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Identification of Two Types of Iranian Alfalfa Gene Pool-Based on Agro-Morphological Traits

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Abstract: There are two types of alfalfa landraces in Iran. The first is temperate landraces such as Hamedani, Gharah Younjeh and the second is sub-tropical landraces such as Bami, Nikshahri and Yazdi. Do they have different gene pools and could they be identified using agro-morphological traits. A total of 332 landraces which had been collected from different parts of Iran were planted in the experimental field. During 1998-2002, agro-morphological traits were evaluated according to IPGRI descriptors. One way ANOVA analysis showed that the variance among accessions within provinces is not significant for mostly of traits, whereas it was significant among provinces. Cluster analysis revealed that there are five clusters which are grouped in two main clusters. The first main cluster consisted of landraces of central to Northern provinces. The other main cluster consisted of landraces of central to Southern provinces. The results of t-test among quantitative traits in two main clusters and distribution of collected accessions of each province in the produced plot based on two first factor of factor analysis, confirmed the cluster analysis results. Therefore these results revealed that those accessions which were collected from Northern parts of the country were genetically different from Southern accessions. As a result, there are two types of gene pools for alfalfa landraces in Iran. Also this study showed that using agro-morphological traits could be used for distinguishing between two types of gene pools of alfalfa landraces in Iran.

Key words: Gene pool, alfalfa, agro-morphological traits, Iran

INTRODUCTION

Alfalfa is the world's most important forage crop adapted to a wide range of environments. This crop is also the most important forage crop in Iran. It is cultivated in about 600,000 ha of arable land in the country. Its economic value is well known in dairy productions and cloth industries.

Alfalfa originated in Vavilov's Near Eastern Center Asia Minor, Transcaucasia, Iran and Turkistan. In the wild, *M. sativa* and related perennial species are found throughout Eurasia and as far north as Siberia (Hanson, 1988). Parts of Iran around of Caspian Sea and east of Anatolian plateau are centers of origin of primitive or diploid forms of *M. sativa* (Lesins, 1976). The diversity center of its cultivation or tetraploid forms has been tied to center of horse breeding (Lesins, 1976). The fertile valleys in the Zagros and Alborz mountains ranges, as well as the oases in the central to southern Iranian plateau, have been the origin of tetraploid forms of alfalfa. About 2,500 years ago during the war between Persia and Greece alfalfa was brought to Europe. Then this crop was taken to Italy by Romans and then to Spain by Moslems and, after the discovery of the New World, to America (Lesins, 1976).

Agro-morphological traits have been used to classify and study of the genetic diversity in alfalfa germplasm collections, as well as other crops (Shi *et al*., 1997; DeHaan *et al*., 2003; Zaccardelli *et al*., 2003; Casler, 2004). On the nondormant alfalfa from India and the Middel East using agro-morphological traits Wabrton and Smith (1993) have reported that there was regional diversity in the materials on the other hand Indian accessions were different from Middle East ones. Crochemore *et al.* (1998) showed that wild populations of both subspecies (*M. sativa* subsp. *sativa* and subsp. *falcata* [*M. falcata*]) were clustered together and characterized by slow regrowth after cutting, no growth during the winter, late spring growth and prostrate growth habit. It was showed that in the alfalfa accessions, from 50 countries, the resistance to *Peronospora trifolium* associated with geographical collected site (Skinner and Stuteville, 1992). Variation among and within Italian alfalfa ecotypes by means of bio-agronomic characters and amplified fragment

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length polymorphism analyses have been studied (Zaccardelli *et al*., 2003). By means of bio-agronomic characters the two ecotypes could be distinguished by the differences in persistency after 15 cuttings in 3 years cultivation and by the seasonal distribution of the dry matter yield (Zaccardelli *et al*., 2003).

There are two types of alfalfa landraces in Iran. The first is cold temperate alfalfa landraces such as Hamedani, Gharah Younjeh and the second is sub-tropical alfalfa landraces such as Bami, Nikshahri and Yazdi. It is believed that these two kinds of alfalfa landraces have a common origin. This hypothesis says that alfalfa has gone from Hamedan to other parts of Iran. Then, in each region, it has assigned the name of that place. On the other hand, Hamedani alfalfa is considered as an Iranian alfalfa parent. Do the cold temperate and sub-tropical alfalfa landraces represent different gene pools and could they be identified using agro-morphological traits? Since, so far, there was no any article that studied the relationship between these two gene pools on alfalfa landraces, objectives of this article are to determine if the cultivars represent different gene pools and if the gene pools could be identified using agro-morphological traits as much as possible.

MATERIALS AND METHODS

Plant data and materials: A total of 332 alfalfa accessions that had been collected from different parts of Iran and are conserved at the Iranian National Plant Gene Bank were planted in a systematic experimental design in the experimental field in Karaj (35° 52′ N latitude; 50° 59′E longitude and 1520 m altitude), Iran in September 1998. These accessions were collected from 4 to 2500 m of geographical altitude, 28° 40′ N to 37° 38′ N geographical latitude and 44° 50′ E to 59° 48′ E geographical longitude within the country. The accessions from each province as well as the whole collection were randomized within blocks in the experimental design. Thirty accessions were planted in each block with 1 m distance between two blocks. Each accession was planted in two rows, 2 m long and 50 cm wide. Distance between plants within rows was 10 cm. The field was irrigated in intervals of eight days from spring to fall. During three years the agro-morphological traits were characterized according to IPGRI (1984) descriptors. For each trait (exception for percentage characters) 10 plants within accession randomly were characterized. Then, the mean of three years data with different cuts in the year were used in data analysis. Growth habit was scored as prostrate, semi-erect or erect forms. Height was recorded at 10% flowering after each cutting. Leaf area meter was used for determination of area leaflet, in 30 leaflets from node 4 in each accession. Regrowth rate was recorded as plant height 15 days after each cutting and then divided by 15. Fall dormancy was recorded as plant height one month after the last cutting in autumn and then each 5 cm of plant height was assigned a score of 1. Percentages of petal colors in each accession were recorded by counting all of plants in plot. To calculate leaf/steam ratio, 1 kg of forage was harvested, after detaching leaves and stems, drying in an oven (55°C, 24 h) and recording weights. The 1000-seed weight was recorded in terms of gram. The seed coat colors (yellow, green-yellow and brown) were scored. Alfalfa weevil susceptibility and mildew susceptibility were scored (1 = non-susceptible, to 9 = very susceptible).

Statistical methods: Coefficients of variation and Shannon indices (Shannon, 1948; Dong *et al*., 2001) were computed to estimate the diversity for quantitative and qualitative traits, respectively. Spearman rank correlations coefficients were computed between trait pairs. The geographic passport data only used in the correlation analysis but not in the cluster or factor analysis. The One-way ANOVA was used for each trait within and among provinces. The mean of each trait per province was used as a datum in the cluster analysis based on Ward's method. Due to the extensiveness of the Khorasan province, it was divided into two parts; northern and southern Khorasan. Student's t-test was computed to compare means of quantitative traits in the accessions with cold temperate origin (Hamedan, Zanjan, Tehran, north of Khorasan, Lorestan, Markazi and Kordestan provinces) against accessions with sub-tropical and hot dry origin (Esfahan, Kerman, south of Khorasan, Fars, Khuzestan and Yazd provinces). The factor analysis was performed on means of traits according to the PCA method and varimax rotation (Manly, 2005). These analyses were performed by SPSS version 11.5.

RESULTS

Shannon and CV indices showed that in most of the traits there were high diversity in the both qualitative and quantitative characters (Table 1). Dark-blue color of petal percentage and forage moisture traits represented the highest and the lowest diversity with 56.9 and 4.8%, respectively. Among qualitative traits, the most and least diversity were shown in leaf hairiness and growth habit, respectively.

There were significant correlations between most of pairwise traits. Significant correlations also were observed between geographical coordinates and some agro-morphological traits (Table 2).

Table 1: Statistical parameters of distribution and center tendency and variation indices of Iranian alfalfa landraces

Statistical parameters	Traits* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Mean	2.3	8.6	5.3	4.7	32.5	22.9	5.7			1.1	233.7	52.5	8.4	81.3		51.9	7.8	
Std. deviation	0.3	0.8	1.5	1.7	18.5	3.2	2.0			0.4	50.0	24.4	1.1	3.9		11.2	1.6	
Range	1.6	5.0	8.0	8.0	100.0	23.0	10.0	2.00	8.00	2.4	388.0	100.0	8.0	21.6	100	53.0	10.0	2.00
Shannon								0.20	0.76						0.21			0.16
CV%	14.7	9.3	28.5	35.4	56.9	14.1	35.4			33.4	21.4	46.5	13.6	4.8		21.6	20.7	

*1: 1000 seed weight (g), 2: Alfalfa weevil susceptibility[1], 3: Central leaflet length[2], 4: Central leaflet width[3], 5: Dark:blue color of petal %, 6: Days to 10% flowering after cutting, 7: Fall dormancy score[4], 8: Growth habit[5], 9: Leaf hairiness, 10: Leaf stem ratio, 11: Leaflet area (mm^2), 12: Light:blue violet color of petal%, 13: Mildew susceptibility[6], 14: Forage moisture %, 15: Petiole hairiness[7], 16: Plant height at 50% flowering, 17: Regrowth rate and 18: Seed coat colour[8]

1: 1 = Resistance to 9 = Susceptible
2: 1 = Very short to 9 = Very large
3: 1 = Very narrow to 9 = Very wide
4: Each 5 cm of plant height is assigned a score of 1
5: 1 = Prostrate, 2 = Semi-erect 3 = Erect
6: 1 = Resistance to 9 = Susceptible
7: 1 = Nile 2 = Spars 3 = Dense
8: 1 = Yellow 2 = Green-yellow 3 = Brown

Table 2: Correlation coefficients between pairwise agro-morphological traits in alfalfa landraces collection of Iran

Traits	D50F	Traits* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-0.138															
2	-0.370**	0.075														
3	0.027	0.241**	0.11													
4	0.024	0.078	0.053	0.335**												
5	0.178**	-0.059	-0.084	0.251**	0.094											
6	-0.009	-0.096	-0.042	-0.249**	-0.247**	-0.752**										
7	-0.385**	0.234**	0.237**	-0.254**	-0.186**	-0.648**	0.195**									
8	-0.254**	0.094	0.223**	-0.420**	-0.076	-0.210**	0.157*	0.207**								
9	0.120*	0.052	-0.004	-0.420**	-0.161**	-0.055	0.106	0.060	0.741**							
10	-0.029	0.114	0.061	-0.106	-0.036	-0.094	0.069	0.127*	0.085	0.269**						
11	0.088	0.012	-0.199**	-0.140*	0.005	-0.116	0.083	0.065	0.092	0.054	0.013					
12	-0.067	-0.086	0.118	-0.421**	-0.314**	-0.301**	0.254**	0.280**	0.196*	0.218*	0.046	0.043				
13	0.082	-0.137	-0.101	0.191*	0.113	0.064	-0.114	-0.044	-0.168*	-0.217**	0.019	0.017	-0.186			
14	0.270**	0.402	0.048	0.418**	0.266**	0.375**	-0.264**	-0.444**	-0.397**	-0.224**	-0.02	-0.236**	-0.473**	0.108		
15	0.109	-0.137	-0.129*	-0.505**	-0.290**	-0.237**	0.287**	0.128*	0.314**	0.414**	0.11	0.156*	0.391**	-0.234**	-0.486**	
Altitude	-0.032	0.150	0.104	0.416**	0.137*	0.189**	-0.173**	-0.130*	-0.300**	-0.354**	-0.11	-0.130*	-0.068	0.135	0.269**	-0.481**

*Significant at 0.05, **Significant at 0.01; D50F = Days to 50% flowering, +: 1 = Leaf area, 2 = Plant height at 50% flowering, 3 = Days to 10% flowering after cut, 4 = White color of petal %, 5 = Light-blue violet colour of petal%, 6 = Dark-blue color of petal%, 7 = Red-violet color of petal %, 8 = Fall dormancy[a], 9 = Regrowthratecm day^{-1}, 10 = Alfalfaweevilsusceptibility[b], 11 = Leaf/steam ratio, 12 = 1000 seed weight m, 13 = Mildew susceptibility[b], 14 = Latitude, 15 = Longitude

a: 1 = dormant to 9 = non dormant
b: 1 = resistant to 9 = susceptible

One-way analysis of variance was performed, to describe the variation within and among provinces (regions) for each trait in the accessions (Table 3). Since the white color of petal trait didn't exist in the accessions from all provinces, so F-value for this trait couldn't be calculated in some provinces. F-value of analysis for most of characters was not significant within province whereas, it was significant between provinces except for alfalfa weevil susceptibility character (Table 3).

The results of cluster analysis based on quantitative traits revealed five clusters which came under two main clusters (Fig. 1). In the first main cluster, which consisted of the first three clusters, are accessions which were collected from Markazi, Hamedan, Lorestan, North of Khorasan, Kordestan, Tehran and Zanjan provinces. The second main cluster, with two clusters, consisted of those accessions which were collected from Booshehr, Esfahan, Fars, Kerman, Khuzestan and Yazd provinces (Fig. 1). Those provinces which came in the first cluster locate in the northern parts of the country with cold temperate climate whereas the second main cluster revealed the provinces with hot desert to sub-tropical climates (Table 4). The meteorological data in the Table 4 are the average of 30 years data which recorded in the metrological sites near by collection sites. Table 5 shows the mean of agro-morphological characters of Iranian alfalfa landraces in the province and region.

Since the cluster analysis discriminate two main clusters based on origin of germplasms, student's t-test was done between means of each pairwise quantitative traits in order to confirm the cluster analysis results (Table 6).

Table 3: F-value of one-way ANOVA for comparison of variance into different agro-morphological traits of accessions within and between provinces

	Traits*												
Groups	1	2	3	4	5	6	7	8	9	10	11	12	13
Within Provinces													
Booshehr	0.2	1.7	18.7*	0.1	1.0	0.2	0.2		4.0		1.6	4.7	
Esfahan	0.4	4.3	2.4	1.6	0.2	0.2	2.5	1.4		3.1	2.0	0.2	
Fars	2.6	1.0	0.4	9.0	0.4	1.7	1.2		0.3	1.1	0.8		
Hamedan	0.1	0.9	0.1	0.1	0.2	0.4	0.4	0.5	0.2	2.0	1.1	0.2	1.10
Kordestan	1.7	1.6	0.4	0.5	1.8	0.5	0.6	1.5	3.4	1.5	0.8	0.5	1.00
Kerman	0.3	3.1*	3.0*	0.2	1.2	0.2	0.5	2.4	4.3	0.5	1.5	115.6	1.30
Khuzestan	0.4	0.1	0.1	0.9	1.7	0.1	2.3	0.4	0.5	0.2	0.6	9.6	
Lorestan	1.9	4.0	1.3	2.6			1.5		0.3	1.0	1.0		0.40
Markazi	0.7	2.9*	1.5	2.2	0.9	0.4	3.08*	1.3	1.2	3.4**	4.38**	8	0.30
North of Khorasan	1.8	6.0		0.1		10.9	0.5		62.5	11.1	4.3	0.4	
South of Khorasan	1.9	1.9	3.3*	0.7	0.8	0.9	0.5	0.3	0.9	2.5	0.7	0.6	
Zanjan	0.7			2.2		1.3	0.5			1.6	0.5		
Yazd	1.7	1.9	3.0	0.4	1.2	0.4	1	3.2	0.9	2.1	1.7	1.3	3.20*
Between Provinces													
All of provinces	1.1	3.2**	9.2**	3.3**	3.2**	2.7**	3.6**	3.3**	1.6*	5.3**	2.9**	5.8**	3.20**

*Significant at 0.05, **Significant at 0.01; *1: Alfalfa weevil susceptibility, 2: Days to first 50% flowering, 3: Days to 10% flowering after cut, 4: Dark blue color of petal %, 5: Fall dormancy, 6: Leaf stem ratio, 7: Light blue violet color of petal%, 8: Mildew susceptibility, 9: Plant height at 50% flowering, 10: Regrowth rate cm day^{-1}, 11: Red-violet color of petal %, 12: 1000 seed weight m and 13: White color of petal %

Table 4: The average of 30 years some meteorological data of collection sites in the provinces

	Traits*					
Province	1	2	3	4	5	6
Southern ones						
Booshehr	20.10	30.00	0.00	4.80	45.40	297.40
Esfahan	9.40	23.80	69.60	-14.10	44.10	104.70
Fars	9.90	27.80	43.90	-4.20	42.60	293.60
Kerman	16.60	26.90	48.90	-19.50	44.50	107.90
Khorasan S	9.50	23.90	51.20	-11.30	41.40	167.90
Khuzestan	18.80	33.00	0.10	2.50	49.90	237.60
Yazd	11.70	26.50	1.20	-4.00	53.00	111.30
Mean of southern ones	13.70	27.40	30.70	-6.50	45.80	188.60
Northern ones						
Hamedan	3.64	19.21	119.19	-21.26	38.00	303.41
Khorasan N	6.64	19.37	83.91	-13.96	38.89	275.56
Kordestan	5.40	17.40	108.70	-29.50	41.00	402.65
Lorestan	9.10	25.20	59.25	-14.60	44.20	490.80
Markazi	6.88	20.45	96.40	-18.47	39.93	309.60
Zanjan	5.40	18.30	103.10	-20.00	39.00	303.20
Mean of northern ones	6.20	20.30	93.50	-19.60	40.40	356.40

*1: Average of minimum temperature in °C, 2: Average of maximum temperature in °C, 3: No. of days with minimum temperature equal 0°C and below, 4: Temperature records lowest in °C, 5: Temperature records highest in °C and 6: Annually total of precipitation in mm

Table 5: The mean of agro-morphological characters of Iranian alfalfa landraces in the province and region

	Traits*												
Province	1	2	3	4	5	6	7	8	9	10	11	12	13
Southern ones													
Boshehr	237.3	59.0	24.8	0.0	42.5	30.0	27.5	6.0	8.0	8.8	0.9	2.0	9.0
Esfahan	239.6	55.5	21.9	0.0	43.6	41.8	14.6	5.5	8.1	8.7	1.1	2.5	8.3
Fars	235.6	51.9	21.7	0.0	46.5	31.5	25.0	5.6	7.4	8.7	1.0		9.0
Hamedan	240.0	51.6	24.6	1.1	60.0	27.6	11.3	4.2	6.8	8.6	1.0	2.2	8.6
Kerman	244.3	50.7	21.5	0.4	51.3	35.8	12.5	6.7	8.8	8.9	1.1	2.6	6.7
Khorasan S	239.7	50.8	21.9	0.0	55.0	32.7	12.4	5.5	8.2	8.8	1.3	2.4	8.0
Khozestan	235.7	53.2	21.3	0.0	48.3	40.0	11.7	7.3	8.3	8.5	1.4	2.6	8.0
Yazd	244.4	49.3	20.8	1.4	41.5	38.1	19.2	6.0	8.6	8.8	1.3	2.4	8.7
Mean of southern ones	239.6	52.7	22.3	0.4	48.6	34.7	16.8	5.8	8.0	8.7	1.1	2.4	8.3
Northern ones													
Khorasan N	243.2	54.4	22.3	12.0	49.0	29.0	12.5	4.5	6.4	8.4	1.2	2.3	8.3
Kordestan	238.0	58.1	25.6	3.7	59.0	26.0	11.2	4.1	7.1	8.9	1.0	2.1	8.8
Lorestan	242.5	52.3	25.2	3.3	70.0	18.3	8.3	6.0	7.7	8.2	1.0	1.8	8.0
Markazi	241.4	50.7	24.6	1.8	65.7	25.8	6.9	4.3	7.1	8.7	1.0	2.3	8.6
Tehran	237.9	57.7	23.6	6.0	54.5	28.0	11.5	5.4	7.8	8.5	1.0	1.9	8.4
Zanjan	248.0	59.2	26.3	10.8	71.7	11.7	5.8		6.7	8.3	1.4		
Mean of northern ones	241.8	55.4	24.6	6.3	61.6	23.1	9.4	4.9	7.1	8.5	1.1	2.1	8.4

*1: Days to first 50% flowering, 2: Plant height at 50% flowering, 3: Days to 10% flowering after cut, 4: White color of petal %, 5: Light-blue violet color of petal%, 6: Dark-blue color of petal %, 7: Red-violet color of petal %, 8: Fall dormancy, 9: Regrowth rate cm day^{-1}, 10: Alfalfa weevil susceptibility, 11: Leaf stem ratio, 12: 1000 seed weight m and 13: Mildew susceptibility

Table 6: Results of t-test between mean of quantitative traits in two main cluster (cold temperate and sub-tropical) shown in the cluster analysis

Traits	t-value	Significant (2-tailed)	Mean difference
1000 seed weight	13.758	0.046	2.2624
Alfalfa weevil susceptibility	131.000	0.005	8.7333
Days to 10% flowering after cutting	13.485	0.047	23.0740
Days to 50% flowering	309.200	0.002	241.3823
Fall dormancy score	6.797	0.093	5.7358
Forage moisture	59.174	0.011	81.3791
Leaf/steam ratio	13.488	0.047	1.1154
Leaflet area	40.167	0.016	236.0359
Mildew susceptibility	54.218	0.012	8.4604
Plant height at 50% flowering	37.224	0.017	52.2785
Regrowth rate	11.776	0.054	7.7562

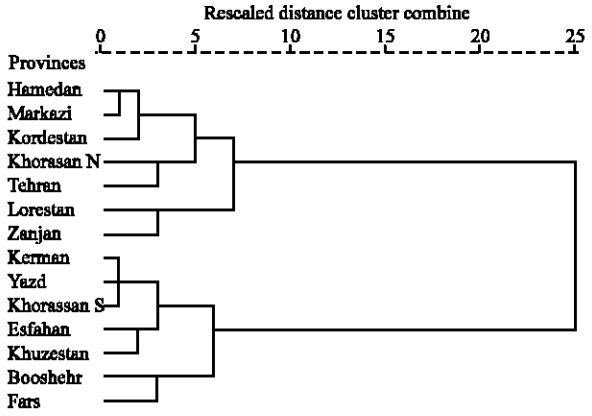

Fig. 1: The produced dendrogram based on mean of quantitative agro-morphological traits of alfalfa accessions in each province in ward method

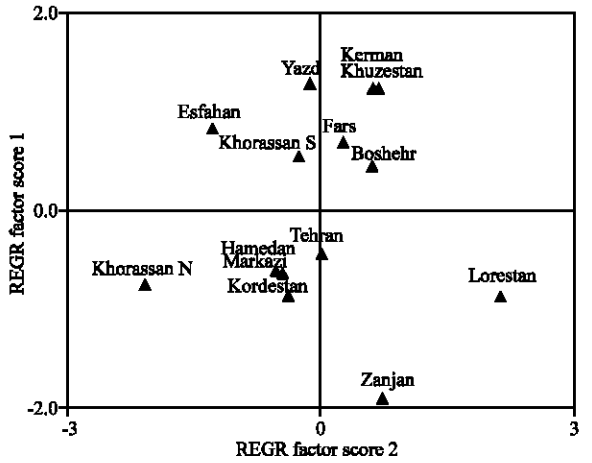

Fig. 2: The provincial dispersion of Iranian alfalfa landraces in the produced two-dimensional plot based on the first and second factors derived through factor analysis

According to factor analysis, 71.3% of community variation was showed in the first four factors. The first and second factors revealed 43.2% of the variation. The first factor impressed on fall dormancy and regrowth rate traits with 0.965 and 0.864 coefficients, respectively. Whereas, the second factor influenced the percent of light-blue violet and red-violet color of petal with 0.950 and 0.788 coefficients, respectively. Dispersion of provinces has been shown in the two dimensional plot derived from the first and second factors in the factor analysis (Fig. 2).

DISCUSSION

Shannon and CV indices (Table 1) showed that there were high diversity in the both qualitative and quantitative characters. Therefore, the existence of high diversity in these materials can be implied on different genetic resources in the Iranian alfalfa collection.

The negative significant correlation was shown between geographical latitude of collection site and fall dormancy ($r = -0.397$, $p<0.01$). Therefore, the accessions with cold temperate origin (in the high latitudes) in comparison with sub-tropical ones (in the low latitudes) showed the low score in the fall dormancy character. Besides, the significant positive correlation between regrowth rate and fall dormancy traits ($r = 0.741$, $p<0.01$) revealed that cold temperate accessions had also low regrowth rate (Table 2). The existence of significant correlation between characters could confirm the performing of cluster and factor analysis.

One-way analysis was not significant for the most of agro-morphological characters when were compared the accessions within each province (Table 3). Therefore, it can be said that the variance was small in each trait for accessions within each province. But it was large when were compared the accessions among the provinces (Table 3). On the other hand, F-values from one-way ANOVA analysis was significant (except for alfalfa weevil susceptibility). Since the most of accessions were susceptible to alfalfa weevil then, the F-value was not significant in this character. It is interpreted that the means of most traits are different in the provinces (Table 5). As the result of one-way ANOVA analysis showed, the variance for each character for accessions within each province was small whereas it was large in the accessions among provinces. The results of variance comparison for traits of accessions within and among provinces allow us to use the mean of each trait in the province for cluster analysis. Therefore, we will able to compare the provinces together in the cluster analysis.

The results of cluster analysis revealed five clusters which came under two main clusters (Fig. 1). In the first main cluster, which consisted of the first three clusters,

are accessions which were collected from northern parts of the country with cold temperate climate (Table 6). The second main cluster, with two clusters, consisted of those accessions which were collected from southern parts of Iran with sub-tropic climate (Table 6). These two main clusters may be representative of two main agro climates in the country. The northern region that consisted of Hamedan, North of Khorasan, Kordestan, Lorestan, Markazi and Zanjan provinces had the cold temperate climate with average of minimum and maximum 6.2 and 20.3°C, respectively (average of 30 years). The southern region consisted of Booshehr, Esfahan, Fars, Kerman, South of Khorasan, Khuzestan and Yazd provinces had the hot desert to subtropical climate with average of minimum and maximum 13.7 and 27.4°C, respectively (average of 30 years). The average of 30 year annually precipitation was 356.4 and 188.6 mm in the northern and southern region, respectively. Since the farmers in the country irrigate their alfalfa farms during cropping season, then the difference in the precipitation in two region couldn't be main effective factor on differentiation between two gene pools. The average of numbers of days with minimum temperature equal 0°C or below it in the northern region differed from 59.3 days in Lorestan province to 119.2 days in Hamedan province with mean of 93.5 days in the region, whereas in the southern region this metrological data varied from 0 to 69.6 days in Booshehr and Esfahan provinces, respectively, with a mean of 30.7 days in the region. This metrological data had high effect on evolution of the germplasm, especially on the fall dormancy score and regrowth rate characters because the northern germplasm had low fall dormancy score and regrowth rate (Table 4) but the southern ones showed high grade of these characters (Table 4). The means of fall dormancy and regrowth rate were 4.9 and 7.1 in the northern region, respectively and 5.8 and 8 in the southern region, respectively. Then the northern ones go to fall dormancy in fall faster than southern ones. The southern germplasm continued to grow until the environmental condition would be unsuitable for plant growth by coming fall frost. These germplasms also started to growth in the spring faster than northern ones.

According to the cluster analysis in the first cluster, Hamedan and Markazi accessions were closer together in comparison with Kordestan ones. One reason for this result can be the exchange of accessions between Hamedan and Markazi provinces. The second cluster consisted of accessions from Tehran and North of Khorasan provinces. The accessions of Lorestan and Zanjan provinces came together in the third cluster. Zanjan province has a cold temperate climate whereas Lorestan province has both the cold temperate and sub-tropical climate, but it should be noted that those accessions had been collected from Lorestan province originated from northern and mountainous area of this province, from Boroojerd and Khoramabad areas, which have cold temperate climate (Table 4). In the fourth cluster, came together the accessions of Kerman, Yazd and South of Khorasan and also Khuzestan and Esfahan provinces. In case of genetic relationships in this cluster, the accessions from Kerman, Yazd and south of Khorasan were closer together in comparison with Khuzestan and Esfahan accessions. This is important to emphasis that accessions from Esfahan province were collected from places with warm temperate climate which is almost similar to Khuzestan province climate. The fifth cluster revealed the accessions from Fars and Booshehr. Booshehr has a sub-tropical climate (Table 4) and also those accessions which had been collected from Fars province, originated from southern and south western parts of the province, Fasa, Firoozabad and Kazeroon cities, which had a similar climate to Booshehr province (Table 4). As it is seen in the Fig. 1, two main clusters which are representative of two main climates are segregated at distance 25. Therefore, the cluster analysis could differentiate two climates, cold temperate and sub-tropical, which locate in the Northern and Southern parts of Iran, respectively. The t-test results for pairwise traits in two main clusters (Table 6) confirmed the cluster analysis results. On the other hand, two main clusters were in accordance with the two gene pools in Iranian alfalfa.

That the cluster analysis based on quantitative traits could discriminate alfalfa germplasms according to their origin is in accordance with Wabrton and Smith (1993) and Crochemore *et al*. (1998). Also Furuta *et al*. (1999) showed that morphological characters corresponded with geographical distribution in the Argentina alfalfa. In other crops such as sorghum, it has been shown that the collected accessions from the same agro-climatic sites have appeared in the same cluster (Abbasi, 2003; Ayana and Bekele, 1999). In wild relatives of lentil has also been reported that the variation of agro-morphological traits is accordance with geographical variation (Fergosen and Robertson, 1999). In *Lolium temulentum* also it has been shown that geographical variation is accordance to genetic variation (Senda *et al*., 2004). A geographical pattern of variation in the USDA soybean germplasm collection based on morphological and physiological traits has been reported by Perry and McIntosh (1991). Geographical distribution and differences among provinces in frequency of distribution of stress tolerant morphotypes has been reported in durum wheat landraces from Turkey (Damania *et al*., 1997). Vellend and Waterway (1999) have reported that there was a strong positive correlation between geographic distance and genetic

distance among population of northern sedge, *Carex rariflora* and the three geographic regions were genetically distinct from each other.

Results of factor analysis showed that a big part of community variation was impressed (43%) by fall dormancy score, regrowth rate, light-blue violet and red-violet of petal colors in the first and second factors. Then, these characters had major effect on classification of the Iranian alfalfa germplasms. Dispersion of accessions and provinces confirmed the cluster analysis results in the derived plot from two first factors of factor analysis (Fig. 2A and B). As shown, two types of gene pools in the cold temperate and sub-tropical alfalfa accessions are completely distinguished. Those provinces which came in the first and second sectors of plot (up on X-axis), including Khuzestan, Fars, Kerman, Esfahan, south of Khorasan and Yazd, belong to sub-tropical regions (Table 4). Whereas the provinces with cold temperate, including Lorestan, Zanjan, Tehran, north of Khorasan, Hamedan and Markazi, revealed in the third and fourth sectors of plot (below the X-axis). Although this plot shows that there are variations within each of two types of main gene pools, but the two main gene pools are completely different from each other. Since the first factor was affected by the regrowth rate and fall dormancy traits, it confirmed again that the germplasms with sub-tropical origin in comparison with cold temperate ones had high score in those traits. Results of the factor analysis in the plot also confirmed the produced dendrogram in the cluster analysis. Because the collected materials from Kordestan, Markazi and Hamedan whom in cluster analysis revealed in the first cluster, in the plot came together in the third sector too. Also Zanjan and Lorestan which in the cluster analysis were grouped in the third cluster, in the plot came in the fourth sector together in spite of their far distance. In the cluster analysis Fars and Booshehr provinces came in the fifth cluster and in the plot these two provinces were appeared in the first sector too.

Based on the obtained results from the cluster analysis, t-test on two main clusters, factor analysis and correlation analysis, two types of gene pools in Iranian alfalfa landraces (tetraploid alfalfa) were distinguished. The landraces from those provinces which have cold temperate climate including Hamedan, Markazi, Zanjan, Lorestan, north of Khorasan and Tehran provinces

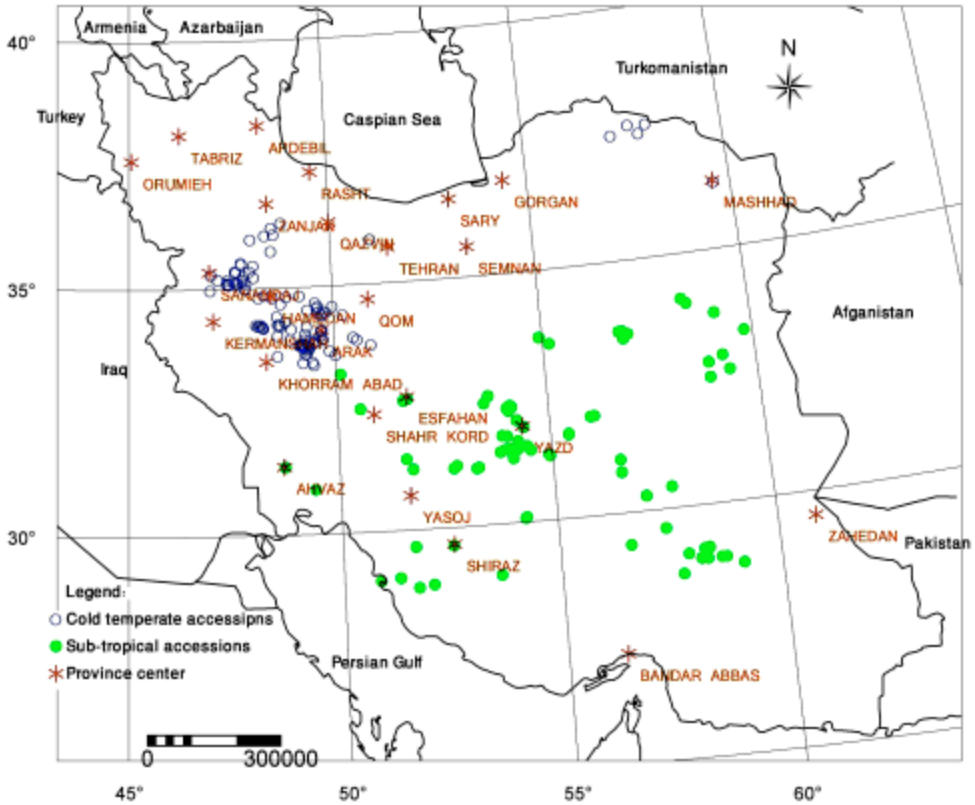

Fig. 3: Geographical distribution on two types of gene pools in Iranian alfalfa landraces

constitute one gene pool. These provinces are located above 34°N latitude in the fertile valleys of Zagros and Alborz mountains ranges. According to Lesins (1976) theory this place has been one of the first sites of cultivation of tetraploid alfalfa. The extended provinces in the center of Iran plateau to south (below 34°N latitude) consist of Esfahan, Fars, Yazd, south of Khorasan, Booshehr and Khuzestan which have hot desert to sub-tropical climate constitute the second main alfalfa gene pool. Also the oases of these places according to Lesin's theory have been another center for cultivation of Iranian tetraploid alfalfa landraces (Fig. 3).

The results showed that the collected germplasms from these two gene pools were agro-morphologically different from each other (Table 3, 5 and 6). The southern germplasms in comparison with northern ones represented more 1000- seed weight, regrowth rate after each cutting and percent of red-violet petals (Table 2 and 5). Whereas in the northern germplasm, days to 10% flowering after each cutting, percent of light blue-violet and white color of petal % were more in comparison with southern germplasms (Table 2 and 5). Besides, the southern accessions had higher score in fall dormancy trait in comparison to the northern materials. Therefore, the northern landraces go to fall dormancy faster than southern ones. The result of this study should be considered by alfalfa breeders in the selection of parents in their breeding programs.

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