbasi	130.6bcd	63.9efg	1.97 b	2.9b.e	26.85ab	9.20a.e	29.2ab	1.21ij	3.0ab	3.07a.d	7.35bc	11.90c.f	0.41bcd	45.61bcd	9.04abc	36.87abc
Diableverde	122.0cde	60.1g	1.62d	3.2abc	28.95ab	8.50cde	39.4a	2.90b	1.5b	3.35abc	5.38f	19.55a	0.34d	51.18a	10.60ab	38.64ab
Dilburnu	124.4b.f	69.7 bc	1.62d	2.85b.e	29.1ab	8.75b.e	27.0abc	1.46fi	1.6b	3.29abc	3.21i	13.35b.f	0.47 b	41.34d.g	7.64bc	35.71abc
Dönemeç	135.0bc	67.0cde	1.76 c	2.9b.e	23.75b	8.95b.e	22.8bc	2.37 c	2.2b	3.20abc	6.94d	10.40def	0.45bcd	42.00d.g	8.01bc	34.11abc
Ercis	143.5b	66.9cde	2.10a	2.95b.e	24.95ab	9.75a.e	25.2bc	2.08e	2.4b	2.64cg	8.10a	9.60ef	0.43bcd	41.15d.g	8.75abc	33.58bc
Gülgören	126.2b.e	71.9abc	1.46fg	2.75b.e	24.3ab	9.75a.e	22.4bc	1.17 k	1.6b	1.98g	2.58j	12.25b.f	0.46 bc	37.11ghi	6.69c	32.48bc
Gülsinberk	98.8fg	76.3a	1.35h	2.85b.e	26.65ab	11.00ab	27.4abc	2.22cd	1.9b	3.23a.d	1.50k	16.25a.d	0.43bcd	34.57 hi	6.34c	31.04c
Hidirköy	111.8d	62.4fg	1.72c	2.7 cde	28.35ab	10.85abc	34.4ab	1.72f	2.2b	2.37efg	8.11a	16.00a.d	0.37bcd	47.34abc	8.81abc	35.87abc
Kasimoglu	119.3g	59.8g	1.72c	3.1a.d	25.7 ab	10.70a.d	29.4ab	1.43ghi	4.8a	2.68cg	4.71g	13.70a.f	0.38bcd	49.74ab	11.72a	40.33a
Kayseri	115.5c.f	73.4ab	1.51e	3.25ab	26.5ab	9.00b.e	33.8ab	1.29ij	2.8ab	3.01b.e	5.52f	17.90abc	0.35cd	36.75ghi	8.04b c	31.64c
Köprüler	119.7cde	70.2bc	1.76 c	2.7 cde	24.45ab	8.35de	32.2ab	1.58fgh	3.0ab	2.81b.f	4.71g	14.70a.f	0.37bcd	39.52e.h	8.32abc	32.88bc
L 1312	111.1d.g	66.9cde	1.44g	2.85b.e	26.35ab	8.35de	33.1ab	1.57fgh	1.7b	3.94a	3.43hi	18.40ab	0.36bcd	43.24c.f	8.14bc	35.68abc
Ladak	128.1bcd	67.7 b.e	1.63d	2.9b.e	29.75a	9.40a.e	31.0ab	1.98e	3.3ab	3.28a.d	7.10cd	15.25a.f	0.43bcd	43.68cde	8.24bc	33.96 bc
Mahmudiye	93.4g	70.4bc	1.36 h	2.65de	27.45ab	10.90abc	33.3ab	1.58fgh	2.9ab	2.65c.g	4.39g	19.65a	0.37bcd	38.13f.i	7.17 bc	32.60bc
Mollakasim	119.3cde	68.0b.e	1.72c	3.05a.e	25.5ab	11.40a	31.2ab	1.67 fg	1.9b	2.94b.e	5.99e	14.60a.f	0.36bcd	42.12d.g	8.75abc	36.74abc
Otluca	135.2bc	69.5bcd	1.61d	3.25ab	29ab	8.10e	35.3ab	2.20cd	2.6b	3.06 b.e	7.54b	17.45abc	0.37bcd	41.63d.g	7.22bc	31.19c
Otluyazi	164.7a	69.8bc	1.97 b	2.55e	26.8ab	8.85b.e	27.5abc	1.16 k	2.6ab	2.62c.g	6.14e	11.15def	0.44bcd	39.26 e.h	7.64bc	31.89c
Savas	98.4fg	76.4a	1.38h	2.85be	27.9ab	7.65e	30.7 ab	1.26 ij	2.4b	2.55d.g	3.68h	17.80abc	0.41bcd	33.93i	7.06 bc	32.95a
Mean	120.8	68.6	1.62	2.9	26.7	9.36	29.37	1.81	2.6	2.93	5.40	14.76	0.41	41.30	8.25	34.10

The Ercis germplasm had the highest in P concentration. The Ca/P ratio for Mahmudiye was higher than observed the other germplasm due to relatively low P and high Ca concentration. The Ca/P ratio is important in livestock nutrition as it affect the availability of the nutrients, bone development and animal physiology[27]. The relatively lower Ca/P ratio in Adigüzel (9.35) and Ercis (9.60) germplasm might be more suitable genetic resources than the higher Ca/P ratios ones. A phosphorus deficiency in tissue, however, is one of the most prevalent minerals deficiencies for livestock[18,28]. The ratio of K/Ca+Mg ranged from 0.34-0.66 meq, Adigüzel germplasm had the higher K/Ca+Mg ratio than the others genotypes. Hypocalcaemia and hypomagnesaemia may affect lactating cows grazing small grain lush pastures under certain conditions, but they are uncommon in young growing animals[25]. When the ratio K/Ca+Mg exceeds 3 meq, the incidence of hypomagnesaemia increases in a cow herd[29] by more than 15 %. The ratio K/Ca+Mg decreases with advance of plant maturity[26].

There were significant phenotypic correlations between some of the mineral and cell wall properties (Table-4). A significant negative correlation observed between CP and DM (-0.296*), Ca/P ratio (-0.503**) and positive correlation with P (0.761**). The DM content was negatively correlated almost all properties except OM (0.974**), highly negative significance were measured between P (-0.479**), Zn (-0,419**), NDF (-0.971**), ADL (-0.792**) and ADF (-0.819**). There were highly positive significant correlation between P and Zn (0.616**), NDF (0.418**) and negative correlation with OM (-0.412**) and Ca/P ratios (-0.488**). The significant correlation observed between Mg and K (0.346*), Fe (0.597**), Mn (0.434**), Zn (0.347*) and Ca/P ratios (0.355*). Highly significant correlation were between K and Ca (0.563**) and Ca/p ratios (0.533**). There was not any significant correlation between Na and the observed properties. On the other hand, Ca significantly correlated with Mn (0.347*), Ca/P ratios (0.856**) and negatively correlated with K/Ca+Mg ratios (-0.864**). There is significant positive correlation between Fe and Mn (0.455**) and Zn (0.386). Cu had significance correlation only with ADL (0.438**). Mn had positive significant correlation with ADL (0.357*) and negatively correlated with K/Ca+Mg ratios (-0.357*). There are positive significant correlation between Zn and NDF (0.415**), OM (-0.419**) and K/Ca+Mg ratios (-0.308*). We observed a greater proportion of significant phenotypic correlation between minerals (Table-3) in this study than reported in earlier studies[18,30].

Diableverde, Kasimoglu and Hidirköy had the higher NDF contents 51.11, 49.71 and 47.31%, respectively. The lowest NDF were observed Savas cultivar (33.93 %). The average ADL measurement was 8.25 % and the Kasimoglu genotype had the highest lignin concentration (11.71 %).

TABLE-4
SIMPLE CORRELATION COEFFICIENT OF CHEMICAL MINERALS AND CELL-WALL CONCENTRATION IN ALFALFA CULTIVARS AND ECOTYPES

	CP	DM	P	Mg	K	Na	Ca	Fe	Cu	Mn	Zn	NDF	ADL	ADF	Ca/P
CP	1														
DM	-0.296†	1													
P	0.761‡	-0.479‡													
MG	-0.021	-0.051	-0.003	1											
K	-0.152	0.021	-0.132	0.346†											
Na	-0.160	-0.165	0.035	-0.005	-0.060	1									
Ca	-0.119	-0.169	0.016	0.424†	0.563‡	0.116									
Fe	-0.031	-0.101	-0.063	0.597‡	0.205	-0.026	0.163	1							
Cu	0.058	-0.239	0.088	0.083	0.019	0.134	-0.043	-0.015							
Mn	-0.043	-0.071	-0.060	0.434‡	0.195	-0.171	0.347†	0.455‡	-0.185	1					
Zn	0.482	-0.419‡	0.616‡	0.347†	0.134	0.012	0.249	0.386†	0.185	0.136					
NDF	0.260	-0.971‡	0.418‡	0.182	0.127	0.142	0.290	0.186	0.211	0.205	0.415‡	1			
ADL	0.203	-0.792‡	0.309†	0.079	-0.164	0.174	0.165	0.014	0.438‡	-0.016	0.238	0.754‡			
ADF	0.078	-0.819‡	0.273	0.036	-0.072	0.221	0.135	-0.008	0.223	0.103	0.094	0.814‡	0.815‡	1	
OM	-0.259	0.974‡	-0.412‡	-0.187	-0.114	-0.137	-0.274	-0.202	-0.220	-0.197	-0.419‡	-0.997‡	-0.755‡	-0.807‡	
Ca/P	-0.503‡	0.127	-0.488‡	0.355†	0.533‡	0.080	0.856‡	0.175	-0.113	0.357†	-0.098	0.000	-0.051	-0.043	
K/Ca+Mg	0.007	0.185	-0.144	-0.409	-0.153	-0.214	-0.864‡	-0.139	0.094	-0.388†	-0.308†	-0.264	-0.200	-0.186	-0.701‡

ADF observation ranged from 31.19 to 40.32 % as a result of this Kasimoglu genotype had the higher ADF percentage (40.33 %). But the identification and development of high yielding, highly digestible cultivars are complicated by the negative relationship between digestibilities and forage yield[6]. Single linkage dendogram indicated that Gülsinberk, Savas and Mahmudiye genotype and cultivar differed than the other genotypes. On the other hand Diableverde, Kasimoglu and Adigüzel much more differed as a single grouped than the remained genotypes (Fig. 1). A wide range of variation for digestibility could be found at the individual level, as for the others traits. NDF reflects the bulkiness of a forage because forage fiber is bulky, there is a limit to amount of NDF that will fit into an animals rumen (first stomach) when that limit is reached she will stop eating. High quality forage digested more completely and has higher energy values. The chemical composition of alfalfa (*Medicago sativa* L) and two grasses, reed canary grass (*Phalaris arundinacea* L.) and brome grass (*Bromus inermis* Leys.), studied by Thender and Westherlund[31]. They stated that chemical composition of the whole plant consequently changes, as the chemical composition differs between various anatomical parts. In addition to these changes, there are also changes in the cell-wall composition during growth. These differences were larger in alfalfa leaf and stems. Data on quality changes of leaves and stems of modern alfalfa cultivars subject to varying harvest regimes. Leaf NDF concentration and digestibility typically decline slowly with increasing maturity, while stem NDF and ADF concentration increase[32,33].

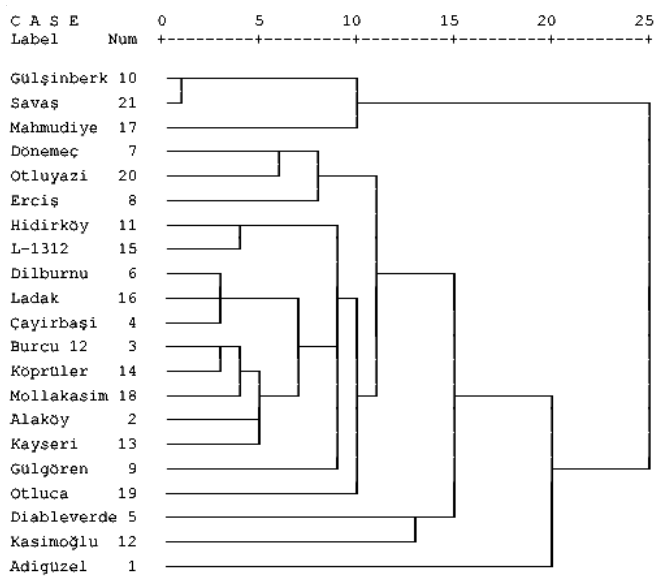

Fig. 1. Single linkage dendogram of the alfalfa cultivar and genotypes

In conclusion, there were considerable variation in chemical concentration between genotypes and cell-wall composition of the alfalfa entries. The research indicated that Savas and Diableverde cultivars, Gülsinberk, Mahmudiye, Kasimolu and Adigüzel genotypes were the most variable either cultivar or genotypes. As a result of this alfalfa breeders can be include those genotype for increasing their mineral concentration or energy content of their breeding program.

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