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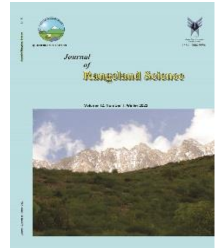

Research and Full Length Article:

Study of Diversity for Yield and Quality Traits in Alfalfa (*Medicago sativa* L.) and Determination of the Best Population for Cultivation in Dryland Farming in Iran

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Abstract. Alfalfa (*Medicago sativa* L.) is one of the most important forage crops, the so-called queen of forage plants, duo to its good quality, high digestibility, and its adaptability to different climates. In this study, 51 alfalfa populations were provided from the Research Institute of the Forests and Rangelands Gene Bank, Tehran, Iran. Seeds were sown based on an augmented design using six control genotypes in the agriculture and natural resources research center, Kermanshah, Iran in 2017. In the flowering stage, plants were cut and forage yield and chemical, quantitative and morphological traits were measured in all of 51 entries. Data were analyzed for descriptive statistics, correlation, factor analysis and cluster analysis. The Result of analysis of variance revealed no significant differences among replications (for replicated genotypes) for all traits except shoot height, leaves/stems, calcium, potassium, and total ash. The results of means comparison showed a significant variation between genotypes for the most studied traits. The highest and lowest forage yield with average values of 10089 and 1824 kg/h was obtained in Sharab-Urmia (Es-053) and Torbati1 (Es-032), respectively. The high protein content with average values of 19.46% was obtained in Sirjan1 (Es-026). Forage yield was positively correlated with stem number (r=0.50**), leaf weight (r=0.95**) and shoot weight (r=0.92**). Cluster analysis Ward method classified all alfalfa based on the all traits into four clusters. Populations in cluster 4 had higher overall mean values for both yield and quality traits. According to the Principle component analysis (PCA), the four components, namely the quality, yield, plant height and Mg+Zn components account for 40, 20, 10 and 7% (In total 77%) of data variance, respectively. The 5 top genotypes as FAO 1 (KR-3003), Cody 2 (Es-058), Italy 2 (Es-75), Kazagi2 (KR-615) and Mashhad 2 (Es-067) were recommended for improved breeding synthetic variety.

Key words: Alfalfa, Biodiversity, Nutritional value, Forage yield

Introduction

Forage plants are valuable because they maintain wild and domesticated herbivores and sustain the delivery of meat, milk, and other commodities. Forage plants contain different quantities of fiber, lignin, minerals, and protein and vary in their tissue proportion that can be digested by herbivores. These quality components are essential determinants of consumer growth rates, reproductive success, and behavior. The determination of forage production in natural habitats is the most important factor for grazing management.

Alfalfa (*Medicago sativa* L.) is one the most important forage legume in temperate regions of the world and is cultivated in 35 M/ha in the world. The area under alfalfa cultivation in Iran is 623,000 ha (Agricultural Statistics of the Crop in 2019). It is famous as the "queen of forages" for its high nutritive value, productivity, and adaptation to a range of environments, good quality, vitamins, and pigments (Russell, 2001; Radovic *et al*., 2009). Therefore, different varieties to cultivate in different farming systems and climates are necessary (Vivianne *et al*., 2018; Porqueddu *et al*., 2017). The *Medicago* genus has 34 annual and 51 perennial species (Small and Jomphe, 1988). The diversity of plant species in an environment enhances the stability and productivity of the ecosystem. However, the important point is that intra-species diversity can play an essential role in the rate of production of an ecosystem (Milić *et al*., 2011, 2014).

In Iran, three climates are considered for alfalfa's growth including dry, semi-dry, and humid regions. The most susceptible regions for alfalfa plantation by enough water are dry and then, semi-dry regions and finally, humid ones.

Forage quality is evaluated based on the consumption rate by animals and high digestion of present feed stuff. Usually, high crude protein (CP) and water soluble carbohydrates (WSC) and low NDF (neutral detergent fiber) and ADF (acid detergent fiber) are the most important quality traits. Also, CP, dry matter digestibility (DMD) are important factors for determining and optimizing forage quality (Marita *et al*., 2020; Farshadfar, 2016). Also, high values of WSC are necessary for supplying the required energy in animals' biochemical reactions. In pure stand and mix cropping of some perennial forages in Turkey showed that The sole alfalfa had higher yield, CP, and other quality contents than the other treatment (sole and mix cropping) (Sayar *et al*., 2014). DM yield had positive correlation with plant height, stem number, (Jafari *et al*., 2003a; Davodi *et al.*, 2011; Jafari *et al.*, 2012). In contrast, forage yield had negative correlations with leaf shoot weight ratio, DMD, CP and WSC (Davodi *et al.*, 2011). Mousavi *et al.* (2010) in a study of genetic diversity in 140 domestic and foreign genotypes of alfalfa found positive correlations between DM yield with both stem number and plant height. They grouped genotypes into 8 clusters. However, their cluster analysis did not separate Iranian genotypes from foreign genotypes. This research was conducted to determine the pattern of variation and correlation between forage yield and quality traits in 51 alfalfa populations, and to determine the best populations for dryland farming system in Iran.

Materials and Methods

This study was carried out at Islamabad agricultural station in the agriculture and natural resources research center of Kermanshah, Iran in 2017 (46º 59' E longitude and 34º 08' N latitude; 1260 m above sea level; mean annual rainfall of 485 mm; the mean temperature 20˚C; loamy soil). 51 accessions were obtained from the Gene Bank of the Research Institute of

Forests and Rangelands of Iran (Table1). Seeds were sown based on an augmented randomized complete block design with three replications and six controls. Each plot consists of four rows with 2m length, 65 cm between rows, and the distance between plots was 75 cm. Plant density has been calculated based on 25 kg/h seeds. No fertilizer was applied to the soil. Weeding was controlled by hand. Forage was harvested three times a year given as kgh^{-1}. The quality traits such as Dry Matter Digestibility (DMD), Crude Protein (CP), Acid Detergent Fiber (ADF), total ash, Crude Fiber (CF), and Water Soluble Carbohydrates (WSC) were measured using NIR (Perten 8620) (Jafari *et al*., 2003b). The atomic absorption device was used to measure chemical traits (Mn, Zn, Fe, Cu, Ca, P, K, Na). Morphological characters, plant height, stem number, shoot weight, leaf weight, DW (dry weight), WW (fresh weight), leaf/shoot weight ratio were recorded. MINITAB 16 Software was used for statistical analysis, correlation, cluster analysis, and principal component analysis.

Table 1. List of alfalfa genotypes and their origin

No.	Genotype code	origin	No.	Genotype code	origin	No.	Genotype code	origin
1	ES-211	Isfahan	18	ES-011	Tehran	35	ES-031	Mashhad 1
2	ES-178	Simer	19	ES-074	Italy	36	ES-066	Zardasht 2
3	ES-027	shahrood	20	ES-034	Birjand	37	ES-053	Sharab-urmia
4	ES-037	Isfahan 2	21	ES-083	Flaverjan	38	ES-004	Nikshahr
5	ES-199	Codi1	22	ES-046	Khansar	39	ES-030	Varamin
6	ES-024	Yazdi	23	ES-058	Kodi2	40	ES-010	Neyshaboor
7	ES-054	Kristar	24	ES-009	Gorgan1	41	ES-025	Sabzevar
8	ES-026	Sirjan 1	25	ES-052	Ranjer	42	ES-075	Italy 2
9	KR-2197	Kazagi1	26	ES-036	Kerman	43	ES-012	Foreign
10	KR-3003	Fao1	27	ES-082	Local1	44	KR-615	Kazagi2
11	ES-035	Jirofti	28	ES-065	Zardasht1	45	ES-088	Kebrit
12	ES-023	Damghani	29	ES-006	Sirjan2	46	ES-008	Isfahan4
13	ES-056	Bami	30	ES-051	Maooya	47	ES-067	Mashhad2
14	ES-049	Kermanshah	31	ES-050	Gorgan2	48	ES-096	Local2
15	KR-2421	Australia	32	ES-061	Fereidoon	49	KR-3002	France
16	ES-047	Kashan1	33	ES-040	Hamand	50	ES-014	Kashan2
17	ES-032	Torbati1	34	ES-126	Isfahan3	51	ES-2566	Fao2

Results

Result of analysis of variance revealed no significant difference between check genotypes that control block soil fertility gradients for all of traits except shoot height, leaf shoot ratios, calcium, potassium, and total ash. This indicated that there were similarities in soil fertility slopes for all of 51 populations. (Table 2.). Result showed Es-053 with 10089 kgh^{-1} had the highest forage yield and Es-032 with 1824 kgh^{-1} had the lowest forage yield. Es-026 with 19.46% had the greatest protein content and KR-2421 with 11.87% had the lowest protein content. The lowest CF was belonged to Es-026 genotypes with value of 22.72%. The lowest amount of ADF (26.77%) was belonged to the Es-061 genotype. Es-061 given as 28.34% had the lowest NDF. Kodi2 (Es-058) and KR-2421 populations with values of 68.93% and 51.51% had the highest and lowest DMD%, respectively.

Descriptive statistics such as mean, maximum, minimum, standard error, standard deviation and coefficient of Variation (CV%) on average values of 51 accessions of alfalfa are presented in Table 3. The highest CV with a value of 63.71% was related to forage, fresh weight, which indicates that there is a great variation between different alfalfa genotypes. The lowest CV with a value of 3.28% belongs to the number of days to flowering.

Because forage dry yield is one of the main indicators for selecting the superior alfalfa cultivar, the amount of dry forage varied from 1824 to 10089 kg h^{-1}. The CV of forage dry yield was 31.80% that is relatively high, and indicates a high amount of diversity for the selection of the superior genotypes. The CV values for quality traits such as CF, ADF, NDF and CP were low with values of 14.31, 12.88, 16.69 and 10.63%, respectively. This indicates a low power of choice for the quality traits (Table 3).

Range of traits for 10 top populations of alfalfa for chemical, quantitative and morphological traits and DM yield out of 51 populations is presented in Table 4. The genotype Es-058 (Codi2) in addition to superiority in forage yield in terms of CP was also one of the best population, followed by Es-023 (Falavarjan). The populations of Falavarjan and Codi2 were the best populations in terms of forage yield and quality that should be considered in the selection of superior cultivars.

Considering the sum of ranks of yield component, the top 5 alfalfa population was recommended to introduce as a synthetic alfalfa cultivar; KR-3003 (Fao1), Es-058 (Kodi2), Es-075(Italy 2), KR-615(Kazagi2), Es-067(Mashhad2) (Table 4).

Table 2. Results of analysis of variance among check genotypes (n=6) to control block soil fertility gradients

Traits	Abbreviation	S.O.V (MS)		
		Replication (DF=2)	Treatment (DF=5)	Error (DF=10)
Fresh Weight	WW	4742^{ns}	229.7^{ns}	3238.5
Dry Weight	DW	1373.255^{ns}	297.7^{ns}	702.03
Plant height	Height	37.167^{ns}	26.76^{ns}	21.23
Leaf stem weight ratio	LS	0.279*	0.062^{ns}	0.062
Crude Fiber	CF	22.857^{ns}	6.856^{ns}	6.093
Crude Protein	CP	2.027^{ns}	0.509^{ns}	0.894
Acid Detergent Fiber	ADF	20.730^{ns}	15.866^{*}	5.801
Neutral detergent fiber	NDF	4.730^{ns}	5.531^{ns}	7.988
Dry Matter Digestibility	DMD	18.000^{ns}	12.438^{ns}	5.447
Calcium	Ca	0.137*	0.062^{ns}	0.022
Phosphorus	P	0.0001^{ns}	0.0001^{ns}	0.0001
Potassium	K	0.103*	0.043^{ns}	0.016
Total ash	ASH	1.579*	0.423^{ns}	0.337
Sodium	Na	0.0001^{ns}	0.0001^{ns}	0.0001
Magnesium	Mg	0.001^{ns}	0.004^{ns}	0.004
Copper	Cu	17.495^{ns}	2.860^{ns}	4.849
Manganese	Mn	2.068^{ns}	16.163^{ns}	9.271
Zinc	Zn	24.899^{ns}	16.489^{ns}	8.956
Iron	Fe	827.171^{ns}	4212.9^{ns}	2845.6

*, ns = significant at 5% and non significant.

Table 3. Descriptive statistics such as mean, maximum, minimum, standard error, standard deviation and Coefficient of Variation (CV%) on average values of 51 accessions of alfalfa

Variable	Minimum	Maximum	Mean	SE Mean	StDev	CoefVar
Maturity (day)	77.39	89.39	85.99	0.40	2.82	3.28
Plant Height (cm)	42.06	73.86	55.15	1.10	7.87	14.27
Stem No.	11.56	91.22	43.83	2.47	17.64	40.25
Fresh Weight (FW) (kg/h)	5172.50	66911	14979.2	1336.2	9542.7	63.71
Dry Weight (DW) (kg/h)	1824	10089	5733.0	256	1828.2	31.89
Leaf Weight (LW) (kg/h)	21.79	113.11	63.75	3.04	21.71	34.05
Stem Weight (SW) (kg/h)	14.69	89.57	50.91	2.41	17.23	33.85
Leaf stem weight ratio (LS)	0.83	2.30	1.28	0.05	0.34	26.44
CP(%)	11.87	19.46	16.57	0.25	1.76	10.63
CF(%)	22.72	40.72	29.81	0.60	4.27	14.31
ADF (%)	26.77	44.97	33.97	0.61	4.38	12.88
NDF (%)	28.34	52.98	42.36	0.99	7.07	16.69
DMD (%)	51.51	68.93	62.55	0.59	4.24	6.78
ASH (%)	6.98	12.55	9.89	0.17	1.24	12.55
Ca (%)	1.08	2.42	1.79	0.04	0.32	17.75
P (%)	0.14	0.23	0.19	0.00	0.02	10.83
K (%)	1.55	2.91	2.05	0.04	0.30	14.64
Mg (%)	0.05	0.32	0.18	0.01	0.08	42.96
Na (ppm)	0.02	0.06	0.04	0.00	0.01	30.75
Cu (ppm)	6.96	19.27	12.44	0.45	3.22	25.90
Mn (ppm)	23.35	49.11	39.46	0.86	6.15	15.58
Zn (ppm)	13.89	32.58	25.15	0.50	3.54	14.07
Fe (ppm)	115.10	633.20	237.30	13.30	95.10	40.09

Table 4. Range of traits for 10 top populations of alfalfa for chemical, quantitative and morphological traits and DM yield out of 51 populations of alfalfa

Variable	Ranges	10 top populations name
Maturity	89.39-88.22	Es-067, Es-088, Es-058, Es-082, Es-065 Es-051, Es-061, Es-040,Es-031, Es-066
Plant Height	73.86-61.86	Es-034, Es-035, KR-2197, Es-051, Es-074, Es-040, Es-032, KR-2421, Es-056, KR-3003
Stem no	91.22-59.22	Es-075, Es-053, Es-036, Es-010, KR-3002, Es-058, Es-052, Es-088, Es-061, Es-008
Fresh Weight	5172-66911	Es-031, Es-075, Es-52, Es-053, KR-3003, Es-058, Es-074, Es-066, KR-615
Dry Weight	1824-10089	Es-053, Es-052, Es-075, Es-058, KR-3003, Es-034, Es-067, Es-066, KR-615, Es-050
Leaf Weight	113.11-84.49	Es-075, Es-053, KR-615, Es-052, KR-3003, Es-058, Es-034, Es-067, Es-004, Es-066
Stem Weight	89.57-69.19	Es-053, Es-052, Es-058, Es-088, Es-050, Es-036, Es-066, Es-075, Es-061, KR-3003
LS	2.30-1.48	KR-615, KR-3002, Es-004, KR-2197, KR-2566, Es-075, Es-008, Es-010, Es-032,
CP (%)	19.46-17.76	Es-026, Es-058, Es-035, Es-050, Es-011, Es-012, Es-065, Es-047, Es-067, Es-054
CF (%)#	22.72-25.8	Es-026, Es-023, Es-065, Es-006, Es-082, Es-035, Es-012, Es-046, Es-061, Es-050
ADF (%)#	26.77-29.97	Es-061, Es-058, Es-026, Es-023, Es-065, Es-082, Es-012, Es-050, Es-046, Es-083
NDF (%)#	28.34-34.4	Es-061, Es-058, Es-065, Es-082, Es-075, Es-088, Es-083, Es-067, Es-053, Es-074
DMD (%)	68.93-66.26	Es-058, Es-061, Es-026, Es-023, Es-065, Es-050, Es-012, Es-082, Es-046, Es-035
ASH (%)	12.55-10.79	Es-035, Es-023, Es-065, Es-026, Es-046, Es-058, Es-083, Es-050, Es-047, Es-010
Ca(%)	2.42-2.09	Es-035, Es-023, Es-026, Es-083, Es-065, Es-047, Es-012, Es-011, Es-050, Es-006
K (%)	2.91-2.30	Es-011, Es-010, Es-023, Es-009, Es-065, Es-058, Es-035, Es-047, Es-050, Es-040
P (%)	0.23-0.21	Es-026, Es-034, Es-006, Es-009, Es-035, Es-047, Es-011, Es-061, Es-058, Es-032

#= For CF, ADF and NDF the genotypes selected based on the lower values

Correlation between traits

The Correlation between different traits is shown in Table 5. There was a significant positive correlation between traits forage, fresh and dry weight (r=0.92). Forage yield was positively correlated with stem number (r=0.50), leaf weight (r=0.95), and shoot weight (r=0.92), respectively (Table 5).

The NDF was positively correlated with the Mg (r=0.64) and ADF (r=0.48). The ADF was positively correlated with CF (r=0.87). The significant correlation was found between Mn and Ca elements (r=0.68). Mn showed a positive and significant correlation with all elements except Mg ($P<0.05$). Cu showed a significant positive correlation with Ca, P, K, and Mg. There were positive and significant correlations between K with Ca and P ($P<0.01$). Ca had a positive and significant correlation with P, K, Na, Cu, Mn, and Fe. Leaf/stem ratios LS had a positive and significant correlation with CP and Ash content (r>0.50). The high amount of leaf due to high protein content plays an important role in alfalfa's quality. CP was negatively correlated with CF and ADF, and positively correlated with DMD, Ca, P.K. Cu, Mn and Ash.

Cluster analysis

Based on The Ward cluster analysis, 51 entries were divided into 4 clusters (Fig. 1 and Table 6). Populations in cluster 4 averaged well above the overall mean for both yield and quality traits. However, The results of cluster analysis did not well group the populations with different geographical origins in the same cluster.

Among the top ten populations in terms of forage dry matter, eight populations (five populations belong to cluster 4 and three populations belong to cluster 3. In addition, clusters 3 and 4 do not differ significantly in terms of forage production and other yield components, but cluster 3 had low quality values than cluster 4. The comparison between clusters for yield and quality traits (Table 6). For most qualitative traits, there was a significant difference between clusters. But there was no significant difference between clusters 3 and 4 for dry forage yield. The top populations of alfalfa in cluster 3 were Ghazafi2, Fao1, Australia, Zardasht2, Kazagi2, Fao2, and cluster 4 including Italy, Flaverjan, Kodi2, Ranger, Local1, Zardasht1, Fereidon, Sharab-Urmia, Italy2, and Mashhad2.

Table 5. Correlation between quality traits and yield

Traits	Height	Stem	WW	DW	LW	SW	LS	CP	CF	ADF	NDF	DMD	Ca	P	K	Na	Mg	Cu	Mn	Zn	Fe
Stem	-0.40																				
WW	0.29	0.37																			
DW	0.19	0.50	0.92																		
LW	0.15	0.49	0.90	0.95																	
SW	0.19	0.44	0.81	0.92	0.75																
LS	-0.10	0.14	0.10	0.08	0.34	-0.29															
CP	-0.07	0.07	-0.14	-0.06	-0.22	0.15	-0.51														
CF	0.06	-0.18	0.07	0.03	0.20	-0.19	0.54	-0.78													
ADF	0.16	-0.21	0.07	0.01	0.16	-0.18	0.44	-0.83	0.87												
NDF	0.27	-0.36	-0.35	-0.30	-0.19	-0.41	0.28	-0.40	0.45	0.48											
DMD	-0.15	0.19	-0.08	-0.02	-0.17	0.18	-0.46	0.88	-0.88	-1.00	-0.48										
Ca	0.05	-0.04	-0.16	-0.16	-0.31	0.05	-0.51	0.73	-0.79	-0.81	-0.31	0.82									
P	0.04	0.07	-0.11	-0.01	-0.13	0.14	-0.34	0.81	-0.60	-0.68	-0.22	0.72	0.63								
K	0.02	0.05	-0.09	-0.07	-0.16	0.06	-0.32	0.63	-0.43	-0.53	-0.13	0.56	0.61	0.54							
Na	-0.05	0.03	-0.09	-0.16	-0.27	0.00	-0.35	0.53	-0.57	-0.60	-0.41	0.60	0.66	0.46	0.52						
Mg	0.10	-0.14	-0.38	-0.29	-0.24	-0.32	0.13	0.13	-0.10	-0.14	0.64	0.14	0.22	0.23	0.23	-0.08					
Cu	0.39	-0.14	-0.13	-0.11	-0.22	0.03	-0.34	0.58	-0.45	-0.49	0.20	0.52	0.62	0.64	0.53	0.27	0.58				
Mn	-0.27	0.12	-0.14	-0.14	-0.22	-0.03	-0.24	0.60	-0.64	-0.70	-0.41	0.70	0.66	0.42	0.32	0.46	0.04	0.36			
Zn	-0.14	0.31	0.10	0.18	0.17	0.17	-0.03	0.33	-0.32	-0.37	-0.17	0.37	0.15	0.23	0.25	0.02	0.00	0.13	0.39		
Fe	0.16	0.10	0.07	0.05	-0.06	0.16	-0.15	0.44	-0.54	-0.50	-0.45	0.50	0.49	0.38	0.22	0.46	-0.21	0.24	0.44	0.26	
ASH	0.02	0.07	-0.09	-0.05	-0.22	0.17	-0.52	0.82	-0.85	-0.87	-0.44	0.88	0.92	0.64	0.61	0.61	0.11	0.58	0.72	0.28	0.61

*,**=the values between (0.30 to 0.40) and the values higher than 0.40 are significant at 5 and 1%.
Abbreviation of traits is explained in Table 2 and 3.

Table 6. Means Comparison of between clusters for quantitative and qualitative traits

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Plant Height (cm)	50.22b	58.48a	60.29a	55.12ab
Stem no	41.66b	40.93b	35.56c	54.09a
Fresh Weight	214.41b	221.40b	347.06a	396.58a
Dry Weight	99.30b	103.11b	125.48a	149.23a
Leaf Weight	57.20b	54.23b	77.02a	79.78a
Stem Weight	42.10c	48.23b	48.46b	69.45a
Leaf stem ratio	1.31ab	1.15b	1.53a	1.16b
CP	16.36a	17.60a	12.74b	17.39a
CF	31.15ab	27.58b	37.38a	27.12b
ADF	35.74b	31.51b	42.01a	30.72b
NDF	43.03a	46.39a	49.11a	32.67b
DMD	61.00a	65.01a	54.32b	65.58a
Ca	1.634b	2.004a	1.358c	1.950a
P	0.186a	0.207a	0.151b	0.196a
K	1.915b	2.254a	1.723b	2.117a
Na	0.033b	0.042a	0.027c	0.046a
Mg	0.174b	0.258a	0.157b	0.102c
Cu	11.19b	15.89a	8.28c	11.72b
Mn	40.13a	40.70a	27.81b	42.69a
Zn	24.99a	26.26a	21.61b	25.92a
Fe	191.56 c	244.01b	165.26c	304.62a
ASH	9.33a	10.65a	7.76b	10.75a

Means of rows with similar values has no differences based on Duncan's method (P<0.05).

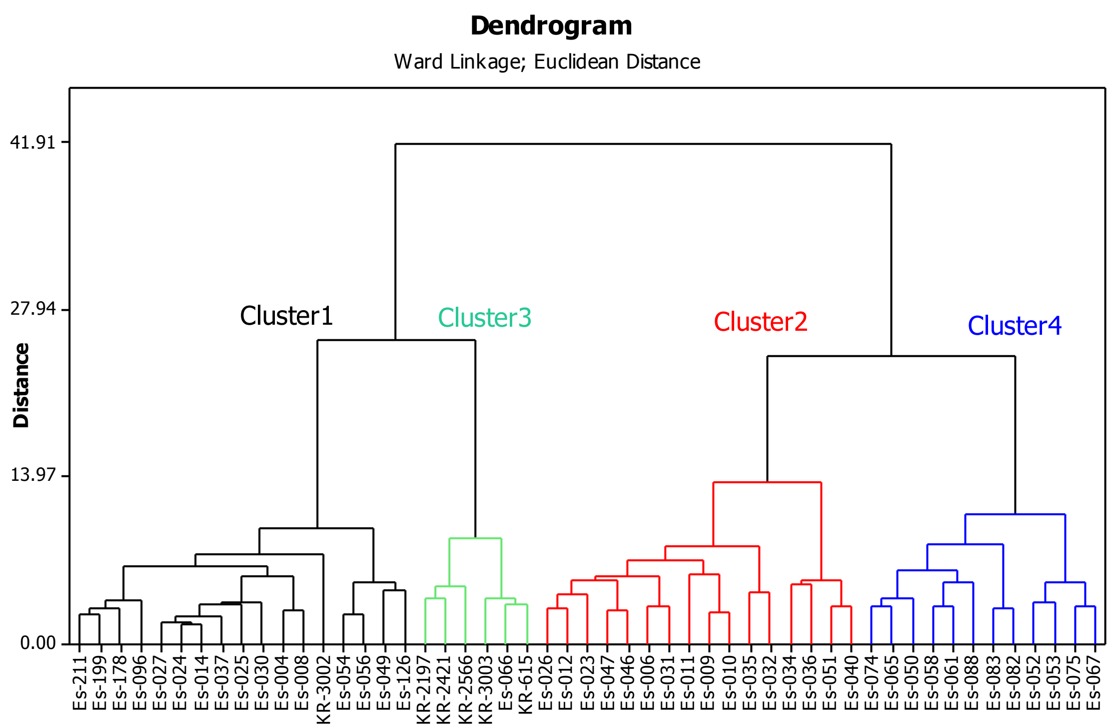

Fig. 1. Dendrogram of the alfalfa populations based on the all traits using the Ward method.

Principal Components Analysis (PCA)

The factor analysis was performed based on principal components extraction and varimax rotation method. Table 7 shows the contribution of different traits to each component. The four components represented 77% of total variance for four first components. PCA1, which accounted for 40% of variation, was associated with CP, DMD, Ca, P, K, Na, Cu, Mn, Fe, ASH, CF, ADF, Leaf stem weight ratio (LS). This component was regarded as quality component. PCA2 which accounted for 20% of the variation was named as productivity component since it was related to fresh weight, dry weight, leaf weight, shoot weight and stem number. The third and forth components were named plant height and (Mg and Zn), respectively (Table 7 and Figs. 2 and 3)

According to Fig. 2, the traits measured well were discriminated against four separate groups. The cluster 3 had low quality and cluster 4 had high productivity (Fig. 2). The Biplot diagram of 51 alfalfa populations based on the first and second components of PCA showed that the populations at the center of the biplot were more similar and less diverse. The plants that are far from the center of the plot and near the margins had a more genetic variation. Therefore, they could be used more in breeding programs. The results of cluster analysis did not well group the populations with different geographical origins in the same cluster and there was low agreement between cluster and PCA analysis (Fig. 3).

Table 7. Results of principal component analysis for Eigenvectors, Eigenvalues, variance percent and accumulated variance for four main factors using PCA analysis

Variable	PCA1	PCA2	PCA3	PCA4
CP	**0.90**	0.00	0.05	0.05
DMD	**0.95**	-0.07	-0.05	0.12
Ca	**0.90**	0.12	0.10	-0.11
P	**0.76**	0.02	0.24	0.10
K	**0.66**	0.07	0.23	0.10
Na	**0.69**	0.04	-0.18	-0.27
Cu	**0.62**	0.24	0.65	0.09
Mn	**0.74**	0.02	-0.28	0.18
Fe	**0.58**	-0.19	-0.12	-0.24
ASH	**0.95**	-0.03	0.03	-0.07
CF	**-0.89**	0.08	0.07	0.01
ADF	**-0.94**	0.09	0.07	-0.13
Leaf stem weight ratio	**-0.55**	-0.03	-0.04	0.47
Fresh weight	-0.13	**-0.91**	0.25	-0.11
Dry weight	-0.09	**-0.95**	0.27	0.05
Leaf weight	-0.27	**-0.89**	0.24	0.19
Stem weight	0.15	**-0.89**	0.25	-0.12
Stem No.	0.11	**-0.61**	-0.24	0.51
NDF	-0.45	0.52	**0.55**	0.26
Plant height	-0.06	-0.05	**0.75**	-0.48
Mg	0.13	0.49	0.49	**0.51**
Zn	0.35	-0.26	-0.07	**0.51**
Eigenvalues	8.62	4.42	2.18	1.60
% Variance	0.40	0.20	0.10	0.07
Cum Var %	0.40	0.60	0.70	0.77

* Numbers with underlines have more value in the main components

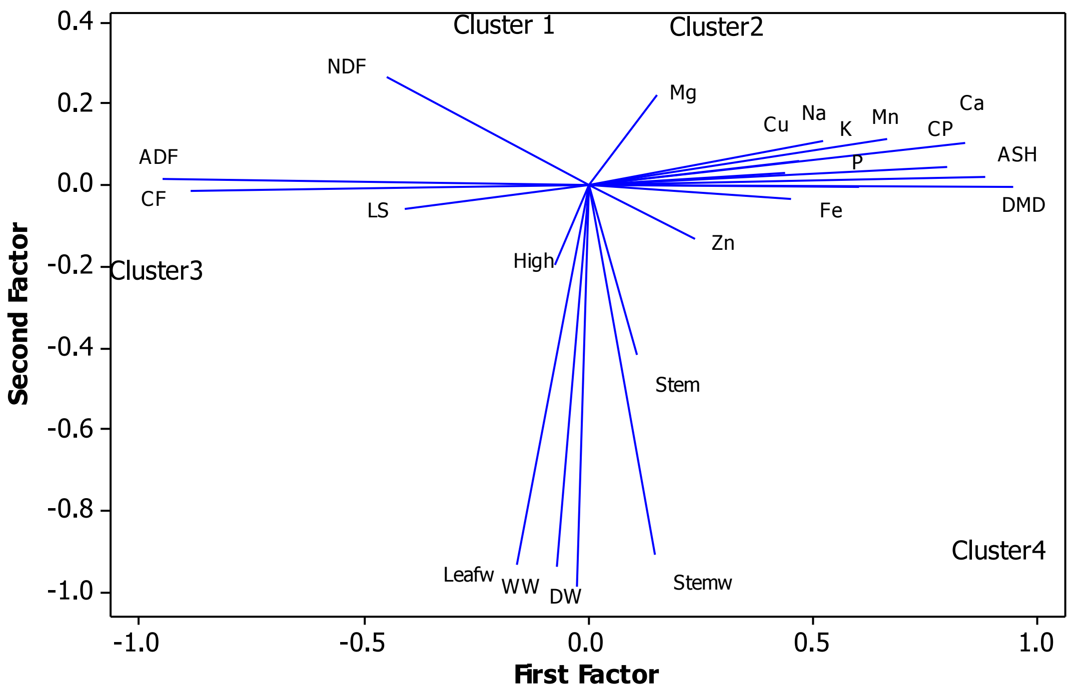

Fig. 2. Biplot diagram relationships between traits based on the first and second component of PCA analysis

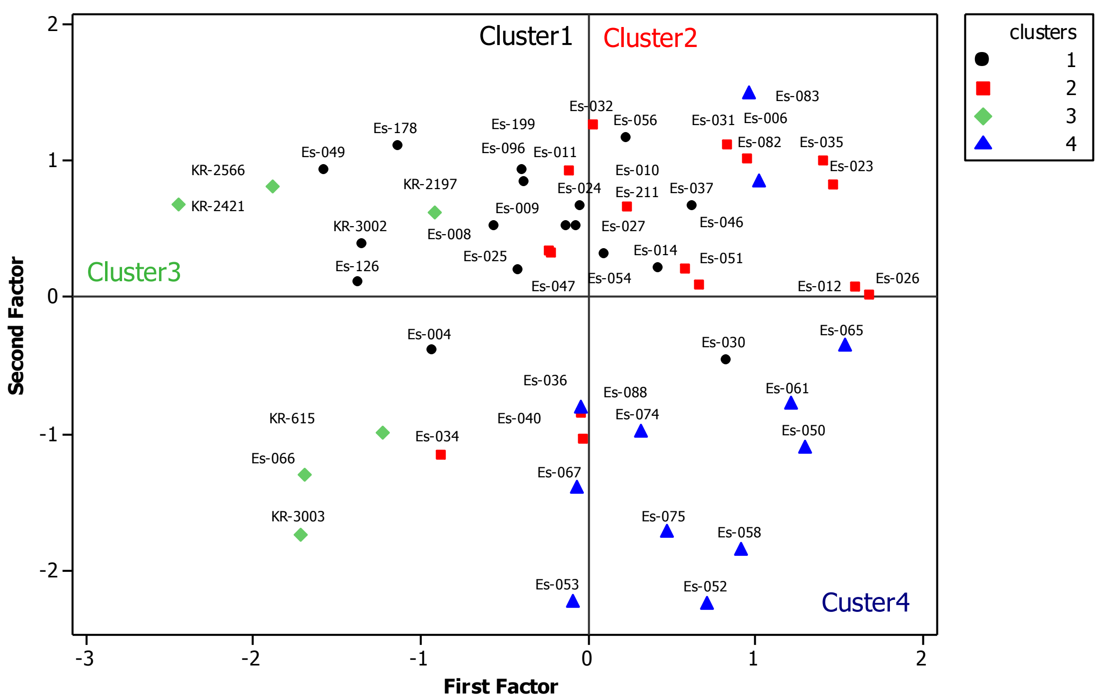

Fig. 3. Biplot diagram of alfalfa populations based on the first and second component of PCA analysis

Discussion

Alfalfa is one of the worldwide-grown perennial forage crops with high yields and nutritive value. It is characterized by high protein content and is often preferred by livestock compared to grasses. It is considered high-value cash crop and has value as a cover crop incorporated into cropping systems. Due to the high adaptation of alfalfa in different climates, genotypes with optimal forage yield in each climate have been released. Yield components that directly and indirectly affect forage production and the correlation between them should be studied. More study should be done about relationships between alfalfa's quality and chemical composition (Bouton, 2012; Hakl *et al*., 2019). In this study, there was no significant difference among replications for all of traits except the for K, Ca, and ASH ($p<0.05$). There was also no significant difference between check genotypes that control block soil fertility gradients (Table 2.). This indicated that there were similar soil fertility slopes for all of 51 populations.

The mean value for the studied traits showed great range and coefficient of variation, indicating considerable diversity among alfalfa genotypes. Breeding for enhanced quality in alfalfa (*Medicago sativa*) frequently involves selecting a higher leaf stem weight ratio, multifoliolate leaves, or short-internode stems. A similar result has been reported by researchers. Three populations selected for such alternative morphologies and a reference population were evaluated for forage yield, leaf-to-shoot ratio, and high protein and low fiber concentrations in leaves, stems, and whole plants. The latter population had higher shoot protein (12.9%) and lower shoot NDF value (58.7%) than other populations (Pecetti *et al.*, 2017). Martiniello *et al.* (1994) observed extended phenotypic diversity in attributes such as dry weight, the leaf to shoot weight ratio, plant height among 54 samples, and populations of *Medicago arboreta* species. The relationships between different morphological, physiological, quality traits, chemical compounds, and forage yield in alfalfa have been extensively studied. Joann *et al.* (2012, 2014) stated that alfalfa stem digestibility, lignin, and polysaccharide composition affect energy availability for livestock and biofuel conversion. Amanda *et al.* (2020) studied the effect of stem and leaf forage nutritive value and reduced lignin content in alfalfa. Reduced lignin alfalfa (*Medicago sativa* L.) can improve alfalfa forage quality, forage morphology, and biomass allocation.

A meta-analytic study including 164 studies on the distribution of biomass under drought stress conditions showed that both shoot and leaf weight decreased. As a result, the leaf-to-shoot ratio did not fluctuate much under drought stress conditions (Eziz *et al*., 2017). In alfalfa, the leaves are more palatable and have a higher digestibility than the stem. Leaf to shoot weight ratio is an important and effective factor in alfalfa forage quality (Moore and Undersander, 2002). El-Hifny *et al*. (2019) studied the yield component of forage and seed yield variation of alfalfa cultivars in response to the planting date. They showed that fresh forage yield had the greatest influence on protein content. Moreover, most mineral had a negative relationship with morphological traits. A negative correlation was observed between forage yield and most of traits except LS, CF, ADF, Zn and Fe. The highest correlation was revealed between LS vs. Ca ($r=-0.51$), LS vs. Ash ($r=0.57$), CP vs. CF ($r=-0.78$), CP vs. NDF ($r=-0.83$), CP vs. DMD ($r=0.88$), CP vs. Ca ($r=0.74$), CP vs. P ($r=0.80$), CP vs. Ash ($r=0.82$). Relation among CF vs. DMD ($r=-0.88$), CF vs. Ca ($r=-0.79$), CF vs.Ash ($r=-0.84$) were also high. The greatest correlation coefficient with r=0.92 was between Ash and Ca. A positive correlation has been reported between leaf to shoot ratio and forage quality in alfalfa (Davodi *et al.*, 2011). Previous results demonstrate that protein content variation exists among high-yielding alfalfa cultivars, suggesting that genetic gain could be achieved for both yield and protein content (Veronesi *et al*., 2010). Significant differences were registered in the content of crude fiber, ADF, and NDF that were caused by genetic factors (Riday and Brummer, 2004; Guines *et al*., 2002).

It is necessary to make this complex structure more abstract and interpretable by multivariate analysis, especially the primary component analysis, to investigate the role of each trait in the variation among genotypes and the contribution of each genotype to the established diversity. Annicchiarico *et al*. (2020) studied the effect of adaptation on Italian alfalfa cultivars. They found growing specific-adapted cultivars provided an estimated average advantage of 12.9% across subregions relative to the best-performing widely-adapted cultivar.

According to the PCA, 77% of the total variance were explained by the first four PCAs. Quality traits as CP, DMD, Ca, P, K, Na, Cu, Mn, Fe, ASH, CF, ADF and LS had the highest amount of variance in the first component (quality component). Dry forage, fresh forage, shoot weight and leaf weight had the highest amount of variance in the second component (yield component). Plant height had higher variance in the third component (Plant height) and Mg and Zn had the highest variance in the fourth component.

Based on the dispersion chart of traits based on PCA1 vs. PCA2 component and dendrogram resulted from that, it could be said that there was a different trend between qualitative and chemical and morphological traits. So, The results of cluster analysis did not well group the populations with different geographical origins in the same cluster and there was low agreement between cluster and PCA analysis (Fig. 3).

Conclusion

There is enough variation among alfalfa specimens for yield, quality, and mineral traits. These results provide a good opportunity to select and study the relationships between different traits. Cluster analysis Ward method classified 51 alfalfa populations based on the all traits into four clusters. Populations in cluster 4 had higher overall mean values for both yield and quality traits. According to the principle component analysis (PCA), the four components, namely the quality, yield, plant height and Mg+Zn, components account for 40, 20, 10 and 7% (In total 77%) of data variance, respectively.

The maximum distance was between KR-615 (Kazagi2) and Es-035 (Jirofti) populations with 169.5. These two populations belong to two different climates in Kazakhstan and Iran. As a result, the genetic distance between them is likely to be high. The minimum distance between the populations Es-027 (Shahrood) and Es-024 (Yazdi) was 2.60, which belong to different climates (Shahrood and Yazd in Iran). Considering the ranking of yield component, the 5 top genotypes such as FAO 1 (KR-3003), Cody 2 (Es-058), Italy 2 (Es-75), Kazakh2 (KR-615) and Mashhad 2 (Es-067) were recommended for improved breeding synthetic variety.

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بررسی تنوع عملکرد و کیفیت علوفه یونجه (*Medicago sativa* L.) و شناسایی جمعیت‌های پر محصول جهت کشت در دیمزارهای کم بازده ایران

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چکیده. یونجه (*Medicago sativa* L.) یک گیاه علوفه‌ای است که بدلیل کیفیت خوب، قابلیت هضم بالا و سازگاری آن با آب و هوای مختلف اصطلاحاً به ملکه گیاهان علوفه‌ای مشهور است. در این پژوهش، ۵۱ ژنوتیپ یونجه تهیه شده از بانک ژن موسسه تحقیقات جنگل‌ها و مراتع کشور مورد آزمایش قرار گرفت. گیاهان بر اساس طرح آزمایشی بلوک کامل تصادفی آگمنت با سه تکرار و شش کنترل کشت شدند. این آزمایش در سال ۱۳۹۶در مرکز تحقیقات کشاورزی و منابع طبیعی کرمانشاه اجرا گردید. عملکرد علوفه ، صفات مورفولوژیکی و کیفیت علوفه در مرحله گلدهی اندازه‌گیری شدند. تجزیه آماری داده‌ها شامل داده‌های توصیفی، ضریب همبستگی، تجزیه به مولفه های اصلی و تجزیه کلاسترانجام گرفت. نتایج آنالیز واریانس نشان داد که اثر تکرار در (ژنوتیپ های تکراری) برای صفات ارتفاع ساقه، نسبت برگ/ساقه، کلسیم، پتاسیم و خاکستر معنی‌داری بود (p<۰/۰۵). نتایج مقایسه میانگین، تنوع قابل توجهی در بین ژنوتیپ‌ها برای صفات مورد بررسی را نشان کرد. رقم ارومیه (Es-053) با تولید ۱۰۰۸۹ کیلو گرم در هکتار بیشترین عملکرد علوفه و رقم تربتی (Es-032) با ۱۸۲۴ کیلو گرم در هکتار کمترین عملکرد علوفه را دارا بود. ژنوتیپ سیرجانی (Es-026) با ۱۹/۴۶ درصد بالاترین مقدار پروتئین خام را داشت. ضریب همبستگی بین عملکرد علوفه خشک با صفات تعداد ساقه، وزن برگ و وزن ساقه به ترتیب ۰/۵۰، ۰/۹۵ و ۰/۹۲ بود. بر اساس تجزیه کلاستر به روش ward ، ۵۱ رقم یونجه به چهار گروه تقسیم شدند. ژنوتیپ های واقع در کلاستر ۴ دارای میانگین بیشتری برای عملکرد علوفه و صفات کیفی بود. طبق تجزیه به مولفه های اصلی ، ۴ مولفه اصلی اول به ترتیب ۴۰، ۲۰، ۱۰ و ۷ درصد و در مجموع ۷۷ درصد از کل واریانس را تبیین گردید. مولفه اول به عنوان مولفه کیفیت، مولفه دوم به عنوان مولفه عملکرد و مولفه سوم به عنوان مولفه ارتفاع و مولفه چهارم به نام منیزیم و روی نامگذاری شدند. بر اساس رتبه‌بندی ارقام با نتایج برتر علوفه و اجزای عملکرد و کیفیت علوفه ۵ ژنوتیپ برتر که عبارتند از فائو۱ (KR-3003)، کدی۲ (Es-058)، ایتالیا۲ (Es-75)، قزاقستانی۲ (KR-615) و مشهد۲ (Es-067) جهت اصلاح واریته ترکیبی توصیه شدند.

کلمات کلیدی: یونجه، تنوع زیستی، ارزش تغذیه، عملکرد علوفه