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EVALUATION OF YIELD AND MORPHOLOGY TRAITS IN 72 GENOTYPES OF SAINFOIN (*ONOBRYCHIS VICIIFOLIA* SCOP) THROUGH FACTOR ANALYSIS

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ABSTRACT

Seventy two genotypes of sainfoin (*Onobrychis viciifolia* Scop) were sown using lattice design under irrigation condition in Alborz, station Karaj, Iran during 2009-10. Forage dry matter (DM) yield, leaf to stem ratio, growth condition, stem density, plant height, flowering time and resistance to sainfoin powder mildew were studied over 2 years. The analysis of variance showed significant differences for all traits ($P<0.01$). The correlation coefficient between DM yield and growth condition, plant height and stem density was positive and significant. Resistance to disease had negative significant relationship with late-growing, plant height, stem density and forage yield. In addition, Ward cluster analysis was done and divided into 4 genotype groups. In coordinate axis, 1st and 2nd factors demonstrated a good agreement between cluster and factor analysis.

Key words: Forage yield, Cluster analysis, Factor analysis, Morphological traits, Correlation.

INTRODUCTION

Sainfoin (*Onobrychis viciifolia*) is a leguminous perennial herb belongs to the Fabaceae family in Southern Europe and in temperate regions of Western Asia as a forage crop. Sainfoin is a plant with many erect or sub erect, hollow stems, 60-80cm height or more, arising from basal buds on branched stock (Sancak, 1999). The flowers produce large amounts of nectar and commonly used for honey production (Elçi, 2005). The root system consists of deep tap root with a few main branches and numerous fine lateral roots bearing most of the rhizobial nodules (Tamas and Savatti, 2006). The forage legume sainfoin is an important crop well adapted to the dry and semi-arid regions where the cultivation of alfalfa is restricted by the environmental conditions. It has grown well on soils with low phosphorus (Elçi *et al.* 1997). It contains condensed tannins which reduce its potential to produce bloat and improve protein digestion by grazing animals (Rumball and Claydon, 2005).

High levels of variability among sainfoin genotypes were encountered at agronomic level by Michelana and Hycka (1988) and Delgado *et al.* (2008). Sainfoin leaves, roots, flowers, and stems were attacked by a wide range of insect pests and diseases such as powdery mildew (Wallace, 1968; Hewitt and Burleson, 1976; Morrill *et al.* 1984). The extension of sainfoin is limited by powdery mildew and decreased forage yield during second harvest (Majidi, 2010). Tosun (1988) reported that 36 landrace of sainfoin populations were under irrigation and three varieties namely; Khoyvar 2, Khosroshahr and Gavani produced the highest DM yield 3.96, 3.83 and 3.81 ton ha^{-1}, respectively, in total of two harvests.

Correlation study occurs due to character association, improvement in respect of one character may have been obtained at the expense of other characters (Turk and Celik, 2006). Mirzaii Nodushan *et al.* (1998) studied 10 varieties of sainfoin in normal situation and reported significant differences among varieties for flowering time and height plant but traits of fresh and dry matter (DM) yield were not significant. Factor analysis was used to reduce the number of variables changes into some

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hidden factors, as well as to identify principal components of the yield, to classify traits on the basis of intra-relations between them and to investigate genetic diversity (Azizi *et al.* 2001).

The objectives of the present work are; 1. To estimate correlation among DM yield and morphological traits 2. To investigate and choose the variability in different populations, and 3. To specify genetic variation model and genotype grouping based on forage yield and morphological traits, cluster analysis and factor analysis of this species.

MATERIALS AND METHODS

The research was carried out at the Alborz Station located in Karaj, Iran. The experimental soil was clays, non saline and had neutral pH. Average annual temperature, relative humidity and precipitation were 19^{0}C, 41.3% and 251.2 mm respectivly in 2009-10. The experiment layout was based on lattice design with two replications. A number of 72 genotypes were provided from natural resource gene bank. Genotypes were sown in 2m rows with 0.5m distance. Plots were fertilized with 60kg.ha^{-1} N and 60 kg.ha^{-1} P_2O_5 at sowing. First irrigation was applied immediately after cultivation and next irrigations were scheduled every 7 days. After 4-5 months the following traits were measured:

Flowering Time: Based on number of days from 1st day of spring till 50% of plants began to blossom.

Growth Condition (Freshness): Based on the covering conditions of plots and freshness two weeks after each harvest and classified on range from 1 to 5. Where: 1 poor growth condition and 5 maximum.

Plant Height: Five plants were selected from each plot and their heights were measured in centimeter and averaged it.

Number of Stem (Density): The stems number was counted and averaged it for each plot.

Powdery mildew disease: The plant disease and damaged were evaluated in second harvest depends on responses from 1 to 5 score. 1 sensitive; 5 resistant to disease.

Leaf to Stem Ratio: DM yield of half a square meter was produced and their leaves and stems were separated and weighted after drying in 100 ?C at oven through over night.

Dry Matter (DM) Yield: One forage sample was dried from each plot in 100^{0}C oven. The DM yield was calculated after weighting.

Following the two years of data collection, combined analyses with split plots design in time were carried out. Besides, phenotypic correlation coefficient was calculated among the traits based on the two years data collected. In addition, factor analysis was performed to determine the share of each trait and data reduction. The average of 7 traits on 72 genotypes was used in factors analysis. Cluster analysis with Ward method in Euclidean scale was performed. Dispersion genotypes diagram was depicted on the first and second factor. Software of MINITAB15 and SAS9 were used for data analysis.

RESULTS AND DISCUSSION

Statistical parameters including maximum, minimum, mean, standard error and coefficient of variation for 72 genotypes of sainfoin were shown in Table 1. After inspection data normality, analysis of variance (ANOVA) was performed as lattice design (Table 2) and because of its disadvantage over block design, data analysis continued as block design. There were significant differences for all traits (P<0.01) (Richard, 1995; Majidi, 2010; Mohajer *et al.* 2011). Pearson correlation analysis results are

TABLE 1. Statistical parameters of traits in 72 genotypes of *Onobrychis viciifolia*.

Parameters	Dm Yield	Leaf To Stem Ratio	Stem No. Per M^2	Plant Height	Flowering Time	Powdery Mildew	Growth Condition
Mean	13.5	0.39	31.9	53.4	46.9	2.51	4.15
Minimum	4.6	0.16	13	22.5	30.5	1	1
Maximum	30.6	0.88	46	77.5	53.5	5	5
SE Mean	0.4	0.01	0.7	1.0	0.51	0.13	0.09
St Dev	5.1	0.15	8.2	12.4	6.1	1.58	1.08
%CV	37.5	30.56	25.8	23.3	13.01	32.94	25.98

TABLE 2. ANOVA of traits in 72 genotypes of *Onobrychis viciifolia*.

Source	DF	Growth Condition	Powdery Mildew	Flowering Time	Plant Height	Stem No. Per M^2	Leaf /Stem Ratio	DM Yield
Genotype	71	1.58**	2.90**	73.19**	231.41**	97.79**	0.029**	2642**
Block	1	3.67*	91.84*	2.77	23.92	13.46	0.02	453
Error	71	0.7	0.8121	1.82	44.89	19.06	0.01452	1093

*, ** = significant at the 0.05 and 0.01 probability level, respectively.

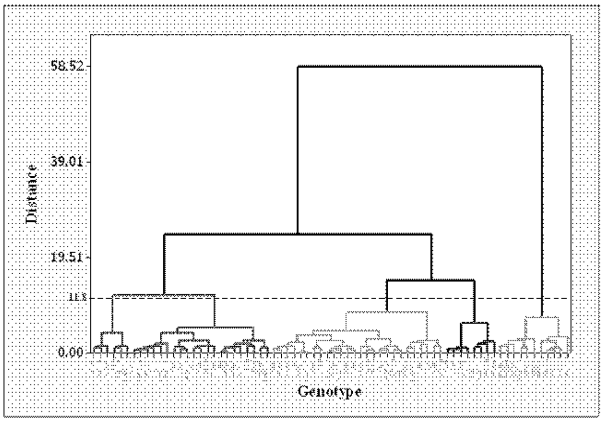

FIG 1. Dendrogram with Ward Linkage and Euclidean Distance in 72 genotypes of *Onobrychis viciifolia*.

shown in Table 3. Correlation coefficients between forage yield and growth condition, plant height and stem density were positive and with resistance to powdery mildew was negatively significant (P<0.01). This demonstrated that yield increased as much as plant height and number of stem increased. The similar results were observed by Turk and Celik (2006). In contrast, the existence of powdery mildew disease caused reduction in forage yield. Moreover, the relationship between growth condition and leaf to stem ratio showed that the genotypes with the better growth condition, would have greater leaf to stem ratio. Disease factor had a negative and significant relationship with stem density, plant height and flowering time (P<0.01) which demonstrated that taller, high density, late flowering genotypes were more sensitive to powdery mildew disease. Flowering time had a direct significant relationship with plant height and stem density which showed that late flowering genotypes have many long stems. Number of stem had also positive and significant relationship with plant height.

In factors analysis, the particular values (variance) of resulting from 1 to 3 factors were more than 1 accounted for 44, 22 and 15 per cent, respectively and justified 81 per cent of total variance (Table 4). After varimax rotation of factors, it was determined that the first factor affected on traits of disease sensitivity, flowering time, plant height and stem density was known as morphological factor. Second factor affected on two traits of forage yield and growth condition and was known as yield factor. Trait of leaf to stem ratio had solely significant

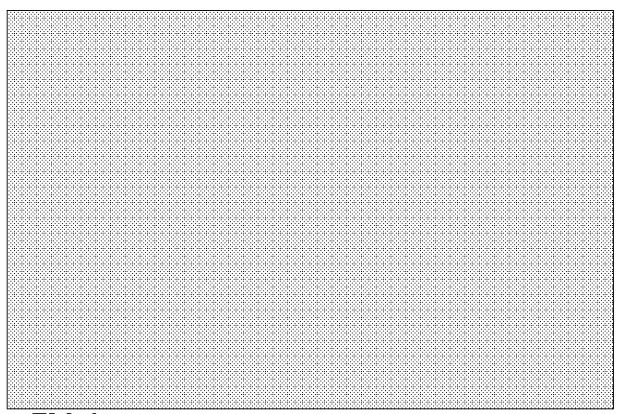

FIG 2. Factor analysis of traits based on 4 clusters of 72 genotypes of *Onobrychis viciifolia*.

TABLE 3. Correlation analysis between forage yield and morphological traits in in 72 genotypes of sainfoin.

Traits	Growth Condition	Powdery Mildew	Flowering Time	Plant Height	Stem No. Per M^2	Leaf To Stem Ratio
Powdery mildew	0.01					
flowering time	0.06	-0.64**				
plant height	-0.04	-0.77**	0.86**			
stem No. per m2	-0.02	-0.70**	0.85**	0.81**		
leaf to stem ratio	0.37*	0.0	0.01	-0.18	0.17	
DM Yield	0.59**	-0.45**	0.14	0.42**	0.38*	0.07

*, ** = significant at the 0.05 and 0.01 probability level, respectively.

TABLE 4. Matrix of coefficients factors after varimax rotation in 72 genotypes of *Onobrychis viciifolia*.

Traits	Factor 1	Factor 2	Factor 3	Communality
Powdery mildew	-0.79*	0.15	-0.05	0.65
flowering time	0.87	-0.01	-0.04	0.76
plant height	0.91	-0.05	0.11	0.84
stem No. per m^2	0.89	-0.05	-0.10	0.80
Growth condition	-0.09	-0.87	-0.15	0.78
DM Yield	0.27	-0.85	0.05	0.79
leaf to stem ratio	0.00	-0.09	-0.99	0.98
Variance	3.07	1.5	1.02	5.6
% Var	0.44	0.22	0.15	0.80

* More valuable coefficients are in bold and underline in comparison with two other factors.

correlation with third factor. In cluster analysis all 7 traits of 72 genotypes were used and grouped into 4 different categories with dendrogram slice in 11.8 of Euclidean distance (Fig1.). Comparison among Clusters mean is illustrated in Table 5 and each cluster characteristics were determined as follow:

Cluster 1 genotypes: sensitive to powdery mildew, good growth condition, late flowering, long-stems and high yield.

Cluster 2 genotypes: poor growth condition, sensitive, taller stems, higher stem density, high yield.

Cluster 3 genotypes: rather resistant, medium height, medium stem number, long-legged, high density, low yield.

Cluster 4 genotypes: resistant to powdery mildew, early flowering, low height, few stem couple with medium yield.

Dispersion of 72 genotypes (in 4 clusters) based on first and second factor is shown in figure 2. First factor that affected flowering time, plant height and stem density. Second factor had high correlation with forage yield and growth condition. Thus regarding the obtained results, the first clusters were determined to have the highest forage yield and fourth cluster were resistant to sainfoin powdery mildew disease. There is meager research information on sainfoin as wide as this project terms different genotypes, thus with cross of these two group genotypes and selection with progeny test, it could be breeding improved sainfoin varieties for high forage yield couple with resistant to powdery mildew disease.

Plant breeders working at the research institutes in Iran have an active germplasm collection in sainfoin. However there is a need for enrichment of these collections by germpalsm introduction and exchange. There is some active centers for *ex situ* conservation of genetic resources of improved varieties, landraces and wild relatives of cultivated crops, but there is no mechanism and legal base for distribution of plant genetic resources. Limiting resources does not fully allow multiplication and maintenance of genetic resources stored in the genbank, Therefore there is not sufficient seed of all varieties available for germplasm exchange within the country and in the international level that limits access of plant breeders to the germplasm to be used for breeding purposes. The use of improved varieties and high quality seeds play a major role in increasing the productivity. Therefore providing farms with certified seed of high yielding well-adapted varieties is a priority task in agricultural development. To achieve this task requires a series of integrated measures, which make up the 'Yield Chain'. The key links in this chain are; breeding of new high-yielding varieties, an efficient variety testing and registration system, maintaining the registered varieties to preserve their genetic features, large-scale production, processing and marketing of seeds, and a quality assurance system to certify these seeds.

As observed for the agronomic traits, morphological traits were highly variable among the accession. Morphological characterisation was also

TABLE 5. Means comparison of traits in cluster analysis in 72 genotypes of *Onobrychis viciifolia* based on ward method.

Cluster	DM Yield	Leaf To Stem Ratio	Stem No. Per M^2	Plant Height	Flowering Time	Powdery Mildew	Growth Condition
1	10841b	0.33b	33.9b	57.6a	49.4a	2.1b	3.5c
2	16513a	0.35b	34.5b	57.4a	49.3a	2.2b	4.7a
3	14542a	0.64a	36.8a	57.3a	48.9a	1.6b	4.4ab
4	11884b	0.39b	17.2c	30.1b	32.9b	4.5a	4bc

The means of the clusters with same small letters were not significantly different as per Duncan's multi-range test at P<0.05.

a challenging task as reproducibility of measurements was crucial but difficult because of the intraaccession variability. A few accessions were characterised by red stems or totally prostrate habit, although these traits were sparsely found in some other individuals. A high morphological variability was also observed among a limited germplasm from central Italy (Negri and Cenci, 1988). Significant differences in morphological traits were seen among accessions of the germplasm collection. Few correlations with geographic and climatic origin were found probably due to the limited accession number analysed. Still, solid correlations were shown on crucial traits, and it appears that geographic origin was a strong driver of *Onobrychis viciifolia* morphological characteristics. A clear division (more than 50% of differences) was found between Eastern European accessions, which were characterised by more inflorescences, and Western European ones characterised by more leaflets. This might reflect general agricultural uses with pasture dominating in Eastern Europe, and hay production, with preference to foliage production, in Western Europe (Carbonero, 2011).

Onobrychis viciifolia in the UK is traditionally used as a hay crop, although it can be cut for silage (Bland, 1971; Sheldrick *et al.* 1987). The leafy 'stubble' can be used for light grazing, but only in the late autumn, to allow the crop time to replenish root reserves (Sheldrick *et al.* 1987). Depending upon the growing conditions, *Onobrychis viciifolia* will yield between 7 and 15 ton.ha^{-1} dry matter, which is 20% lower than *Medicago sativa*. This is due to a lower leaf area index, a more prostrate canopy structure and less efficient nitrogen fixation (Frame *et al.* 1998). Regrowth is slow, and it is important to allow enough time to replenish root reserves to maintain its persistence and longevity. Varieties of second cluster had the highest DM yield with 14 ton.ha^{-1} in this research. Yield of three harvests was one of the main reasons behind high forage yield in the present work, while total annual yield was related to two harvests at the research activity made by Tourchi *et al.* (2007).

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