Genetic diversity and distance among Iranian and European alfalfa (*Medicago sativa* L.) genotypes

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Received: August 2010 Accepted: November 2010

Abstract

Moghaddam, A., G. Pietsch, M. R. Ardakani, A. Raza, J. Vollmann and J. K. Friedel. 2011. Genetic diversity and distance among Iranian and European alfalfa (*Medicago sativa* L.) genotypes. **Crop Breeding Journal 1(1):** 13-28

Alfalfa is the best known fodder crop with high ability of biological nitrogen fixation and drought tolerance in dry, Pannonian region of east Austria. Different morphological and physiological characteristics of 18 alfalfa genotypes from different geographical origins, 8 Iranian ecotypes and 10 European cultivars were evaluated under irrigated and rainfed conditions during 2006-08 cropping seasons. The objectives of this study were to measure genetic distance and divergence among genotypes and to classify them based on morphological and physiological characters. Cluster analysis differentiated Iranian ecotypes and European cultivars from each other under irrigated condition, and when data averaged across two environments (irrigated and rainfed). However, under rainfed conditions small changes occurred in grouping of genotypes due mainly to differential responses of the genotypes to rainfed condition. Considerable genetic distance observed between Iranian and European genotypes. Different crossing programs are recommended between Iranian and European genotypes to develop new alfalfa cultivars.

Key words: Lucerne, Drought stress, Shoot dry matter, Cluster analysis, Organic farming.

Introduction

Alfalfa or lucerne (*Medicago sativa* L.) is the world's most important forage crop (Barnes *et al*., 1988) and the only forage known to be grown before recorded history (Michaud *et al*., 1988). Organic agriculture is often characterized as a natural way of farming, mostly referring to the absence of synthetic chemical inputs, such as chemical fertilizers, herbicides, and pesticides (IFOAM, 2002). Organic farming aims to be self-sufficient in nitrogen (N) through fixation of atmospheric N_2 by legumes,

recycling of crop residues and application of manures or composts. Crop cultivars adapted to organic agriculture systems should have the ability to perform under low input of organic fertilizers, an efficient root system, the ability to interact with beneficial soil microorganisms and to suppress weeds, and the ability to produce a healthy crop and healthy propagules (Lammerts van Bueren *et al*., 2002; Lammerts van Bueren *et al*., 2003). Legume fodder crops such as alfalfa are an essential component of organic systems particularly in arid and semi-arid conditions. Alfalfa is the best known fodder crop with high ability of biological nitrogen fixation (BNF) and drought tolerance in eastern Austria (Pietsch, 2004).

Genetic structure of alfalfa is complex at both individual and population levels because of being autotetraploid, allogamous and a seed-propagated species. Information about germplasm diversity and relationships among elite breeding materials is of fundamental importance in alfalfa breeding programs (Hallauer and Miranda, 1988). This is particularly true for species like alfalfa which suffers severe inbreeding depression. Katepa-Mupondwa *et al.* (2002) stated that researchers have postulated that multi-allelic loci are important in conditioning maximum productivity in autotetraploid alfalfa, and conversely that the loss of multi-allelic loci contributes significantly to inbreeding depression (Carnahan, 1960; Demarly, 1960; Dundier and Bingham, 1975). Therefore, genetic diversity of initial selection materials is essential for successful breeding and development of new cultivars.

For the estimates of genetic diversity, different criteria, such as morphological, agronomic and physiological characters, pedigree records, molecular markers or a combination of criteria are used. Alfalfa is distributed worldwide and grown in highly contrasting environments. This wide geographical adaptation enhances genetic variation and provides the opportunity to use diverse gene pools in breeding programs (Tucak *et al*. 2008). Cluster analysis can be applied to measure genetic distance and divergence between genotypes which can be used in planning of crossing in alfalfa breeding programs (Bauchan *et al.*, 1993; Riday *et al.,* 2003; Dehghan-shoar *et al.*, 2005; Tucak *et al*., 2008).

The objective of this study was to evaluate different morphological and physiological characters in 18 alfalfa

ecotypes and cultivars from different geographical regions under irrigated and rainfed organic farming conditions of dry, Pannonian region of east Austria for grouping and estimating genetic distance and divergence between genotypes.

Materials and Methods

Site and experiment description

To estimate genetic distance and divergence, 18 alfalfa genotypes including eight Iranian ecotypes and 10 European cultivars (Table 1) were evaluated under organic conditions during 2006-08 cropping seasons. This study was carried out in two separate experiments; irrigated (normal) and rainfed (drought stress) condition at two different organically managed fields, Gross-Enzersdorf (48º12' N, 16º33' E) and Raasdorf (48º15' N, 16º37' E), at the research station of the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria.

The farm management practices were organic, stockless and no organic manures were applied. The soils were a Calcaric Phaeozem (WRB) from loses with a silty loam textures. The long-term average of annual precipitation (1971-2000) was 520 mm. The amount of precipitation, average temperature and applied irrigation water from March to September in 2007 and 2008 cropping seasons are shown in Fig. 1.

Both experiments were hand seeded in May 2006. The first experimental year was considered as establishment year. During the establishment, plots were hand clipped one time in September 2006. The seeding density was 25 kg ha^{-1}, adjusted by the germination rate of the genotypes. The field plots, in both experiments, were laid out in a α-lattice design with two replications. Each replication consisted of three incomplete blocks and each incomplete block consisted of six plots. Each plot consisted 12 rows of two meters long in rainfed trial at Raasdorf and eight rows of 1.5 m long in irrigated trial at Gross-Enzersdorf. Row spacing in both trials was 12.5 cm.

In irrigated trial, soil moisture content was monitored weekly by four Frequency Domain Reflectometry (FDR) probes in 15, 40, 80 and 120 cm soil depths. These devices were installed in one plot in each incomplete block including genotypes 1, 9 and 18 in one replication. Plots were irrigated by drip irrigation system.

Table 1. Iranian alfalfa ecotypes and European alfalfa cultivars and their origins.

Ecotype/cultivar	Origin	Ecotype/cultivar	Origin
1- Mohajeran	Iran-West	10- Verko	Hungry
2- Khorvande	Iran-West	11- Vlasta	Czech Republic
3- Famenin	Iran-West	12- Monz42	Slovakia
4- Ghargholog h	Iran-Northwest	13- Fix232	Slovakia
5- Ordobad	Iran-Northwest	14- NS- Banat	Serbia
6- Shorakat	Iran-Northwest	15- Sanditi	Netherlands
7- Ghara-aghaj	Iran-Northwest	16- Alpha	Netherlands
8- Hokm-abad	Iran-Northwest	17- Plato	Germany
9- Sitel	Netherlands	18- Niva	Czech Republic

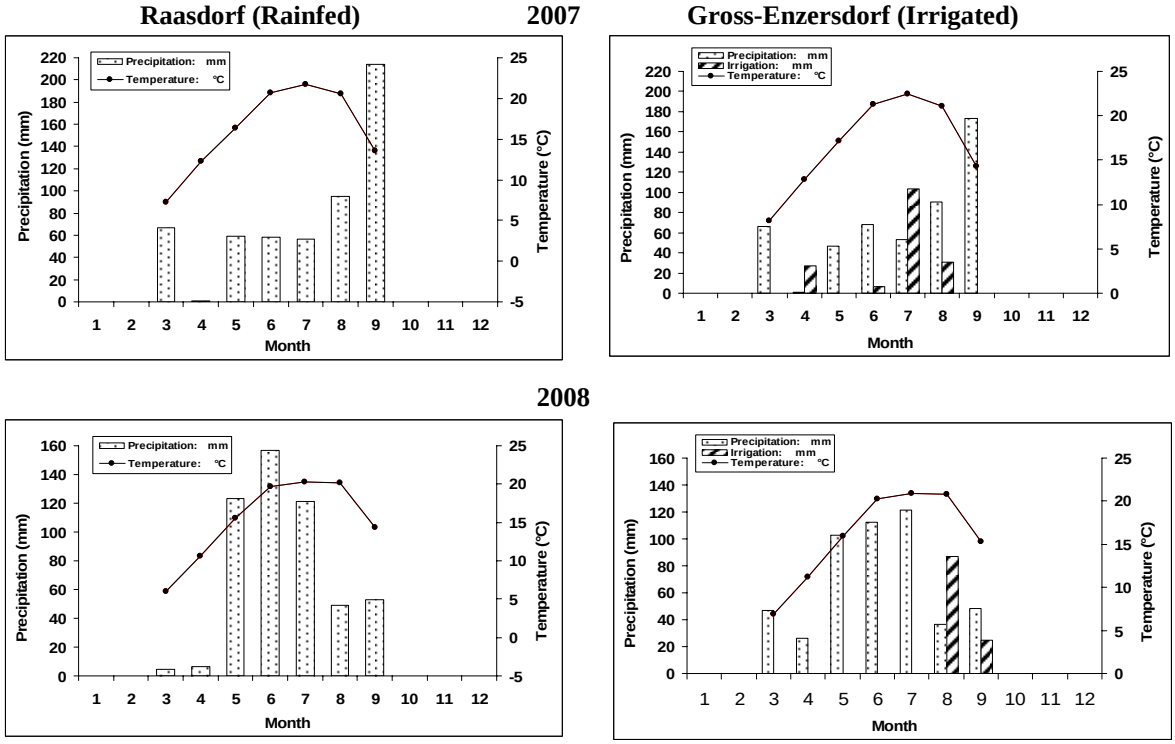

Fig. 1. Monthly precipitation (mm), average temperature (°C) and applied irrigation water (mm) from March to September in 2007 and 2008 growing seasons.

Data collection and analysis

Plots were hand clipped three times a year at 30-40 % of flowering using a garden scissor to a five centimetres stubble height in both locations. Seven characteristics; crop re-growth (cm) (CR), plant height (cm) (PH), number of stem m^{-2} (STN), leaf to stem ratio (LSR), leaf area index (LAI), shoot dry matter (t ha^{-1}) (SHDM), protein content (%) (CP) and root dry matter

(t ha^{-1}) (RODM) were measured and recorded. Fresh shoot yield data was adjusted to a dry matter basis by sub-sampling approximately 200 g of fresh shoot from 0.5 m^2 of the plots at each harvest, and drying the samples at 60 °C for 72 h. Annual shoot dry matter production was determined by summing the yield data over the harvests within each year. Roots were sampled using a soil corer of nine cm diameter. Two samples were taken in each plot down to 30 cm depth and fresh roots, after washing, were dried at 60°C for 72 h. Root dry matter was recorded only at the third harvest in each year. Crop re-growth was measured18-20 days after each harvest based on the average of plant height in three spots per plot. Plant height, number of stems m^{-2}, leaf to stem ratio and leaf area index were measured at harvest time each year and the average of harvests in each year was used in data analysis. STN and LSR were determined in a sub-sample of 0.25 m^2 in each plot. LAI was measured using LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE), before each harvest. Nitrogen content was determined by an isotope ratio mass spectrometer (IRMS-ThermoQuest Finnigan DELTAplus) in the laboratory of the Department of Chemical Ecology, University of Vienna. Protein content based on shoot dry matter was calculated by multiplying N content by a factor of 6.25.

Data were analyzed based on repeated measure analysis of variance based on an α-lattice design by PROC MIXED in SAS software (SAS Institute, 2004). A linear mixed model was used, where location (L), Replication (Rep) and genotype (G) were considered as fixed effects, and incomplete block within replication [iblock (rep)] and year (Y) were considered as random effects and repeated measure, respectively. Denominator degrees of freedom (DDF) for F-tests were calculated usingthe Kenward-Roger (KR) method. Mean comparisons were adjustedfor the *p*-values (α =0.05) using ADJUST=SIMULATION option in SAS software. A SAS macro was used to find letters display for all pairwise mean comparisons (Piepho, 2009). Adjusted least square (LS) means of genotypes at each location (average over years) and across both locations (average over years and locations) for above mentioned characters were used in the cluster analysis using SPSS software (Ver. 15, SPSS Inc., USA). The Ward's

clustering method was adopted and Euclidean distance used as the dissimilarity measure among genotypes (Crossa *et al.*, 1995). Data were standardized by transforming values to Z scores for each character before analysis.

Results

Combined analysis of variance for the characteristics showed significant differences among locations (L) (except for CP), years (Y), and genotypes (G). Two-way interaction effects G × L, G × Y were also significant for CR, PH, STN, SHDM, RODM and CP; and non-significant for LAI and LSR (Table 2). The mean values of genotypes for different characteristics under rainfed (RF) and irrigation (IR) conditions are presented in Table 3.

Table 2. Combined analysis of variance for different morpho-physiological characteristics in tow locations (irrigated and rainfed conditions) in 2007 and 2008 cropping seasons.

	CR	**PH**	**STN**	**LAI**	**LSR**	**SHDM**	**RODM**	**CP**
S. O. V.								
Location (L)	***	***	***	***	**	***	**	ns
Year(Y)	***	***	***	***	***	***	**	***
Genotype(G)	**	***	***	***	***	***	***	***
L ×Y	**	ns	**	***	*	**	ns	***
G × L	**	*	***	ns	ns	***	***	**
G ×Y	*	*	***	ns	ns	*	***	**
G × L × Y	**	*	***	ns	ns	***	ns	ns

*, ** and ***: Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.
ns: Non-significant.
CR=Crop re-growth; PH= Plant height; STN= Stem number per m^2; LAI= Leaf area index; LSR= Leaf to stem ratio; SHDM = Shoot dry matter;
CP= Shoot crude protein; and RODM= Root dry matter.

Eighteen alfalfa genotypes were classified into three clusters in irrigated conditions (Fig. 2a and Table 4). First cluster included all European cultivars, second cluster consisted of seven Iranian ecotypes and the third cluster only one Iranian ecotype, Khorvande (Fig. 2a).

Table 3. Mean comparison for different morpho-physiological characteristics in alfalfa ecotypes/cultivars in irrigated and rainfed conditions in 2007 and 2008 growing seasons.

Ecotype/	CR (cm)		PH (cm)		STN		LSR		SHDM (t ha^{-1})		RODM (t ha^{-1})		CP %	
Cultivar	**IR**	**RN**	**IR**	**RN**	**IR**	**RN**	**IR**	**RN**	**IR**	**RN**	**IR**	**RN**	**IR**	**RN**
Mohajeran	31.3bc	18.1a	88.8b	61.3ab	1183.6bcd	1033.6bcd	0.57a	0.85ab	18.4e	9.4ad	7.5abc	7.4ac	21.8ab	23.8cd
Khorvande	33.2bc	17.4a	77.5a	59.2ab	1016.6ac	895.1ac	0.71ab	0.96b	11.4a	7.8a	10.2d	6.8ac	22.5ac	23.1ac
Famenin	29.2ab	19.3a	86.5ab	63.2ab	1148.0ad	990.5bcd	0.61ab	0.73a	15.5bcd	10.3ad	8.4ad	6.2a	21.4a	21.3a
Ghargholog h	28.5ab	16.9a	85.1ab	60.4ab	1100.7ad	1069.4cd	0.67ab	0.80ab	13.6ab	9.8ad	10.5d	8.1ac	22.3ac	22.2ac
Ordobad	28.7ab	17.7a	84.8ab	58.5a	1207.6cd	773.2a	0.66ab	0.77ab	15.1bcd	8.3ab	9.4cd	6.8ac	22.7ac	22.9ac
Shorakat	31.8bc	19.4a	89.8b	61.8ab	1234.7d	999.8bcd	0.63ab	0.76ab	16.3bce	9.8ad	8.4ad	9.0c	22.2ac	22.5ac
Ghara-aghaj	28.9ab	20.0a	89.1b	68.1b	1193.4cd	1101.0d	0.64ab	0.76ab	15.5bcd	11.5cd	6.9ab	7.2ac	22.5ac	21.5ab
Hokm-abad	27.3ab	19.6a	84.4ab	63.3ab	1152.4ad	982.5bcd	0.63ab	0.80ab	14.3ac	8.4ac	9.2bd	7.7ac	23.1ac	22.5ac
Sitel	29.2ab	18.6a	86.0ab	65.8ab	1005.0ab	954.5ad	0.79ab	0.90ab	17.4de	12.3d	8.2ad	6.5ab	22.9ac	21.9ad
Verko	26.9ab	17.2a	86.8ab	60.5ab	1130.7ad	875.1ac	0.79ab	0.92ab	17.4ce	9.7ad	8.1ad	5.9a	23.1ac	22.9ac
Vlasta	27.8ab	20.9a	86.5ab	67.9ab	1118.3ad	1003.0bcd	0.76ab	0.84ab	16.4bce	11.7de	7.2abc	8.9bc	23.5bc	23.0ac
Monz 42	25.6ac	17.8a	89.9b	63.0ab	1042.3ac	931.8ad	0.78ab	0.90ab	15.5bce	9.1ace	8.2ad	6.4ab	22.8ac	22.8ac
Fix 232	28.4ab	18.9a	87.2b	65.3ab	1080.4ad	884.4ac	0.80ab	0.93ab	17.2de	10.6bcd	10.4d	5.5a	22.2ac	23.5bcd
NS_Banat	31.8bc	20.0a	84.1ab	64.7ab	987.3a	957.2ad	0.78ab	0.87ab	16.1bce	10.9ad	10.6d	6.5ab	22.8ac	22.6ac
Sanditi	28.8ab	20.2a	89.1b	66.3ab	1024.6ac	993.4bcd	0.76ab	0.85ab	15.3bcd	11.6de	6.2a	6.8ac	22.8ac	23.5bcd
Alpha	26.3ac	17.9a	85.7ab	60.3ab	1094.3ad	1038.7bcd	0.78ab	0.90ab	16.8ce	9.5ad	5.9a	5.7a	22.3ac	24.0c
Plato ZS	22.9a	18.3a	84.1ab	64.2ab	1082.1ad	862.9ab	0.79b	0.87ab	17.0ce	10.9bcd	6.4a	6.7ac	24.0c	24.0c
Niva	28.2ab	19.3a	86.5b	66.3ab	1027.4ac	865.2ab	0.73ab	0.86ab	16.5ce	11.1bcd	7.0abc	6.5ac	22.9ac	23.6cd
SE	1.10		1.70		31.23		0.04		0.62		0.44		0.35	

Means, in each column, followed by at least one letter in common are not significantly different at the 5% probability level-using Duncan's Multiple Range Test.
IR=Irrigated; RN=rainfed; CR=Crop re-growth; PH= Plant height; STN= Stem number per m^2; LAI= Leaf area index; LSR= Leaf to stem ratio; SHDM = Shoot dry matter; CP= Shoot crude protein; and RODM= Root dry matter.

Apart from Khorvande, the seven Iranian ecotypes in cluster two were characterized by fast crop re-growth after cutting, greater stem no. m^{-2}, high root biomass, lower leaf and protein content of shoot biomass and lower shoot dry matter as compared to European cultivars in the first cluster (Table 4).

Alfalfa genotypes were grouped into four clusters in rainfed conditions (Fig. 2b and Table 4). The first cluster consisted of three European cultivars; Plato ZS, Niva and Fix 232, which are characterized by high shoot dry matter, high leaf and protein content of shoot material as well as taller stems and lower root dry matter (Table 4). The second cluster included three Iranian ecotypes; Mohajeran, Khorvande and Ordobad, and three European cultivars; Verko, Monz42 and Alpha (Fig. 2b). This cluster was characterized by slow crop re-growth after cutting, short stems, lower number of stem m^{-2} and lower LAI, shoot and root dry matter, but higher leaf and protein content in shoot biomass (Table 4). The third cluster contained three Iranian ecotypes; Shorakat, Hokmabad and Ghargholog, which were characterized by higher root dry matter and higher stem no. m^{-2}, however, for other characteristics were lower or equal to the mean of all clusters (Table 4). The fourth cluster consisted of two Iranian ecotypes; Famenin and Ghara-aghaj and four European cultivars; Sitel, Vlasta, Sanditi and NS-Banat, which could be described by rapid crop re-growth, tall stems, reasonable stem no. m^{-2}, higher LAI , higher shoot dry matter, greater root dry matter than grand mean, and lower shoot protein content (Table 4). Considering results of cluster analysis and mean values obtained under rainfed conditions (Fig. 2b and Tables 3 and Table 4) genotypes in the fourth cluster can be grown in rainfed organic farming system.

Based on average values across two locations (irrigated and rainfed conditions), genotypes could be classified into three clusters (Fig. 2c and Table 4). The first cluster contained all European cultivars; Sitel, Verko, Vlasta, Monz42, Fix 232, NS-Banat, Sanditi, Alpha, Plato ZS, and Niva, which were characterized by higher shoot dry matter and protein content and relatively taller stems, but lower crop re-growth, stem no. m^{-2} and root dry matter as compared with the total mean.

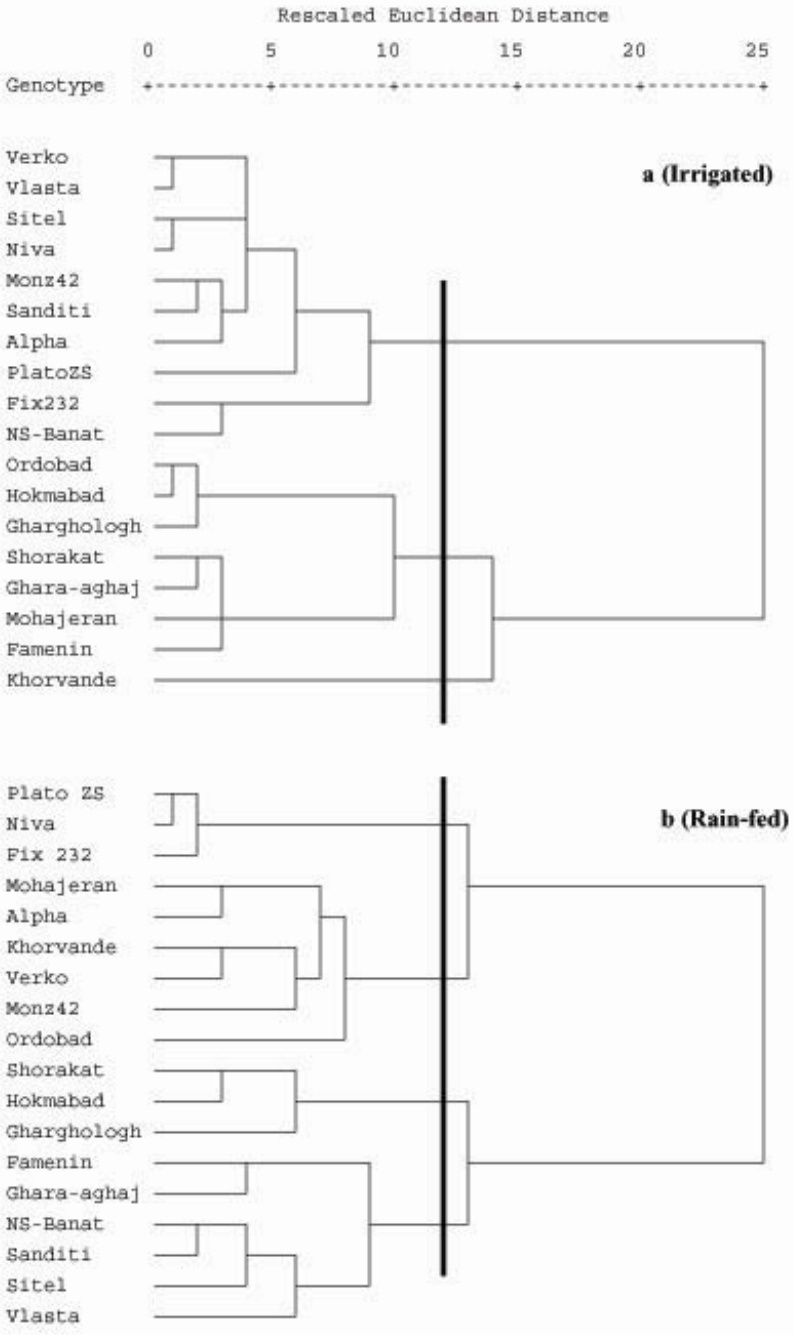
Rescaled Euclidean Distance
0
5
10
15
20
25
Genotype
Verko
Vlasta
Sitel
Niva
Monz42
Sanditi
Alpha
PlatoZS
Fix232
NS-Banat
Ordobad
Hokmabad
Ghargholog h
Shorakat
Ghara-aghaj
Mohajeran
Famenin
Khorvande
a (Irrigated)
Plato ZS
Niva
Pix 232
Mohajeran
Alpha
Khorvande
Verko
Monz42
Ordobad
Shorakat
Hokmabad
Ghargholog h
Pamenin
Ghara-aghaj
NS-Banat
Sanditi
Sitel
Vlasta
b (Rain-fed)

Fig. 2. Continued

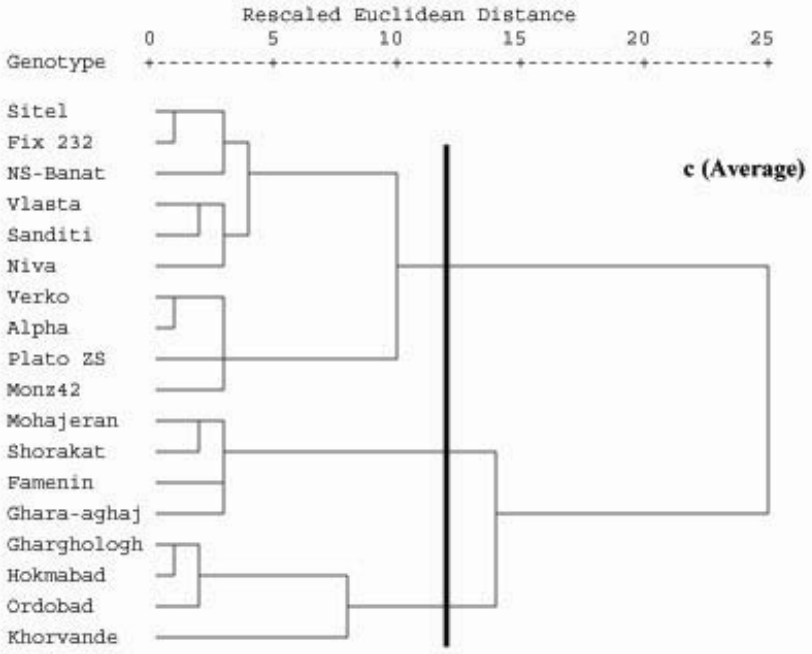

Fig. 2. Dendrogram of cluster analysis for 8 Iranian alfalfa ecotypes and 10 European alfalfa cultivars grown under irrigated (a) and rain-fed (b) conditions and averaged over the two irrigation regimes. The Ward's clustering method was used to generate the dendrogram.

The second cluster included four Iranian ecotypes; Mohajeran, Famenin, Shorakat and Ghara-aghaj, which were described by rapid crop re-growth, taller stems, greater stem no. m^{-2}, relatively higher shoot dry matter, but lower LAI, leaf to stem ratio and shoot protein content. The third cluster comprised of four remainder Iranian ecotypes; Ghargholog h, Hokmabad, Ordobad and Khorvande, which could be defined only by higher root dry matter. Considering the extent of variation within each cluster, genotypes in cluster 1 or 2 were wider adaptation and can be grown in both irrigated and rainfed organic farming systems.

Ecotypes Hokmabad and Ghargholog h were classified in the same cluster in all cases as well as Famenin and Ghara-aghaj, indicating their higher genetic similarity, based on studied morpho-physiological traits.

Table 4. Number of alfalfa genotypes (N), mean and standard deviation of mean for morpho-physiological characteristics in each cluster for irrigated, and rainfed conditions and average of two locations.

Irrigated

Cluster	Trait	CR (cm)	PH (cm)	STN	LAI	LSR	SHDM (t ha^{-1})	RODM (t ha^{-1})	CP (%)
1	Mean	27.6	86.6	1059.2	4.6	0.8	16.6	7.8	22.9
	N	10	10	10	10	10	10	10	10
	Std. Deviation	2.4	1.9	48.8	0.2	0.02	0.7	1.6	0.5
2	Mean	29.4	86.9	1174.3	4.3	0.6	15.6	8.6	22.3
	N	7	7	7	7	7	7	7	7
	Std. Deviation	1.6	2.3	44.3	0.1	0.03	1.5	1.2	0.6
3	Mean	33.2	77.5	1016.6	3.7	0.7	11.5	10.2	22.5
	N	1	1	1	1	1	1	1	1
	Std. Deviation	-	-	-	-	-	-	-	-
Total	Mean	28.6	86.2	1101.6	4.4	0.7	15.9	8.3	22.6
	N	18	18	18	18	18	18	18	18
	Std. Deviation	2.4	2.9	74.9	0.3	0.1	1.6	1.5	0.6

Rainfed

Cluster	Trait	CR (cm)	PH (cm)	STN	LAI	LSR	SHDM (t ha^{-1})	RODM (t ha^{-1})	CP (%)
1	Mean	18.8	65.3	870.8	2.6	0.9	10.8	6.2	23.7
	N	3	3	3	3	3	3	3	3
	Std. Deviation	0.5	1.1	11.8	0.0	0.0	0.3	0.6	0.3
2	Mean	17.7	60.5	924.6	2.4	0.9	9.0	6.5	23.2
	N	6	6	6	6	6	6	6	6
	Std. Deviation	0.3	1.6	101.2	0.3	0.1	0.8	0.6	0.5
3	Mean	18.6	61.8	1017.2	2.4	0.8	9.3	8.3	22.4
	N	3	3	3	3	3	3	3	3
	Std. Deviation	1.5	1.5	46.0	0.2	0.0	0.8	0.7	0.2
4	Mean	19.8	66.0	999.9	2.7	0.8	11.4	7.0	22.3
	N	6	6	6	6	6	6	6	6
	Std. Deviation	0.8	1.9	53.4	0.1	0.1	0.7	1.0	0.9
Total	Mean	18.7	63.3	956.2	2.5	0.8	10.1	6.9	22.8
	N	18	18	18	18	18	18	18	18
	Std. Deviation	1.2	2.9	84.2	0.2	0.1	1.3	1.0	0.8

Average over two locations

Cluster	Trait	CR (cm)	PH (cm)	STN	LAI	LSR	SHDM (t ha^{-1})	RODM (t ha^{-1})	CP (%)
1	Mean	23.3	75.5	997.9	3.6	0.8	13.6	7.2	23.0
	N	10	10	10	10	10	10	10	10
	Std. Deviation	1.6	1.6	38.6	0.1	0.0	0.7	0.8	0.4
2	Mean	24.8	76.1	1110.6	3.4	0.7	13.3	7.6	22.1
	N	4	4	4	4	4	4	4	4
	Std. Deviation	0.6	1.7	32.2	0.1	0.0	0.5	0.7	0.6
3	Mean	23.7	71.6	1024.7	3.2	0.8	11.1	8.6	22.7
	N	4	4	4	4	4	4	4	4
	Std. Deviation	1.1	2.3	61.7	0.1	0.1	1.0	0.5	0.3
Total	Mean	23.7	74.8	1028.9	3.5	0.8	13.0	7.6	22.8
	N	18	18	18	18	18	18	18	18
	Std. Deviation	1.4	2.4	61.5	0.2	0.1	1.3	0.9	0.6

CR= Crop re-growth; PH= Plant height; STN= Stem number per m^2; LAI= Leaf area index; LSR= Leaf to stem ratio; SDHM= Shoot dry matter; RODM= Root dry matter; CP= Shoot protein content

Genetic distances (Euclidean distance) calculated based on morpho-physiological traits among the genotypes are presented in Table 5. A smaller value of distance shows lower genetic diversity or greater genetic similarity. Genetic diversity among Iranian ecotypes was higher than European cultivars (Table 5). This could be due to wider genetic bases of evaluated ecotypes as compared to registered European cultivars. Among Iranian ecotypes, Khorvande had the highest genetic distance from other ecotypes as it was grouped in a separate cluster in irrigated conditions (Fig. 2). In irrigated trial, the most similar pairs of genotypes were Verko and Vlasta followed by Ordobad and Hokmabad, and then Sitel and Niva (Fig. 2a). In rainfed condition, the most similar genotypes were Plato ZS, Niva and Fix232 followed by NS-Banat and Sanditi, and then Khorvande and Verko. Considering averages over two environments (irrigated and rainfed conditions), Sitel and Fix232 showed the highest similarity followed by Gharghologh and Hokmabad, and then Verko and Alpha.

Discussion

In irrigated conditions and based on the average of two environments (irrigated and rainfed conditions), cluster analysis clearly differentiated Iranian alfalfa ecotypes from European alfalfa cultivars (Figs. 2a and c), whereas in rainfed conditions, small changes in grouping of genotypes were observed (Fig. 2b). Herbert *et al.* (1994) reported higher variability among annual medics due to increasing environmental stresses. The differences observed in clustering the genotypes in irrigated and rainfed conditions could be associated with differential responses of the genotypes to drought stress developed in rainfed conditions. The estimated genetic distances, based on characteristics used in the cluster analysis, were higher among Iranian alfalfa ecotypes and European cultivars than within each group of alfalfa genotypes.

The genetic distances and variability observed within Iranian ecotypes were higher than European improved cultivars. The characteristics used in the cluster analysis and estimation of genetic distances included the most important agronomic characteristics considered alfalfa growers. Therefore, different clusters and greater genetic distances represented different gene pool and alleles for these characteristics in more dissimilar genotypes.

Table 5. Genetic distance among Iranian and European alfalfa ecotypes/cultivars estimated based on their morpho- physiological data in irrigated and rainfed conditions.

Genotype		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	IR	7.4																
	RN	3.0																
	AVE	5.8																
3	IR	2.6	5.6															
	RN	4.4	5.4															
	AVE	2.8	6.0															
4	IR	4.4	4.3	2.6														
	RN	2.6	4.0	3.7														
	AVE	3.4	4.4	3.3														
5	IR	3.6	5.3	2.7	2.1													
	RN	3.8	3.3	4.5	4.2													
	AVE	3.3	3.7	3.5	2.4													
6	IR	2.1	6.5	2.4	3.6	2.7												
	RN	3.7	5.1	3.3	3.3	4.6												
	AVE	2.0	5.9	2.6	3.0	3.6												
7	IR	2.5	6.6	2.5	3.3	2.3	1.9											
	RN	5.2	6.8	2.7	4.7	6.6	3.6											
	AVE	2.4	7.3	2.5	4.4	4.7	2.4											
8	IR	4.4	4.8	3.1	1.9	1.5	3.5	2.9										
	RN	2.8	3.8	2.8	3.1	3.7	2.2	3.8										
	AVE	2.6	4.2	3.2	1.6	1.6	2.7	3.9										
9	IR	4.4	6.1	4.3	3.8	3.7	4.4	3.7	3.9									
	RN	4.9	5.3	3.5	4.8	5.8	4.3	3.3	4.3									
	AVE	3.5	6.4	4.0	4.8	4.2	4.1	3.9	4.4									
10	IR	4.2	6.8	4.2	3.8	2.9	3.9	3.0	3.4	2.0								
	RN	3.3	2.2	4.3	3.9	3.2	4.8	5.8	3.9	3.8								
	AVE	3.6	5.4	4.6	4.2	3.3	4.7	4.6	3.6	2.4								
11	IR	4.5	6.8	4.5	4.0	3.0	4.2	2.9	3.3	2.2	1.3							
	RN	5.1	6.5	4.6	5.4	6.7	3.2	3.2	3.9	3.7	5.9							
	AVE	2.8	6.5	4.4	4.5	4.1	3.2	3.3	3.7	2.4	3.0							
12	IR	4.9	7.1	4.4	3.7	3.7	4.6	3.4	3.8	2.4	2.1	2.3						
	RN	4.1	3.6	4.0	4.5	4.5	4.0	4.9	3.5	3.1	2.6	4.7						
	AVE	4.4	6.3	4.7	4.6	4.0	4.9	4.5	4.0	2.8	2.2	2.9						
13	IR	4.4	6.8	4.0	3.5	3.5	4.3	4.0	4.3	2.4	2.5	3.3	2.7					
	RN	3.8	3.8	4.4	5.1	4.7	5.0	5.0	3.9	3.0	2.5	4.7	2.7					
	AVE	3.6	5.9	4.5	4.4	3.9	4.1	4.2	3.9	1.3	2.1	1.9	2.1					
14	IR	5.1	5.0	4.5	3.3	3.8	4.9	4.7	4.1	2.2	3.5	3.6	3.7	2.6				
	RN	3.4	4.3	2.9	4.1	4.6	3.5	3.3	2.6	2.5	3.3	3.3	3.2	2.2				
	AVE	3.3	4.7	4.1	4.0	3.3	3.3	4.4	3.4	2.5	3.3	2.7	3.8	2.2				
15	IR	4.4	6.6	4.2	4.0	3.9	4.3	2.9	4.0	2.2	2.6	2.2	1.9	3.5	3.7			
	RN	4.3	5.6	3.8	5.1	5.9	3.7	3.3	3.7	2.6	4.5	2.4	3.4	2.8	2.1			
	AVE	3.2	6.5	4.3	5.2	4.3	4.1	3.5	4.1	2.2	2.8	1.9	2.6	2.2	3.1			
15	IR	4.2	7.1	3.9	4.3	3.8	4.5	3.1	4.2	2.6	2.2	2.3	2.5	3.2	4.2	2.3		
	RN	2.2	3.0	4.7	3.9	4.4	4.8	5.7	3.8	4.6	2.6	5.7	3.3	2.9	3.4	4.0		
	AVE	3.7	6.0	4.8	4.9	4.0	5.1	4.6	4.1	3.5	1.8	3.6	2.9	3.4	4.4	3.0		
17	IR	6.3	8.0	6.2	5.5	4.8	6.3	4.9	4.6	3.7	2.8	2.5	3.3	4.7	5.2	3.8	3.2	
	RN	3.3	3.9	4.3	4.6	4.1	4.1	5.0	3.6	3.3	2.7	4.2	2.7	1.8	2.6	2.7	3.1	
	AVE	5.0	7.0	6.3	5.9	4.7	6.3	6.0	5.1	3.9	2.4	3.9	3.0	3.5	5.0	3.6	2.7	
18	IR	4.0	5.7	3.5	3.3	3.2	3.8	2.9	3.1	1.7	2.3	2.3	2.6	3.5	3.3	2.0	2.5	3.7
	RN	3.7	4.5	3.9	4.9	4.5	4.1	4.3	3.4	2.9	3.3	3.6	3.1	1.6	1.9	2.1	3.6	1.3
	AVE	3.3	5.6	4.4	4.8	3.5	4.4	4.4	3.8	2.3	2.2	2.9	3.0	2.3	2.8	2.0	3.1	3.2

Genotypes no. 1 to 18 are: Mohajeran, Khorvande, Famenin, Ghargholog, Ordobad, Shorakat, Ghara-aghaj, Hokmabad, Sitel, Verko, Vlasta, Monz 42, Fix 232, NS-Banat, Sanditi, Alpha, Plato ZS and Niva, respectively.
IR= irrigated; RN= rainfed; AVE= average over irrigated and rainfed conditions.

Genetic diversity is a key element in alfalfa breeding programs for development of new cultivars.

Bauchan *et al.* (1993) selected a core collection to use in breeding programs after evaluation and classification of 122 annual Medicago species by cluster analysis. Since alfalfa ecotypes contain great genetic variation for agronomic characteristics, crossing programs might be initiated between diverse Iranian ecotypes such as Khorvande and Mohajeran with the European cultivars to develop high yielding alfalfa cultivars adapted to organic farming systems.

Acknowledgements

The authors are thankful to Dr. Wolfgang Wanek at the Department of Chemical Ecology, the University of Vienna, for measuring the N content of samples and to the staff of the research station of the University of Natural Resources and Life Sciences in Gross-Enzersdorf for support with field work. The technical assistance of C. Gabler and S. Zeidler is also gratefully acknowledged.

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