EVALUATION OF ALFALFA GERMPLASM COLLECTION BY MULTIVARIATE ANALYSIS BASED ON PHENOTYPIC TRAITS

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ABSTRACT

Crop improvement depends largely on the availability of diverse germplasm and their efficient utilization. The evaluation, characterization and screening of genetic resources are considered priorities in alfalfa breeding, as such information is crucial in choosing material for incorporation into breeding activities. The objectives of this research were to explore the extent and pattern of phenotypic variability in the alfalfa collections, to classify the germplasm into similar groups and to identify the main traits contributing to the overall variability. Twenty seven populations and cultivars of alfalfa of different origin were evaluated for thirteen phenotypic traits on the experimental field of the Osijek Agricultural Institute in Osijek - Croatia, during three growing seasons (2004-2006). Collected data were centered and scaled to unit variance and analyzed by principal component (PC) analysis. The first four PCs contributed to 89.02% of the total variability among the populations and cultivars. The yields of green mass and dry matter, vigour, growth habit, plant regeneration and length of central leaflet were the most important traits for the genetic variability, representing 58.21% of the total variability in the first PC variable. The second PC explained 16.24% of the total variability and was associated with number of stems, shape of leaf and width of central leaflet. The cluster analysis performed on the two first components grouped the majority of alfalfa cultivars and populations into three clusters of germplasm. In most cases, clustering did not depend on the country of origin. The present investigation revealed that there was wide phenotypic variability for the majority of the assessed traits in the alfalfa collections.

Key words: alfalfa collection, phenotypic traits, variability, principal component analysis.

INTRODUCTION

Crop improvement depends largely on the availability of diverse germplasm and their efficient utilization. Alfalfa is distributed worldwide and grown in highly contrasting environments. This extensive geographical adaptation promotes genetic variation and gives breeders the possibility of using highly diverse gene pools (Maureira et al., 2004). Cultivated alfalfa (*Medicago sativa* L., 2n = 4x = 32) is an autotetraploid, open-pollinated species characterized by tetrasomic inheritance with multiple allelism and by pronounced inbreeding depression (Barcaccia et al., 1999). Therefore, evaluation, characterization and screening of genetic resources are considered priorities in alfalfa breeding, as such information is crucial in choosing material for the incorporation into breeding activities. In most classical alfalfa breeding programmes, determining the value of new germplasm, as a potential select material, is based upon realized mean values and variability of significant agronomic traits during year-long investigation. Phenotypic characterization is the first step toward the classification of crop germplasm (Smith et al., 1991; Ghafoor et al., 2003).

Multivariate analysis is a very useful method because it reveals the relationships and correlation among variables studies. This type of analysis applied to studies of germplasm collection allows a better understanding of the structure of the collection, identification of more relevant variables, detection of the relationships among accession, as well as identification of possible groups (Martines-Calvo et al., 2008). Principal Component Analysis (PCA) has been widely used in the studies of variability in germplasm collections of many species (Julier et al., 1995; Bennett, 2000; Veasey et al., 2001; Naghavi and Jahansouz, 2005; Zakova and Benkova, 2006; Tucak et al., 2009).

The objectives of this research were to explore the extent and pattern of phenotypic variability in the alfalfa collections, to classify the germplasm into similar groups and to identify the main traits contributing to the overall variability.

MATERIAL AND METHODS

Plant material and experimental design

Twenty seven cultivars and populations of alfalfa of different geographical origin were

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evaluated in this study (Table 1). The research was conducted on the experimental field at the Agricultural Institute Osijek. A total of 108 seedlings of each cultivar and population were grown in jiffy pots for forty days before transplanting into a field in April 2004. The field trial was arranged in a randomised complete block with three replications.

Each plot included 36 spaced plants (0.50 x 0.50 m). Four cuts were performed during plantation year (27/06, 28/07, 30/08, 29/09 in 2004), five cuts during the second growing season (16/05, 13/06, 14/07, 24/08, 03/10 in 2005) and three cuts during the third growing season (15/06, 17/07, 22/08 in 2006).

Table 1. List of alfalfa cultivars and populations evaluated in the present study

No.	Name	Country	Type/species	Origin of seed sources
14	Vuka	CRO	cultivar/*sativa*	Agricultural Institute
15	L-XXV	CRO	breeding population/*sativa*	Osijek
2	Prime	AUS	cultivar/*sativa*	South Australian
3	Genesis	AUS	cultivar/*sativa*	*Medicago*
18	Aurora	AUS	cultivar/*sativa*	Genetic Resource Centre
19	Jindera	AUS	cultivar/*sativa*	SARDI
4	Magali	FRA	cultivar/*sativa*	Dr. Julier Bernadette
5	Maron	FRA	wild population/*falcata*	Institut Nationale
12	Europe	FRA	cultivar/*sativa*	de La Rechearche
20	Mercedes	FRA	cultivar/*sativa*	Agronomique-INRA
21	Malzeville	FRA	wild population/*falcata*	
7	Barbara	ARG	cultivar/*sativa*	Dr. Daniel Basigalup
8	Victoria	ARG	cultivar/*sativa*	Instituto Nationale de
23	Monarca	ARG	cultivar/*sativa*	Technologia
24	Lujan	ARG	cultivar/*sativa*	Agropecuaria-INRA
10	Magnum V	USA	cultivar/*sativa*	Dr. Dan Undersander
11	Blazer XL	USA	cultivar/*sativa*	University of Wisconsin
26	Columbia	USA	cultivar/*sativa*	
1	Pondus	SWE	cultivar/*sativa*	Nordic Gene Bank
17	Vertus	SWE	cultivar/*sativa*	and
9	Sverre	SWE	cultivar/*sativa*	Svalof Weibull AB
6	Radius	POL	cultivar/*media*	IHAR
27	NS Mediana ZMS V	SER	cultivar/*media*	Institute of Field and Vegetable Crops Novi Sad
25	Resis	DEN	cultivar/*sativa*	Nordic Gene Bank
13	Classe	ITA	cultivar/*sativa*	Istituto di Migliramente Genetico Vegetable, Perugia
22	Planet	GER	cultivar/*sativa*	DSV
16	KM Norbert	HUN	cultivar/*sativa*	Fleischmann Rudolf Research Institute

Phenotypic data collection and statistical analysis

During the three-year investigation, thirteen phenotypic traits were observed in this research. The following traits were collected on all individual plants of each cultivar/population in all twelve cuts:

1. Green mass yield (GMY) was obtained to plants, hand cut at approximately 5 cm above the ground and weighed on electronic balance (Ohaus Scout II).

2. To determine dry matter yield (DMY) fresh samples of randomly chosen plants were taken from each plot and placed into paper bags. The samples were weighed and dried at 105°C for 24 h to assess average dry matter content (DMC). DMY were calculated by DMC x GMY/100 formula. Total yearly GMY

and DMY for each plant were determined by summing up the biomass yield (g) from each cut during each year.

3. Number of stems (NS) of individual plants was recorded directly following cuts.

4. Prior to cuts, plant height (PH, cm) was measured from the ground to the top of the inflorescence.

5. Plant regeneration (PR, cm) was assessed within the two weeks after the previous cut by individual measurements of the plant height.

The traits NS, PH and PR are shown as averaged values per plant.

In the second cut of 2005 growing season the longest stem of each plant of all cultivar/population was sampled. The number of internodes (NI) from the bottom to the first inflorescence was counted. These stems and their leafy branches were used to determine leaf to steam ratio (LSR). In the same cut the following morphological traits were described: growth habit (GH) (1 – erect to 9 – prostrate), plant colour (PC) (3 – light green to 7 – dark green), shape of leaf (SL) (3 – elongated to 7– round), length of central leaflet (LCL) (1 – very short to 9 – very long), width of central leaflet (WCL) (1 – very narrow to 9 – very broad), plant vigour (PV) (1 – very fast to 9 – very slow) on all plants of each cultivar/population. The traits were evealuated by combining the UPOV guidelines for distinctness, homogeneity and stability tests in lucerne (UPOV/TG/6/4, 1988) and Forage Legume Descriptors (IBPGR/84/191, 1984).

The data recorded were analysed by ANOVA, using SAS 9.1 software (SAS Institute, 2003). Principal component analysis (PCA) was performed on all traits. As the traits were measured on different scales, the mean observation of each traits were standardised prior to the analysis to eliminate scale differences. Hierarchical clustering was carried out on the coordinates of every plant on the PCs whose eigenvalue was higher than 1.

RESULTS AND DISCUSSION

The ANOVA detected significant differences among alfalfa cultivars and populations for all traits (data not shown). The PCA analysis was applied to identify the traits which were the main source of the variability and to explain the genetic diversity in germplasm collections.

The first four principal components (PCs) gave eigenvalues greater than 1.0 and explained 89.02% of the total variability among the cultivars and populations for all the traits investigated (Table 2).

Jenczewski et al. (1999) analysed gene flow between wild and cultivated alfalfa populations using thirteen quantitative traits and found that the first four PCs contributed 75% of the entire variability among the twenty populations.

Table 2. Eigenvalues and proportion of total variability explained by the first four principal components (PC)

Principal component	Eigenvalue	Variance (%)	Cumulative variance (%)
PC 1	7.58	58.21	58.21
PC 2	2.11	16.24	74.46
PC 3	1.14	8.76	83.21
PC 4	1.03	5.81	89.02

The first PC, which is the most important component, accounted for 58.21% of the total variability and was associated with yields of green mass and dry matter, vigour, growth habit, plant regeneration and length of central leaflet (Figure 1).

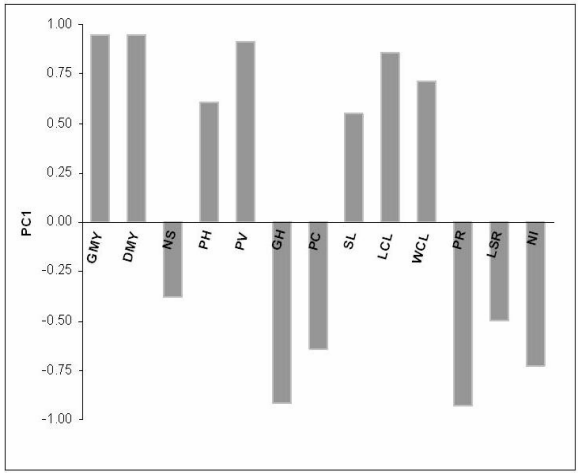

Figure 1. Plot of contribution of the thirteen investigated traits into the first principal component

Similar results were obtained by Prosperi et al. (2006) who studied morphological and agronomical diversity of wild genetic resources of alfalfa. The authors detected that the first PC explained 56.4% of the total variability in the measured traits and was associated with biomass production, which is congruent with our results. But Annicchiarico (2006) found that the first PC included only 35% of the total variation, which is considerable lower than the results reported here. Most probably this difference was related to a higher level of homogeneity among tested alfalfa materials and/or smaller numbers of analyzed traits.

The second PC accounted for 16.24% of the variability. This portion of variation mainly resulted from the variation in number of stems, shape of leaf and width of central leaflet (Figure 2).

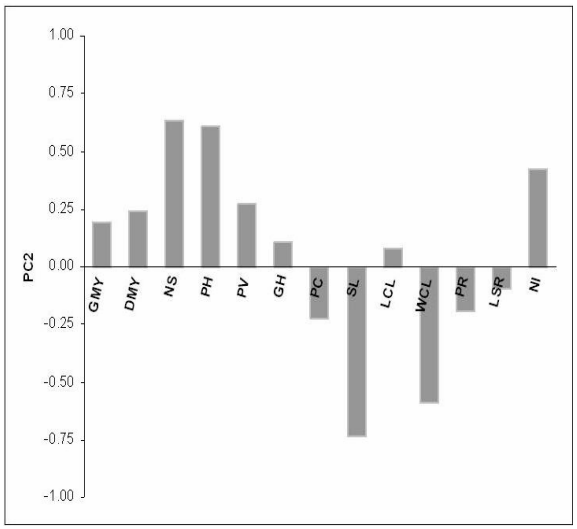

Figure 2. Plot of contribution of the thirteen investigated traits into the second principal component

The cluster analysis performed on the first two components grouped the majority of alfalfa cultivars and populations into three clusters of germplasm (Figure 3). Cluster 1 contained nine cultivars originated from the Australia (Genesis, Aurora, Prime), America (Magnum V, Blazer XL), Argentina (Barbara, Lujan, Monarca) and Hungary (KM Norberth). The cultivars were characterized by the highest yields of green mass and dry matter, tall plants, high number of stems and internodes of plants, the fastest regrowth after cut, and erect growth habit. Most plants in this group had oval leaves of light green colour (large leaf area) and high leaf to stem ratio. Cluster 2 was composed of six cultivars (Europe, Mercedes, Planet, Vertus, Resis, and Columbia) which were characterized by medium to small yields and yield components (plant height, number of stems, regrowth after cut). Most plants in this group had round leaves of dark green colour and favorable leaf to stem ratio. This cluster was mostly formed by cultivars from European countries, with the exception of cultivar Columbia (USA).

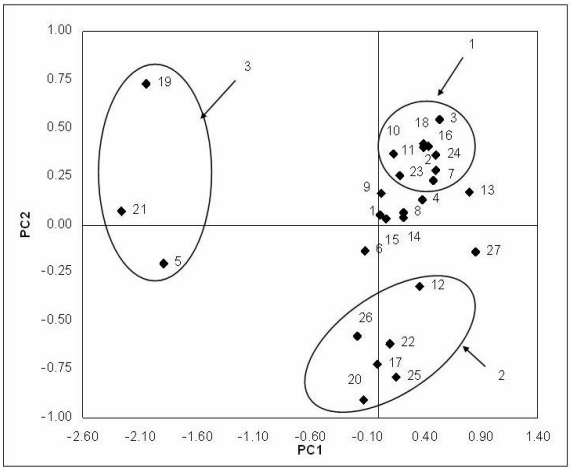

Figure 3. Plot of the two first principal components and positions of the 27 alfalfa cultivars/populations (Number of cultivars/populations corresponds to those in table 1)

These two clusters were separated by the Polish cultivar Radius, the Croatian cultivar Vuka and breeding population L-XXV, the Swedish cultivars Pondus and Sverre, the Argentinean cultivar Victoria, the French cultivar Magali, the Serbian cultivar NS Mediana ZMS V and the Italian cultivar Classe. The intermediate position of these materials was due to some mixed traits.

Cluster 3 was the smallest and composed of only two French populations (Maron, Malzeville) and one Australian cultivar (Jindera). They were characterized by the lowest forage production, prostrate growth habit, very slow regrowth after cut, elongated shape of leaves, the lowest leaf area, and the highest leaf to stem ratio and number of internodes.

The clustering in our research was partly related to the geographic origin, which corresponds to the results obtained by the other authors who studied genetic diversity and

variability of forage crops by PCA analysis using morpho-agronomic traits (Drobna et al., 1999; Sardana et al., 2007; Dias et al., 2007). The groups obtained in the classification depend on the material involved in the study (Crochemore et al., 1998).

CONCLUSIONS

The present investigation revealed a wide phenotypic variability for many of the assessed traits in the alfalfa collections. PCA analysis separated the majority cultivars and populations into three clusters of germplasm. Positive traits for breeding were found in all clusters. Valuable plants from the most promising materials could be used for future activities in our alfalfa breeding programme.

Acknowledgements

This research was supported by the Ministry of Science, Education and Sport of the Republic of Croatia (project "Evaluation of breeding value in alfalfa (*Medicago* spp.) germplasm – 073-0000000-3535).

REFERENCES

Annicchiarico, P., 2006. *Diversity, genetic structure, distinctness and agronomic value of Italian lucerne* (*Medicago sativa* L.) *landraces*. Euphytica, 148 (3): 269-282.

Barcaccia, G., Albertini, E., Tavoletti, S., Falcinelli, M., Veronesi, F., 1999. *AFLP fingerprinting in Medicago spp.: Its development and application in linkage mapping*. Plant Breeding, 118 (4): 335-340.

Bennett, S.J., 2000. *Genetic variation of five species of Trifolium L. from south-west Turkey*. Genetic Resources and Crop Evolution, 47 (1): 81-91.

Crochemore, M. L., Huyghe, C., Ecalle, C., Julier, B., 1998. *Structuration of alfalfa genetic diversity using agronomic and morphological characteristics. Relationship with RAPD markers*. Agronomie, 18 (1): 79-94.

Dias, P. M. B., Julier, B., Sampoux, J. P., Barre, P., Dall'Agnol, M., 2007. *Genetic diversity in red clover (Trifolium pratense L.) revealed by morphological and microsatellite (SSR) markers*. Euphytica, 160 (2): 189-205.

Drobna, J., Zakova, M., Hauptvogel, P., Sedlakova, E., 1999. *Evaluation of Alfalfa Genetic Resources Using Cluster Analysis Based on the Morphological and Agronomic Characteristics*. Lucerne and Medics for the XXI Century. Proceedings of the XIII EUCARPIA *Medicago* spp. Group Meeting, Istituto di Miglioramento Genetico Vegetale, Perugia, Italy: 219-227.

Ghafoor, A., Afzal, M., Anwar, R., 2003. *Diversity in food legumes for sustainable utilization of plant genetic resources*. In: Sustainable utilization of plant genetic resources for agricultural production (Eds.: Anwar, R., Takahashi, J., Bhatti, M.S., Masood, S.) PARC/IPGRI/JICA, Islamabad, Pakistan: 238-250.

IBPGR/84/191, 1984. *International board for plant genetic resources. Forage Legume Descriptors*. Commission of European Communities: Committee on Disease Resistance Breeding and Use of Gene banks, Rome, Italy.

Maureira, I. J., Ortega, F., Campos, H., Osborn, T. C., 2004. *Population structure and combining ability of diverse Medicago sativa germplasms*. Theoretical and Applied Genetics, 109 (4): 775-782.

Martinez-Calvo, J., Gisbert, A. D., Alamar, M. C., Hernandorena, R., Romero, C., Llacer, G., Badenes, M. L., 2008. *Study of a germplasm collection of loquat (Eriobotrya japonica Lindl.) by multivariate analysis*. Genetic Resources and Crop Evolution, 55 (5): 695-703.

Naghavi, M. R., Jahansouz, M. R., 2005. *Variation in the agronomic and morphological traits of Iranian chickpea accessions*. Journal of Integrative Plant Biology, 47 (3): 375-379.

Jenczewski, E., Prosperi, J. M., Ronfort, J., 1999. *Evidence for gene flow between wild and cultivated Medicago sativa (Leguminosae) based on allozyme markers and quantitative traits*. American Journal of Botany, 86 (5): 677-687.

Julier, B., Porcheron, A., Ecalle, C., Guy, P., 1995. *Genetic variability for morphology, growth and forage yield among perennial diploid and tetraploid lucerne populations* (*Medicago sativa* L.). Agronomie, 15 (5): 295-304.

Prosperi, J. M., Jenczewski, E., Angevain, M., Ronfort, J., 2006. *Morphologic and agronomic diversity of wild genetic resources of Medicago sativa L. collected in Spain*. Genetic Resources and Crop Evolution, 53 (4): 843-856.

Sardana, S., Mahajan, R. K., Gautam, N. K., Ram, B., 2007. *Genetic variability in pea* (*Pisum sativum* L.) *germplasm for utilization*. SABRAO Journal of Breeding and Genetics, 39 (1): 31-41.

Smith, S. E., Doss, A. L., Warburton, M., 1991. *Morphological and agronomical variation in North African and Arabian alfalfas*. Crop Science, 31 (5): 1159-1163.

Tucak, M., Popovic, S., Cupic, T., 2009. *Karakterizacija i morfo-agronomska evaluacija germplazme lucerne*. Proceedings of the 44th Croatian & 4th International Symposium on Agriculture, Opatija, 16-20 February, Maric, S & Loncaric, Z (eds.) Faculty of Agriculture University of J.J. Strossmayer in Osijek, Osijek: 400-404.

SAS Institute Inc., 2003. SAS/STAT Software, Version 9.1, SAS Institute, Cary, North Carolina, USA.

UPOV/TG/6/4, 1988. *Guidelines for the conduct of tests for distinctness, homogeneity and stability of lucerne* (*Medicago sativa* L.). International Union for the Protection of New Varieties of Plants, Geneva, Switzerland.

Veasey, E. A., Schammass, E. A., Vencovsky, R., Martins, P. S., Bandel, G., 2001. *Germplasm characterization of Sesbania accessions based on multivariate analyses*. Genetic Resources and Crop Evolution, 48 (1): 79-90.

Zakova, M., Benkova, M., 2006. *Characterization of spring barley accessions based on multivariate analysis*. Communications in Biometry and Crop Science, 1 (2): 124-134.