

# Genetic variation for clonal propagation and trait association with field performance in sainfoin

SAYAREH IRANI, MOHAMMAD M. MAJIDI AND AGHAFAKHR MIRLOHI

Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.  
<http://eagro.iut.ac.ir>

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## Abstract

Clonal plant materials with identical genotypes may be used to precisely detect environmental effects and genotype x environment interactions resulting in a more accurate estimate of genetic parameters in plant genetic analysis. In sainfoin (*Onobrychis viciifolia*), knowledge on genetic variation for clonal propagation and its association with field performance is limited. Eleven natural ecotypes of sainfoin from wide geographical areas of Iran were used to evaluate genetic variation for clonal propagation and its association with related traits. From each ecotype 11–21 genotypes were cloned via cuttings. Then, clones of a hundred genotypes from 10 ecotypes were transplanted to the field. High genetic variation was found between ecotypes of sainfoin for producing viable clones. The mean values for viable clones varied from 50% (Borujen ecotype) to 97% (Najafabad ecotype). The values of within-ecotype coefficient of variation were higher than the genetic coefficient of variation. The highest heritability estimates were obtained for sensitivity to powdery mildew, plant height and number of stems per plant. Dry matter yield (DMY) in the field was significantly and positively correlated with plant height and number of stems per plant, inflorescence length and growth score. An association between DMY and percent of viable clones was found indicating the possibility of selection during the early stages of clonal propagation. According to principal component analysis, Baft and Fereydunshahr ecotypes have potential for improving production of sainfoin if introduced into breeding programs. These issues warrant further study.

## Resumen

Materiales de plantas clonales con genotipos idénticos pueden ser utilizados para detectar con precisión efectos ambientales e interacciones genotipo x ambiente, lo que permite una estimación más precisa de los parámetros en el análisis genético de plantas. En esparceta (*Onobrychis viciifolia*), una especie de polinización cruzada en la familia Leguminosae (Fabaceae), el conocimiento sobre la variación genética para la propagación clonal y su relación con factores de rendimiento a nivel de campo es limitado. En el estudio se utilizaron 11 ecotipos de esparceta procedentes de amplias zonas geográficas de Irán para evaluar dicha variación genética en la propagación clonal y su asociación con características relacionadas con la producción. De cada ecotipo se clonaron entre 11 y 21 genotipos mediante la técnica de propagación por esquejes. En total se trasplantaron en campo los clones de 100 genotipos obtenidos de 10 ecotipos. Se encontró una alta variación genética entre los ecotipos de esparceta para producir clones viables; los valores medios de clones viables variaron de 50% (ecotipo Borujen) a 97% (ecotipo Najafabad). Los valores del coeficiente de variación dentro de un ecotipo fueron más altos que el coeficiente genético de variación. Los mayores valores de heredabilidad se encontraron para las características susceptibilidad al oídio (*Leveillula taurica*), altura de planta y número de tallos por planta. El rendimiento de materia seca (MS) en campo se correlacionó de manera significativa y positiva con la altura de planta y el número de tallos por planta, la longitud de inflorescencia y la calificación del crecimiento. Se encontró una asociación entre el rendimiento de MS y el porcentaje de clones viables lo cual indica la posibilidad de selección temprana durante las primeras fases de propagación clonal. Según el análisis de componentes principales, los ecotipos Baft y Fereydunshahr tienen el potencial de mejorar la producción de esparceta, cuando se introducen en programas de fitomejoramiento.

Correspondence: S. Irani, Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, 84156 8311, Iran.  
E-mail: [sayarehirani@yahoo.com](mailto:sayarehirani@yahoo.com)

## Introduction

Sainfoin (*Onobrychis viciifolia*) belongs to the family Fabaceae, is well adapted to a range of soil and climatic conditions, displays high nitrogen-fixing capacity and nutritive value combined with good palatability and is non bloat-inducing (Goplen et al. 1991; Frame 2005; Delgado et al. 2008). Since sainfoin cross-pollinates, plants multiplied by sexual methods are highly heterogeneous and less useful for genetic analysis. Vegetative propagation is important for preserving uniformity (George and Sherrington 1984). The most widespread method for vegetative propagation of many plant species is by cuttings (Sancak 1999). The capacity of cuttings to produce roots is influenced by many factors, including plant genotype, pre- and post-treatment of the cuttings and the rooting environment (Hartmann and Kester 1983). Low levels of self-pollination have limited the use of inbreeding and inbred lines in breeding programs with sainfoin and some other forage legumes.

Clonal propagation has eliminated the need for inbreeding for developing breeding lines and maintaining parental genotypes. This technique can be used in studies to detect environmental effects and genotype  $\times$  environment interactions, and also to estimate total genetic variance and heritability (Nguyen and Sleper 1983). In this respect, Julier et al. (2000) estimated among- and within-cultivar variance and heritability for dry matter yield, leaf:stem ratio and morphological and quality traits using 11 alfalfa (*Medicago sativa*) cultivars and 7–20 genotypes from cultivars that were clonally propagated. In addition, Bolaños-Aguilar et al. (2000) reported that within-population variance accounted for 69–95% of total variation for seed yield components in alfalfa. Moreover, expression of genes related to vegetative propagation could be assayed by rooting ability and related traits. In this case, Scotti-Saintagne et al. (2005) detected 10 quantitative trait loci (QTL) explaining 4.4–13.8% of phenotypic variance for rooting ability in pedunculate oak (*Quercus robur*). Grattapaglia et al. (1995) showed 10 QTLs for micropropagation response in eucalypts (*Eucalyptus grandis* and *E. urophylla*) (measured as fresh weight of shoots).

One of the most important factors that influence the capacity of cuttings to root is the plant genotype. Data on genetic variation for clonal propagation and its association with other traits in sainfoin are scarce. This study aimed to: determine the ability of Iranian sainfoin ecotypes to produce roots on cuttings and the field performance of the cuttings following transplanting; assess the relationship between their ability to reproduce from

clones and other morphological traits; and make a preliminary selection of promising clones for further research studies.

## Materials and Methods

### *Evaluation of clones in the greenhouse*

Natural ecotypes of sainfoin (800 genotypes) from wide geographical areas of Iran were established in 2009 in a large spaced-plant nursery at Isfahan University of Technology Research Farm. From these, 11 ecotypes were chosen in the fall of 2010 to represent a wide range of phenotypic variation. From each ecotype 11–21 plants (a total of 177 phenotypes) were removed from the field and cloned via cuttings to obtain equal clones during the winter of 2011. For 60 days, the clones (8 clones for each genotype) were grown in a mixture of soil and sand under controlled conditions in a greenhouse, with average minimum and maximum air temperatures of 18 and 26 °C, respectively, 16 h light with 200–400  $\mu\text{E}/\text{S}/\text{m}$  intensity and 70% relative humidity. Seven traits including percent of viable clones, clone growth score (1 for poor growth to 9 for perfect growth; hereafter referred to as ‘clone score’), plant height, number of stems per plant, sensitivity to powdery mildew *Leveillula taurica* (based on the leaf area covered with mildew using a scale of 0–9), fresh matter yield and dry matter yield (DMY) were measured. The greenhouse experiment was set up in an unbalanced completely randomized design, where each genotype was considered as a sample within the ecotypes.

### *Evaluation of clones in the field*

For the field experiment all genotypes of Borujen ecotype and a number of genotypes from other ecotypes were eliminated because of their poor ability to be cloned. Consequently, uniform clones of 100 genotypes from 10 ecotypes were transplanted to the field according to a randomized complete block design with 6 replications in April 2011. One hundred spaced plants in each block were grown at 50 cm spacing between and within rows.

The experiment was conducted at Isfahan University of Technology Research Farm (32°30' N, 51°20' E), Isfahan, Iran. The mean annual temperature and precipitation were 14.5 °C and 140 mm, respectively. The experimental site contained a Typic Haplargid, silty clay loam soil. The soil was non-saline, non-sodic and calcareous, containing 390 g/kg calcium carbonate equivalent, 5.0 g/kg organic C and 0.77 g/kg total N, with pH 8.3. The electrical conductivity and the sodium adsorption ratio of the soil saturated extract were 1.6 dS/m and 1.4 mmol/L<sup>0.5</sup>,

respectively. Plots were fertilized with 200 kg N/ha and 200 kg P/ha prior to planting.

Plant height, inflorescence length, number of stems per plant and growth score (1 for poor growth to 5 for perfect growth) were measured. All traits were recorded at flowering stage, except growth, which was recorded 2 weeks after transplanting. Forage was harvested by hand-cutting plants at approximately 5 cm above the ground. To determine DMY, fresh samples were dried at 72 °C for 48 h.

#### Genetic and statistical analyses

Analyses of variance were performed by the GLM procedure of SAS (SAS 1999). Variances were calculated with the VARCOMP procedure of SAS. Means were compared using the least significant difference (LSD) test at  $P < 0.05$  (Steel and Torrie 1960).

In the greenhouse experiment, broad-sense heritability (H) on an entry-mean basis was defined according to Falconer and Mackay (1996) using the formula:

$$H = \frac{\sigma_E^2}{\sigma_P^2}$$

where:  $\sigma_E^2$  is the genotypic variance and  $\sigma_P^2$  is the phenotypic variance.

In the field experiment, broad-sense heritability (H) was calculated by the following formula (Julier et al. 2000):

$$H = \frac{\sigma_E^2 + \sigma_{G(E)}^2}{\sigma_E^2 + \sigma_{G(E)}^2 + \sigma_\varepsilon^2}$$

where:  $\sigma_E^2$  represents among-ecotype variance,  $\sigma_{G(E)}^2$  represents within-ecotype variance and  $\sigma_\varepsilon^2$  is the error component of variance.

The genotypic coefficient of variation (GCV), within-ecotype coefficient of variation (WECV) and phenotypic coefficient of variation (PCV) were calculated as:

$$GCV = (\sigma_E/\mu) 100$$

$$WECV = (\sigma_{G(E)}/\mu) 100$$

$$PCV = (\sigma_P/\mu) 100$$

where:  $\sigma_E$  is the standard deviation of the genotypic variance,  $\sigma_{G(E)}$  is the standard deviation of within-ecotype variance,  $\sigma_P$  is the standard deviation of phenotypic variance and  $\mu$  is the phenotypic mean (Majidi et al. 2009).

The phenotypic correlation between 2 traits was calculated as described by Falconer and Mackay (1996):

$$r_p = S_{xy}/(S_x \cdot S_y)$$

where:  $S_{xy}$  is the phenotypic covariance for the characters x and y, and  $S_x$  and  $S_y$  are the standard deviations for traits x and y, respectively.

Data for each ecotype were averaged across the replications and then used for factor analysis and principal

component analysis (PCA). Factor analysis was done based on PCA according to Johnson and Wichern (2007). For PCA, the data were analyzed by Statgraphics statistical software (Statgraphics 2007).

## Results

### Greenhouse experiment

Ecotypes were significantly ( $P < 0.01$ ) different for all measured traits in the greenhouse experiment (data not shown). High genetic variation was found between ecotypes of sainfoin for the ability to produce viable clones. Najafabad and Fereydunshahr ecotypes produced the highest percentage of viable clones, while Borujen had the lowest percentage. The mean values varied between 50 and 97% for viable clones and 3 and 7 for clone score (Table 1). The highest and lowest clone scores, and fresh and dry matter yields were observed for ecotypes Fereydunshahr and Borujen, respectively. Sirjan ecotype produced the tallest plants and the highest number of stems per plant. Ecotypes Najafabad and Kabotarabad were the most resistant to powdery mildew (Table 1).

Phenotypic coefficients of variation (PCV) ranged from 25 to 47% and genotypic coefficients of variation (GCV) from 13% for fresh dry matter yield to 42% for sensitivity to powdery mildew (Table 2). The lowest broad-sense heritabilities were observed for viable clones (0.16) and fresh matter yield (0.29). The highest heritability was obtained for sensitivity to powdery mildew (0.78) (Table 2).

### Field experiment

Ecotype and genotype per ecotype were significantly different for all the measured traits in the field experiment at  $P < 0.01$  (data not shown). The greatest plant height, inflorescence length, number of stems per plant, dry matter yield and growth score were observed for ecotypes Baft and Sirjan and Najafabad (Table 1). The within-ecotype contribution (the percent of  $\sigma_{G(E)}^2$  to  $\sigma_E^2 + \sigma_{G(E)}^2$ ) was 80% for plant height, 88% for inflorescence length, 69% for number of stems per plant, 61% for DMY and 71% for growth score. Within-ecotype coefficients of variation (WECV) (range 2–20%) were higher than genotypic coefficients of variation (GCV) (range 0.7% for inflorescence length to 14% for dry matter yield) (Table 2). The lowest broad-sense heritabilities were observed for growth score (0.57), inflorescence length (0.60) and dry matter yield (0.61), while the highest heritabilities were obtained for number of stems per plant (0.80) and plant height (0.74) (Table 2).

**Table 1.** Evaluation of 11 ecotypes of sainfoin for a range of traits in the greenhouse and field.

Ecotype	Greenhouse experiment							Field experiment				
	Viable clones (%)	Clone score	Plant height (cm)	Number of stems per plant	Sensitivity to powdery mildew	Fresh matter yield (g/plant)	Dry matter yield (g/plant)	Plant height (cm)	Inflorescence length (cm)	Number of stems per plant	Dry matter yield (g/plant)	Growth score
Arak	87.5abc	4.28de	18.9d	1.49e	3.90e	47.7de	16.3f	24.0bc	5.06d	6.91c	16.6c	2.85c
Najafabad	97.0a	6.35ab	26.1c	2.28d	1.85f	62.3abc	20.1b-e	25.1bc	5.19abc	8.61b	20.7b	3.50b
Semirom	77.9bcd	6.00bc	24.5c	2.25d	5.98b	63.7ab	20.7bcd	23.0c	5.09cd	6.83c	17.8bc	3.11bc
Sanandaj	88.2abc	5.96bc	25.2c	2.11d	5.55bc	54.8cd	17.8ef	23.5c	5.11cd	6.70c	17.6bc	3.03bc
Kabotarabad	72.0cd	5.36c	27.3bc	3.16b	1.87f	59.0bc	19.5cde	23.8bc	5.21ab	8.80b	19.7bc	3.31bc
Fereydunshahr	94.1ab	7.16a	32.2ab	2.94bc	7.14a	70.7a	24.1a	23.1c	5.12bcd	7.83bc	20.1bc	3.35bc
Isfahan	71.5cde	5.26c	26.4c	2.40d	2.20f	54.4cd	18.1def	23.6c	5.12a-d	7.81bc	18.4bc	3.13bc
Baft	62.5de	5.14cd	28.7bc	3.20ab	4.19e	66.6ab	22.7ab	27.9a	5.22a	10.73a	27.9a	4.43a
Sirjan	71.6cde	5.75bc	36.71a	3.77a	4.25de	63.6abc	21.6abc	26.0ab	5.13a-d	9.10b	20.9b	3.48b
Borujerd	80.0a-d	5.28c	28.3bc	2.61cd	4.90cd	66.9ab	22.1abc	23.6bc	5.16abc	8.01bc	17.7bc	3.03bc
Borujen	50.3e	3.77e	17.1d	1.21e	5.05cd	39.5e	12.9g	-	-	-	-	-

In each column means followed by the same letter are not significantly different according to LSD test at  $P=0.05$ .

Clone score (1 for poor growth to 9 for perfect growth), sensitivity to powdery mildew (based on the leaf area covered with mildew using scale of 0–9), growth score (1 for poor growth to 5 for perfect growth).

**Table 2.** Estimates of variance components, genotypic, within-ecotype and phenotypic coefficients of variation (GCV, WECV and PCV, respectively) and broad-sense heritabilities (H) for different traits in sainfoin ecotypes in the greenhouse and field experiments.

Parameter	Greenhouse experiment							Field experiment				
	Viable clones	Clone score	Plant height	Number of stems per plant	Sensitivity to powdery mildew	Fresh matter yield	Dry matter yield	Plant height	Inflorescence length	Number of stems per plant	Dry matter yield	Growth score
$\sigma_E^2$	144.58	0.70	21.03	0.43	3.20	65.09	7.87	1.70	0.001	1.23	8.63	0.15
$\sigma_{G(E)}^2$	786.65	1.61	46.80	0.55	0.87	162.6	17.96	6.82	0.011	2.78	13.93	0.37
GCV	15.50	15.13	17.37	26.71	42.31	13.57	14.21	5.35	0.73	13.67	14.85	11.70
WECV	36.15	22.92	25.91	30.06	22.12	21.45	21.46	10.71	2.04	20.51	18.87	18.46
PCV	39.33	27.46	31.19	40.22	47.74	25.39	25.73	13.89	2.80	27.52	30.59	28.89
H	0.16	0.30	0.31	0.44	0.78	0.29	0.30	0.74	0.60	0.80	0.61	0.57

$\sigma_E^2$ , among-ecotype variance;  $\sigma_{G(E)}^2$ , within-ecotype variance.

Clone score (1 for poor growth to 9 for perfect growth), sensitivity to powdery mildew (based on the leaf area covered with mildew using a scale of 0–9) and growth score (1 for poor growth to 5 for perfect growth).

### Association between traits, factor analysis and principal component analysis

In the greenhouse experiment, percent of viable clones was positively correlated with clone score, plus fresh and dry matter yields; fresh and dry matter yields were positively and significantly associated with clone score, plant height and number of stems per plant (Table 3). In the field experiment, dry matter yield and growth score were positively and significantly associated with plant height, inflorescence length and number of stems per plant in (Table 3).

Factor analysis was performed on all measured traits (Table 4), and revealed that 2 main factors accounted for 77.4% of the total variability. The first factor, named 'forage yield component in the field', included plant height, inflorescence length, number of stems per plant, dry matter yield and growth score in the field experiment, which explained most of the total

variation. The second factor, named 'forage yield potential in the greenhouse', included clone score, plant height, and fresh and dry matter yields in the greenhouse.

Principal component analysis based on all measured traits (Figure 1) demonstrated that the first 2 components accounted for 53.4 and 23.1% of total variance, respectively. The first principal component (PC1) was related to plant height, inflorescence length, number of stems per plant, dry matter yield and growth score in the field experiment. The second principal component (PC2) was positively correlated with clone score and dry matter yield in the greenhouse experiment (data not shown). Therefore, selection of genotypes with high PC1 and PC2 should increase forage yield. Ecotype Baft had the highest value for the first factor and ecotype Fereydunshahr had the highest value for the second factor. In contrast, ecotype Arak had the lowest values for both the first and second factors (Figure 1).

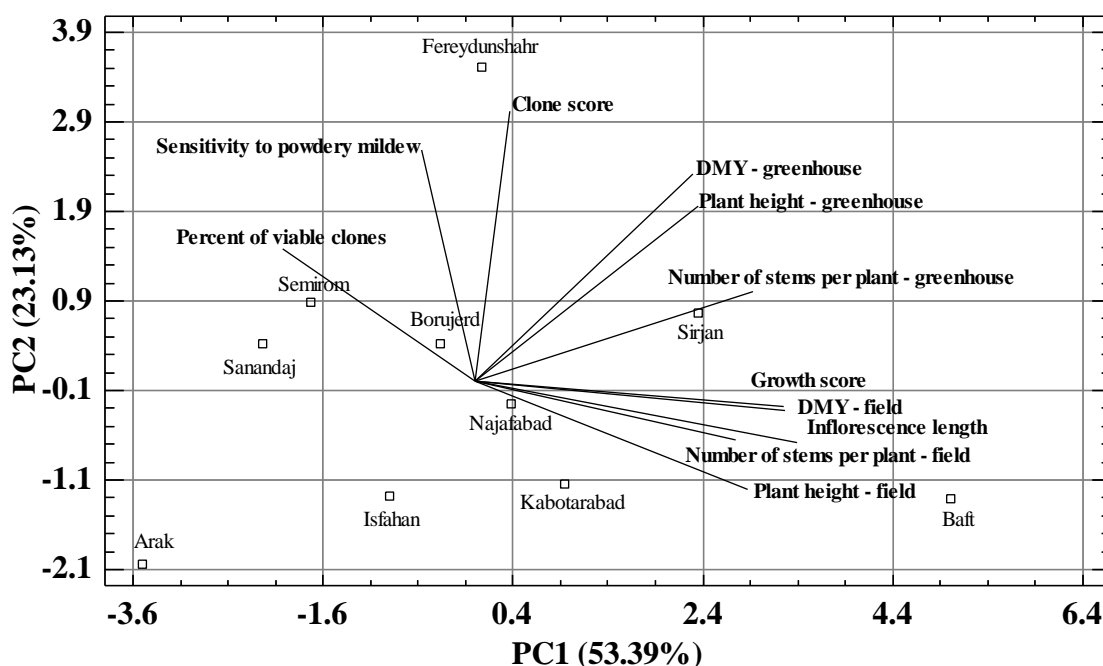
**Table 3.** Correlation coefficients between different traits in sainfoin in the greenhouse and field experiments.

Traits	Greenhouse experiment						Field experiment					
	Viable clones	Clone score	Plant height	Number of stems per plant	Sensitivity to powdery mildew	Fresh matter yield	Dry matter yield	Plant height	Inflorescence length	Number of stems per plant	Dry matter yield	Growth score
Viable clones	1											
Clone score	0.69*	1										
Plant height - greenhouse	0.21	0.67*	1									
Number of stems per plant - greenhouse	0.03	0.51	0.93**	1								
Sensitivity to powdery mildew	0.08	0.24	0.07	-0.08	1							
Fresh matter yield - greenhouse	0.41	0.78**	0.81**	0.77**	0.19	1						
Dry matter yield - greenhouse	0.38	0.74**	0.83**	0.79**	0.21	0.97**	1					
Plant height - field	-0.49	-0.24	0.32	0.48	-0.26	0.21	0.27	1				
Inflorescence length	-0.35	0.06	0.34	0.56	-0.47	0.45	0.41	0.55	1			
Number of stems per plant - field	-0.56	-0.07	0.52	0.73*	-0.34	0.46	0.52	0.86**	0.81**	1		
Dry matter yield - field	-0.48	0.05	0.43	0.61	-0.11	0.49	0.56	0.88**	0.69*	0.92**	1	
Growth score	-0.47	0.08	0.42	0.60	-0.10	0.52	0.55	0.86**	0.71*	0.90**	0.98**	1



**Table 4.** Factor loadings (rotated using the varimax method) and eigen-values of factors for sainfoin ecotypes in the greenhouse and field experiments.

Traits		Factor 1 (Forage yield components in the field)	Factor 2 (Forage yield potential in the greenhouse)
Greenhouse experiment	Viable clones	-0.69	0.18
	Clone score	-0.22	0.83
	Plant height	0.41	0.73
	Number of stems per plant	0.67	0.55
	Sensitivity to powdery mildew	-0.42	0.63
	Fresh matter yield	0.31	0.89
	Dry matter yield	0.36	0.88
Field experiment	Plant height	0.88	-0.03
	Inflorescence length	0.79	0.14
	Number of stems per plant	0.97	0.17
	Dry matter yield	0.89	0.25
	Growth score	0.89	0.26
Eigen-value		6.31	2.97
Explaining proportion (%)		52.6	24.8
Cumulative (%)		52.6	77.4

**Figure 1.** Distribution of the first 2 principal components of different traits (greenhouse and field experiments) in 10 ecotypes of sainfoin.

DMY = Dry matter yield.

## Discussion

This study has demonstrated significant variation among ecotypes for all measured traits, indicating the potential for improvement of productivity by selection within this germplasm. Cloning ability of genotypes is a prerequisite for clonal selection (Annicchiarico et al. 2010) and we

demonstrated high genetic variation among ecotypes for producing viable clones, with Najafabad ecotype displaying the highest (97%) and Borujen ecotype the lowest percentage (50%) of viable clones. These findings are in agreement with the report of Haapala et al. (2004), who demonstrated large variation for producing vigorous cuttings in aspen (*Populus tremula* × *P. tremuloides*).

Similarly, Lamhamedi et al. (2000) compared zygotic and somatic seedlings of white spruce (*Picea glauca*) and reported that clones within each family differed significantly in terms of plant height, root-collar diameter and shoot and root dry mass, when compared with zygotic seedlings.

High variability, heritability and positive association among traits provide potential for improving genotypes in plant breeding programs (Akbar et al. 2003). In the greenhouse experiment, genotypic coefficients of variability ranged from 13 (fresh matter yield) to 42% (sensitivity to powdery mildew). Since only small differences between GCV and PCV were observed for sensitivity to powdery mildew, it appears that the observed variations for the trait were mostly due to genetic factors, with the environment playing only a modest role in the expression of this trait. Number of stems per plant and sensitivity to powdery mildew had high GCV indicating that further selection may improve the ecotypes in terms of these traits.

The higher within-ecotype variance than among-ecotype variance for all the measured traits in the field experiment was in agreement with reports of other researchers (Annicchiarico and Piano 1995; Bolaños-Aguilar et al. 2000; Julier et al. 2000) that within-population variation was larger than among-population variation for morphological traits in forage plants. We demonstrated that the within-ecotype contribution (the percent of  $\sigma_{G(E)}^2$  to  $\sigma_E^2 + \sigma_{G(E)}^2$ ) was 80% for plant height, 88% for inflorescence length, 69% for number of stems per plant, 61% for DMY and 71% for growth score. Julier et al. (2000) reported that within-cultivar variance was 31–70% of the total genetic variance for leaf:stem ratio and quality traits and 57–100% for morphological traits and dry matter yield.

Broad-sense heritability estimates were moderate to low for most studied traits. The low estimates of heritability for dry matter yield were in agreement with those previously reported for alfalfa (Julier et al. 2000), tall fescue (*Festuca arundinacea*) (Amini et al. 2013) and smooth bromegrass (*Bromus inermis*) (Araghi et al. 2014). The high heritabilities displayed for sensitivity to powdery mildew, plant height and number of stems per plant indicate that selection would be effective in improving these traits.

Associations between agronomic traits are of interest to determine whether selection for one trait will affect another (Nair et al. 2004). In the greenhouse experiment, the positive correlations between dry matter yield and ability to produce viable clones, clone score, plant height and number of stems per plant indicate that any increase in one of these latter traits may cause increase in forage yield. The positive significant associations of dry matter

yield and growth score with plant height, inflorescence length and number of stems per plant in the field experiment confirmed the findings of Turk and Celik (2006) that there were significant and positive correlations between dry matter yield, number of stems per plant and plant height in sainfoin. Similarly, Mohajer et al. (2011) found that dry matter yield was positively correlated with seed yield, plant height, number of stems and inflorescence length in sainfoin.

The finding that factor analysis showed 2 factors explaining 77.4% of the total variability augers well for further selection studies. The first factor, 'forage yield components in the field', highlighted forage yield and its components, demonstrating that selection of ecotypes based on this factor should increase forage yield. Including all parameters in a selection program could give better results than selection based only on forage yield. Ebrahimiyan et al. (2012) reported that factor analysis in tall fescue revealed 4 factors which explained 77% of the total variability, their first factor, 'biological yield', emphasizing forage yield and its components. In addition, Ozel et al. (2010) reported that the first 5 factors in cottonwood (*Populus deltoides*) explained 71% of the total variability, with the first and second factors being 'the growth of clones' and 'traits related to morphology'.

In this study, principal component analysis showed that selection of genotypes with high PC1 (related to plant height, inflorescence length, number of stems per plant, dry matter yield and growth score in the field) and PC2 (related to clone score and dry matter yield in the greenhouse) would increase forage yield. Ecotype Baft had the highest value for PC1 and ecotype Fereyduhshahr had the highest value for PC2 and could be introduced for further breeding programs. In contrast, ecotype Arak had the lowest value for both PC1 and PC2 (Figure 1). In agreement with our findings, Prosperi et al. (2006) reported that the first principal component had strong correlation with yield components in alfalfa.

## Conclusion

Sainfoin segregates when plants are multiplied by producing seed during breeding programs. Alternatively, clonal propagation through cuttings allows easy, rapid and reliable multiplication of selected material for genetic analysis (Avcı et al. 2010). Our results showed that clonal propagation of sainfoin provides the opportunity to detect the environmental effects and genotype x environment interactions, through precise estimation of genetic parameters. Although a high genetic variation between and within ecotypes of sainfoin for producing viable clones was evident, clonal materials and identical plant genotypes were

successfully used to estimate the variances and heritability of traits under different environmental conditions. The positive association between percent of viable clones and dry matter yield in the greenhouse suggests there is potential for selection during early stages of clonal propagation. In addition, according to correlation and factor analyses, plant height, inflorescence length and number of stems per plant in the greenhouse and field were identified as the main components influencing forage yield; thus selection for these parameters could indirectly improve the forage yield of sainfoin. Principal component analysis suggests that Baft and Fereydunshahr ecotypes, with the highest values for the first and second factors, could be useful for including in future breeding programs. These issues warrant further study.

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