# EVALUATION OF HERBAGE YIELD, AGRONOMIC TRAITS AND POWDERY MILDEW DISEASE IN 35 POPULATIONS OF SAINFOIN (ONOBRYCHIS SATIVA) ACROSS 5 ENVIRONMENTS OF IRAN

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## ABSTRACT

The present study was carried out to evaluate herbage yield, agronomic traits and powdery mildew disease and their relationships in 35 populations of Sainfoin (Onobrychis sativa L.) across five environments of Iran (Tabriz, Sanadaj, Khoramabad, Zanjan and Samirom)) over 2 years (2010-2011). The experimental layout was a randomised complete block design with three replications. Data were collected for total annual dry matter (DM) vield, flowering date, plant height, stem number, leaf to stem weight ratio (LSR), growth condition, disease infection (DI) and disease severity index (DS). Combined analysis of variance across five environments showed significant differences among locations for all traits except growth condition (P<0.01), and among populations for all of traits except LSR and populations x location for all of traits (P<0.01). In comparisons among populations, Polycross, Esfahan, Faridan, Khomin2, Faridonshar1 and Miandoab with average values of 5.00 to 5.75 ton ha<sup>-1</sup> had higher herbage production over all environments. Among them, the late flowering Polycross had lower DI and DS with average values of 51% and 2.09, respectively and it was introduced as a local variety for cultivation. DM yield was positively correlated with both stem number and growth condition and negatively correlated with LS and DS. Similarly, DS was positively correlated with LSR and DI and negatively correlated with stem number, DM yield and flowering date. The results of PCA analysis showed that the first three components accounted for 34, 22 and 14% of the total variation, respectively. DM yield, stem number, DS and DI in the PCA1, plant height and LS in the PCA2, growth condition and flowering date in the PCA3 components were identified as important traits. Using Ward cluster method the 35 populations were grouped into 4 clusters. The 11 populations in Cluster 1 had late maturity, lower productivity, higher LSR and were recognized as semi-susceptible to sainfoin powdery mildew. Cluster 2 with 19 populations had higher productivity with early flowering and was in the semi-susceptible class. Cluster 3 included populations of lower productivity with early flowering and belonging to susceptible class. Finally Cluster 4 included populations with higher productivity, early flowering and dormant class powdery mildew disease.

Key words: sainfoin (Onobrychis sativa), populations, forage yield, agronomic traits, powdery mildew.

## **INTRODUCTION**

**S** ainfoin (Onobrychis spp.) is a crosspollinated perennial legume used for hay and forage in Iran. So far, 162 species have been described around the world in the genus *Onobrychis*. This genus extends from the Mediterranean region to Caucasia, the Zagros Mountains of Iran and Asia. The genus is concentrated in Iran (60 species) (Rechinger, 1984) and Turkey (52 species), (Emre et al., 2007 and Çelik et al., 2011). Iran and Turkey appear to be the main centres of genetic diversity. It is often grown in conjunction with forage grasses to reduce bloat hazard, as well as to improve soil fertility due to its nitrogen fixing ability (Lu et al., 2000). It contains condensed tannins which reduce its potential to produce bloat and improve protein digestion by grazing animals (Rumball and Claydon, 2005). Forage dry matter (DM) yield is the main goal in sainfoin breeding programs. High levels of variability among sainfoin populations had been reported for herbage DM yield. In Iran, Nakhjavan et al. (2011) in evaluation of 34 populations of sainfoin under irrigation condition obtained annual total DM yield of 10.79 tons ha<sup>-1</sup> in three cuts; similarly, Hosaininejadmir et al. (2011) in assessment of the same material for

Received 07 May 2013; accepted 03 February 2014. First online: 15 May 2014. DII 2067-5720 RAR 2014-383

both seed and herbage production, found DM yield of 3.2 tons ha<sup>-1</sup> in the first cut under swards condition. Mohajer et al. (2011) obtained total DM yield (sum of three cuts) of 7.3 and 6.2 tons  $ha^{-1}$  under spaced plant and sward conditions, respectively. Mohajer et al. (2012) in another experiment evaluated 72 genotypes sainfoin and obtained total annual DM yield of 13.5 tons ha<sup>-1</sup>. In Turkey, Tosun (1988) obtained (DM) yield as much as 3.96 tons ha<sup>-1</sup>. DM yield is a complex trait which is dependent on yield components and is highly influenced by many genetic, as well as environmental factors. Therefore, evaluating genotypic potential in different environments is the important step in breeding programs of sainfoin before selecting desirable ones to commercial cultivation. However the extension of sainfoin, in many parts of Iran is limited by powdery mildew and decreased forage yield during second and third harvest (Majidi, 2010). The plant parts severely affected by this disease and were dried and fell off. The disease caused by Leveillula taurica started in August to September in Iran (Sharifnabi and Banihashemi, 1990 and Ershad, 1995). Although sainfoin is one of the important legume forage cultivated as high quality hay in Iran, the information on the effect of powdery

mildew disease on production and recognition of dormant populations is limited. Therefore, this study was conducted 1) to study variability among 35 populations for forage yield, agronomic traits and powdery mildew disease, and 2) determine pattern of variation for forage yield and powdery mildew disease to identify groups of populations accessions through a multivariate approach.

## MATERIAL AND METHODS

## Sites

The study was conducted in 5 locations (Kurdistan, Samirom, Tabriz, Zanjan and Lorestan) in Iran. Some meteorological characteristics of the tested locations are presented in Table 1. The climate of Kurdistan and Lorestan, using Emberger method, is moderate semiarid (Xerophilous forest zone) with annual precipitation above 400 mm. The climate of Samirom (Esfahan), Tabriz, and Zanjan are cold substeppic zone with annual precipitation between 230-450 mm (Badripou et al., 2006). The Embrothemic diagram shows that drought period of station is for four months of year and wet season starts in October and it continues until May.

Locations	Longitude	Latitude	Altitude	Avera	ge tempe (°C)	Annual	
Locations	(E)	(N)	(m)	Mean	Min.	Max.	rainfall (mm)
Sanandaj (Kurdistan)	46.99	35.32	1373	16.0	5.4	21.4	462.4
Khoramabad (Lorstan)	48.22	33.29	1125	17.2	9.2	24.8	525.0
Tabriz (East Azar)	46.17	38.05	1361	14.0	6.9	18.6	310.0
Smirom (Esfahan)	51.17	30.43	2400	12.3	3.5	17.7	398.1
Zanjan (Zanjan)	48.29	36.41	1663	11.0	3.9	18.5	283.0

Table 1. Some meteorological characteristics of the research locations

## **Experimental design and management**

Seeds of 35 sainfoin populations were provided from natural resources gene bank (Research Institute of Forests and Rangelands, Iran). Seeds were sown in four drilled lines as long as 2 m with 25 cm distance in sward condition, using randomised complete block design with three replications in spring 2008. Irrigation was made according to the plant requirement. Weeds were control mechanically and fertilizing schedule was made based on scientific advices and recommendations.

# Sampling for yield and agronomic traits

In the establishment year, plots were cut once, but no data were measured. In 2009 and 2010 plots were cut three times for DM yield and agronomic traits and powdery mildew disease.

The following traits were measured:

*1. Flowering date:* The number of days from 21 March to the stage at which 50 percent of plants produced flower.

2. *Growth condition:* was measured visually as the percentage of ground cover and longevity of plants in each plot after each cut.

3. *Plant height*: Ten plants of each plot were selected and their heights (in cm) were measured and then their averages were taken.

4. Stem number: The average plant number of one  $m^2$  from each plot was noted down based on counting.

5. Dry matter (DM) yield: At the end of flowering time each plot was harvested, plant material was air-dried and dry weight was expressed in tons ha<sup>-1</sup>. Thus, this represents the above-ground biological yield.

6. Leaf to stem weight ratio (LSR): Estimate of leaf stem ratio was based on separation and weighing of leaf and stem in the sub samples.

7. Disease severity index (DS): Twenty leaves of each plot were selected, the powdery mildew disease were evaluated in cuts 2 and 3 based on method of Horsfall and Cowling (1978), in which, 1=resistance, 2=dormant, 3=semi-susceptible and 4=susceptible.

8. Disease infection (%) (DI): Twenty leaves of each plot were selected, and the powdery mildew disease were evaluated in cuts 2 and 3 based on method of Horsfall and Cowling (1978), in which, 1-10%=resistance, 11-25%=dormant, 36-55%=semi-dormant, 56-75%=semi-susceptible, 76-100% = susceptible.

# Statistical analyses

The total annual DM yields, summed over three cuts over two years per plot, and the values of other traits were averaged over years and consequently were used for combined analysis over 5 locations. Data was analysed using combined analysis of variance over 5 locations. Phenotypic correlations among characteristics were estimated for all pair-wise combinations. All variables were used in principal component and cluster analysis. The variables were standardized for cluster analysis (Ward method). The SAS9 software (SAS Institute Inc.) was used for ANOVA and Minitab 16 for multivariate analysis.

# **RESULTS AND DISCUSSION**

# Analysis of variance and means comparisons

Combined Analysis of variance across five environments showed significant differences among locations for all of traits, except growth condition (P<0.01), among populations for all of traits except LS, and populations x location for all of traits (P<0.01) (Table 2).

 Table 2. ANOVA of traits for 35 populations of Sainfoin evaluated across 5 environments of Iran averaged over 2 years (2010-2011)

 Plant
 DM

SOV	DF	Stem No./plant	Plant height (cm)	Leaf/Stem ratio	DM yield (t/ha)	Growth condition	Flowering date (day)	Disease severity index	Disease infection
Location	4	167828**	31915**	12015**	37.1**	0.53	3342**	38.1**	34258**
Error1	10	57861	180.8	128.5	9.35	3.01	21.52	0.70	92.41
Population	34	10173*	86.0**	33.24	4.06**	0.78**	46.58**	1.42**	625.7**
POP X LOC	136	9146*	72.0**	46.22*	2.71**	0.92**	28.75**	0.53**	325.9**
Error1	341	7237	44.8	34.53	1.32	0.47	7.46	0.32	111.37
CV%		25.38	9.86	12.32	16.26	19.23	4.66	16.29	14.62

In comparisons between populations, Polycross, Esfahan, Faridan, Khomin2, Faridonshar1 and Miandoab with average values of 5.00 to 5.75 tons ha<sup>-1</sup> had higher herbage production over all of environment. Among them, the late flowering Polycross, had lower disease infection (DI) and severity (DS), with average values of 51.02% and 2.09, respectively and it was introduced as a local variety for cultivation. The overall means of locations for DM yield were 6.47, 7.19, 7.89, 7.03 and 6.76 tons ha<sup>-1</sup> for Samirom, Kordestan, Lorestan, Tabriz and Zanjan. The average DM ratios for cuts 1, 2 and 3, were 36, 34 and 30%, respectively (data not shown). The overall mean of DM yield in 35 genotypes in current experiment  $(4.67 \text{ tons ha}^{-1})$  (Table 3) was similar to Liu et

al (2008) who obtained 4.53 tons ha<sup>-1</sup> annual yield of sainfoin monoculture in UK. But, it was lower than that reported by Mohajer et al. (2011) and Nakhjavan et al. (2011) with average values 6.2 and 10.79 tons ha<sup>-1</sup>, respectively, and it was higher than that reported by Tosun (1988) in Turkey who obtained (DM) yield as high as 3.96 tons ha<sup>-1</sup>.

For flowering date, populations of Damavand and Marand with 55 days up to 50% flowering was known as earliest and Polycross with 64 days up to 50% flowering was known as latest maturity ones. For plant height, Khoramabad and Oshnevia with average values of 56.36 and 62.94 cm and for stem number, Ardebil and Sarab with average values of 248 and 326 and for LSR, Polycross and Songor with average values of 57.82 and 47.17%, for growth condition, Azarshar and Silvana with average values of 2.14 and 3.13 score, for DS, Oshnevia and Marand with average values of 1.99 and 3.74 score, for DI, Oshnevia and Silvana with average values of 29.21 and 87.37% had lowest and highest vales, respectively (Table 3).

*Table 3.* Mean of traits in 35 populations of Sainfoin evaluated across 5 environments of Iran over 2 years (2010-2011)

Cluster	Name	Stem (No m <sup>-2</sup> )	Plant height	Leaf/ Stem	DM yield	Growth	Flowering date (day)	Disease severity	Disease infection
			(cm)	ratio	$(\text{tons ha}^{-1})$		( )/	index	
1	Aligodarz3	296.8	57.80	56.48	4.52	2.81	60.40	3.02	74.97
1	Ardebil	248.0	61.02	53.31	4.18	2.80	61.58	3.35	74.13
1	Asadabad	275.0	57.36	55.51	4.36	2.78	60.84	3.06	71.91
1	Azna2	296.2	59.42	55.55	4.51	2.66	59.98	3.17	82.44
1	Bonab	288.5	56.36	56.17	4.75	2.98	60.23	2.94	71.76
1	Divandara	295.7	62.94	54.14	4.79	2.75	59.51	3.12	72.61
1	Faridonshar2	266.5	59.98	54.69	4.38	2.82	59.40	3.04	78.93
1	Khalkal	274.0	59.77	53.02	4.20	2.81	61.51	3.16	82.76
1	Sanadaj	282.2	60.91	56.04	4.70	2.88	60.08	3.20	79.50
1	Songor	289.7	57.27	57.82	4.50	2.90	62.44	3.34	84.06
1	Varzaghan	255.5	59.50	55.05	4.69	2.71	61.94	3.26	76.26
2	Ahar	280.8	58.70	54.30	4.44	3.06	57.18	3.21	67.68
2	Aligodarz1	306.0	57.73	54.14	4.43	3.01	57.88	2.94	75.18
2	Aligodarz2	313.9	60.20	51.76	4.97	3.04	59.82	3.28	84.04
2	Arak	278.0	60.01	52.31	4.45	3.02	58.65	3.16	65.91
2	Aznal	269.5	57.83	51.91	4.54	3.08	57.29	3.02	65.61
2	Damavand	259.7	62.24	50.55	4.81	2.91	55.95	3.68	70.81
2	Faridan	282.7	57.57	51.80	5.10	3.01	59.38	3.26	71.58
2	Faridonshar1	281.2	59.27	52.26	5.03	2.85	60.40	2.99	69.35
2	Heris	309.8	60.04	52.34	4.57	2.76	63.24	2.93	69.02
2	Esfahan	288.7	60.38	52.24	5.15	2.93	58.42	3.27	60.87
2	Kermanshah	294.3	58.94	52.34	4.77	2.52	58.61	3.12	76.32
2	Khomin1	296.4	59.83	52.38	4.65	3.01	59.61	3.22	64.00
2	Khomin2	303.2	56.74	52.45	5.04	3.10	59.39	3.20	77.47
2	Khonsar1	282.7	59.66	51.73	4.69	3.03	57.40	3.21	71.84
2	Khonsar2	294.2	58.09	54.21	4.82	2.78	59.28	3.23	69.03
2	Khoramabad	306.6	56.36	52.37	4.56	3.02	56.91	2.92	56.13
2	Miandoab	326.4	61.04	52.16	5.00	3.08	58.47	3.05	80.62
2	Ormia	279.8	60.02	52.17	4.98	2.77	58.36	3.25	78.80
2	Sarab	326.8	61.04	52.62	4.83	2.96	62.37	3.08	69.89
2	Silvana	288.9	62.23	52.26	4.72	3.13	57.06	2.67	87.37
3	Marand	253.9	59.52	53.52	3.94	2.42	55.73	3.74	78.78
3	Azarshar	281.4	60.93	49.86	3.79	2.12	58.97	3.38	74.55
4	Polycross	300.2	59.28	47.17	5.75	2.91	64.05	2.09	51.02
4	Oshnevia	307.2	62.94	47.58	4.77	2.79	57.79	1.99	29.21
т	Means	288.0	59.51	52.98	4.67	2.86	59.43	3.11	71.84
	LSD	51.75	4.09	4.47	0.67	0.57	2.27	0.43	12.04
	LOD	51.75	7.07	7.4/	0.07	0.37	2.21	0.45	12.04

#### ALI ASHRAF JAFARI ET AL.: EVALUATION OF HERBAGE YIELD, AGRONOMIC TRAITS AND POWDERY MILDEW DISEASE IN 35 POPULATIONS OF SAINFOIN (*ONOBRYCHIS SATIVA*) ACROSS 5 ENVIRONMENTS OF IRAN

Leveillula taurica was identified as causal fungus of sainfoin powdery mildew (Sharifnabi and Banihashemi, 1990; Ershad, 1995). In the present study, the disease was not observed in the first cut and it was low in the second cutting, but in the third cutting, powder mildew disease was more. All populations showed different ranges of the disease symptomss except late flowering Polycross and early flowering Oshnevia, which were recognized as tolerant. Naseri and Marefat (2008) found that the cool nights of September, caused disease outbreaks in semiarid area of Zanjan, Iran

# Relationships between yield and agronomic traits

The results of correlation among traits are shown in Table 4. DM yield was positively correlated with both stem number and growth condition (P<0.01) and negatively correlated with LSR (P<0.05) and DS (P<0.01). This demonstrated that yield

increases as much as plant height and number of stem increase. These results were in agreement with Turk and Celik (2006) and Mohajer et al. (2011). Similar to the present study, Mohajer et al. (2011) also found negative correlation coefficients between forage yield and resistance to powdery mildew. Since the lower values of DS and DI mean more resistance, in other words the productive genotypes were more resistant to powdery mildew disease. There was a negative and significant relationship between plant height and LSR (P<0.01) indicating taller stems had lower leaf stem ratio. LSR was positively correlated with both DS and DI (P<0.01) indicating leafy plants were more susceptible to powdery mildew disease. Similarly, DS was negatively correlated with stem number, DM yield (P<0.01), and flowering date (P<0.05), which demonstrated that taller, high density and late flowering genotypes were more resistant to powdery mildew disease (Table 4).

*Table 4.* Correlation analysis between herbage yields, agro and yield components in 35 populations of *Onobrychis sativa* evaluated across 5 environments of Iran over 2 years (2010-2011)

Traits	Stem (No./ plant)	Plant height (cm)	Leaf / Stem ratio	DM yield (ton/ha)	Growth condition	Flowering date (day)	Disease Severity index
Plant height (cm)	-0.02						
Leaf/Stem ratio	-0.18	-0.41*					
DM yield (ton/ha)	0.45**	0.01	-0.36*				
Growth condition	0.35*	-0.19	0.01	0.49**			
Flowering date (day)	0.17	-0.10	0.17	0.21	-0.08		
Disease severity index	-0.46**	-0.01	0.38*	-0.46**	-0.27	-0.35*	
Disease infection	-0.18	-0.09	0.58**	-0.28	-0.07	0.06	0.53**

\*significant at the 0.05 probability level; \*\* significant at the 0.01 probability level.

# **Classification of populations**

Principal component analysis is often used prior to cluster analysis to determine the relative importance of variables classification (Jackson, 1991). Eigenvalues from the first, second and third principal component axes, respectively, accounted for 34, 22 and 14 of total variance percent (Table 5). The relative magnitude of eigenvectors from the PCA1, indicated that DM yield, stem number, DS and DI were important traits for classifying populations into clusters. From the PCA2 plant height and LSR were important classification variables. Eigenvectors from the PCA3 suggest that growth condition and flowering date may be found to improve DM yield (Table 5). Table 5. Eigenvectors from the first three principal component axes for 8 variables used to classify
35 sainfoin populations across 5 environments of Iran over 2 years (2010-2011)

Variable	PC1	PC2	PC3
Stem (No./plant)	0.41	-0.25	0.03
DM yield (ton/ha)	0.46	-0.19	-0.12
Disease severity index	-0.49	0.07	-0.23
Disease Infection (%)	-0.41	-0.31	-0.01
Plant height (cm)	0.08	0.53	0.13
Leaf to stem weight			
ratio	-0.38	-0.50	0.04
Growth condition	0.27	-0.38	-0.58
Flowering date (day)	0.11	-0.36	0.76
Eigenvalue	2.73	1.64	1.12
Proportion	0.34	0.22	0.14
Cumulative	0.34	0.56	0.70

Based on Ward clustering method, 35 entries were subjected to cluster analysis for 8 variables. An arbitrary distance coefficient of 9.5 was chosen to separate the accessions into 4 cluster groups in a dendrogram (Figures 1 and 2).

Populations in Cluster1 (n=11) had lower productivity, higher LSR values and were

semi-susceptible to powdery mildew disease. Populations in Cluster 2 (n=19) averaged well above the overall mean for both DM yield and growth condition and lower LSR, were early flowering and semi-susceptible to powdery mildew disease. Cluster 3 had lower productivity with early flowering and was more susceptible to powdery mildew disease. Finally Cluster 4 had higher productivity with lower LSR values and was dormant to powdery mildew disease (Table 6).

This result suggested that two populations, Polycross and Oshnevia in cluster 4 had good capability to improving new varieties without decreasing DM yield.

Populations from a defined geographic area tended to cluster, but some accessions from a particular area were spread across several cluster groups (Tables 3, 6 and Figures 1 and 2).

Figure 1 shows graphically how populations are classified into four clusters according to the first two principal components.

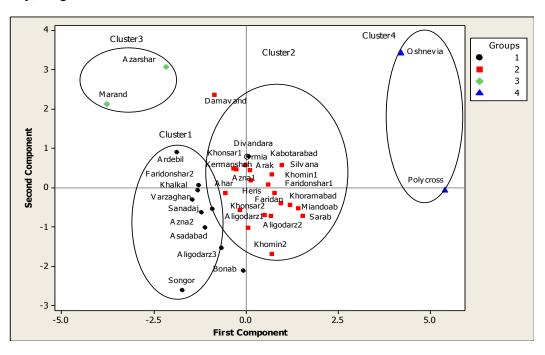
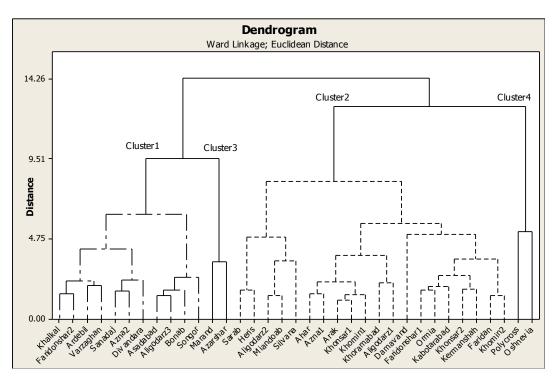


Figure 1. Scatter plot of 35 populations for the first two principal components

The first component separated clusters 3, 1, 2 and 4 from low to high values for DM yield and stems number, and reverse for DS and DI (Figure 1). The second component did not separate Clusters but, it well separated population for LSR, with higher stems and higher leaves weights in above and lower half of biplot, respectively (Figure 1). This result indicated that distribution of accessions based on the first two component scores are in agreement with cluster analysis.

#### ALI ASHRAF JAFARI ET AL.: EVALUATION OF HERBAGE YIELD, AGRONOMIC TRAITS AND POWDERY MILDEW DISEASE IN 35 POPULATIONS OF SAINFOIN (*ONOBRYCHIS SATIVA*) ACROSS 5 ENVIRONMENTS OF IRAN



*Figure 2.* Dendrogram of 35 accessions by analyzing yield, morphological and powdery mildew disease using Ward cluster analysis method

Clusters	Stem (No./plant)	Plant Height (cm)	Leaf/ stem ratio	DM yield (ton/ha)	Growth condition	Flowerin g Date (day)	Disease Severity index	Disease Infection (%)
Cluster1 (n=11)	278.9 a	59.30 a	55.25 a	4.51 b	2.81 b	60.72 a	3.15 b	77.21 a
Cluster2 (n=19)	293.5 a	59.40 a	52.42 b	4.78 a	2.95 a	58.78 b	3.14 b	71.58 a
Cluster3 (n=2)	267.6 a	60.22 a	51.69 b	3.87 c	2.28 c	57.35 b	3.56 a	76.67 a
Cluster4 (n=2)	303.7 a	61.11 a	47.37 c	5.26 a	2.85 ab	60.92 a	2.24 c	40.11 b

Table 6. Means of traits used in classification of the 4 clusters

The means of the clusters with same letters were not significantly different based on DMRT P<0.05 method

## CONCLUSIONS

Herbage yield of sainfoin varied under on-farm and irrigation conditions in cold and moderate semiarid areas in Iran and they could be used to select sainfoin accessions for herbage production and powdery mildew disease. The overall means of top populations as Polycross, Esfahan, Faridan, Khomin2, Faridonshar1 and Miandoab were 5.00 to 5.75 tons ha<sup>-1</sup> over all environments. The late flowering Polycross, with average values of 5.75 tons ha<sup>-1</sup>) and early flowering Oshnevia with moderate production (4.77 tons ha<sup>-1</sup>) were highly resistant to powdery mildew disease infection and they were introduced for future breeding programs as a tolerance source of powdery mildew in sainfoin. Thus by crossing these two genotypes with other productive genotypes and by selection with progeny test, improved sainfoin varieties for high forage yield couple with resistance to powdery mildew disease could be bred. The results indicated that leafy plants were more susceptible to powdery mildew disease. Results suggested that selection should focus on increased DM yield, late flowering, high density and taller stem.

#### Acknowledgement

The authors thank Iranian research institute forests and rangelands (RIFR) for providing genetic resources samples and financial support.

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