doi: 10.17138/TGFT(9)97-108

Research Paper

Evaluation of sainfoin accessions exposed to powdery mildew disease at four locations in Iran

Evaluación de accesiones de esparceta expuestas al oídio (mildiú polvoroso) en cuatro localidades de Irán

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Abstract

In order to evaluate resistance of sainfoin (*Onobrychis viciifolia*) to powdery mildew, seeds of 19 accessions were collected from different parts of Iran and sown at 4 locations, i.e. Kheirabad, Khoramabad, Semirom and Tabriz, in 2014. Accessions were evaluated for powdery mildew severity index (DSI), forage dry matter yield (DM), dry matter digestibility (DMD) and crude protein (CP) and water soluble carbohydrate (WSC) concentrations over 4 years. Based on Duncan's test, accessions 15353 and 3001 showed disease severity index lower than 25% and were nominated as resistant to powdery mildew. Accessions Oshnavieh and Polycross were considered semi-resistant due to their DSI ranging from 25 to 50%. Other accessions were considered susceptible because their DSI was higher than 50%. The resistant accessions (15353 and 3001) with average yields of 3,341 and 3,304 kg DM/ha were ranked as having high DM production, in addition to displaying high DMD plus high CP and WSC concentrations. Severity of powdery mildew infection was linked negatively with all 3 quality traits, i.e. DMD and CP and WSC concentrations. According to Eberhart/Russell regression results, stability of accessions 3001 and 15353 for DSI and DM yield was confirmed across 4 locations. We recommend the use of accessions 3001 and 15353 in future breeding programs to increase resistance to powdery mildew, while at least maintaining yield and quality attributes. Evaluation of other sources of sainfoin germplasm should continue to identify further resistant accessions.

Keywords: Forage; nutritive value, *Onobrychis*; productivity, susceptibility, tropical legumes.

Resumen

En un experimento multilocacional conducido durante 4 años en 4 localidades de Irán (Kheirabad, Khoramabad, Semirom y Tabriz), se evaluaron 19 accesiones de esparceta (*Onobrychis viciifolia*), una leguminosa forrajera, recolectadas en diferentes regiones de Irán. Las evaluaciones incluyeron: susceptibilidad al oídio (*Leveillula taurica/*

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Oidiopsis sp.); rendimiento de materia seca (MS); digestibilidad de la MS; y concentraciones de proteína cruda (PC) y carbohidratos solubles. Las accesiones 15353 y 3001 presentaron un índice de severidad (IS) de la enfermedad inferior a 25% y fueron consideradas resistentes al oídio. Las accesiones Oshnavieh y Polycross presentaron resistencia media (IS entre 25 y 50%). Las demás accesiones presentaron un IS >50% y se calificaron como susceptibles. Las accesiones resistentes (15353 y 3001) presentaron altos rendimientos promedio de MS (3,041 y 3,304 kg MS/ha, respectivamente) además de alta digestibilidad y altas concentraciones de PC y carbohidratos solubles. La severidad de la infección por el oídio presentó relaciones negativas con la digestibilidad y las concentraciones de PC y carbohidratos solubles. El análisis de estabilidad de Eberhart/Russell confirmó la estabilidad de las accesiones 3001 y 15353 para la resistencia al IS y el rendimiento de MS a través de las localidades. Por tanto, se sugiere usar estas accesiones en futuros programas de mejoramiento de esparceta. La evaluación de otras fuentes de germoplasma de esta especie debe continuar para identificar adicionales accesiones resistentes.

Palabras clave: Forrajes, leguminosas subtropicales, *Onobrychis*, productividad, susceptibilidad, valor nutritivo.

Introduction

Common sainfoin, *Onobrychis viciifolia* Scop. (syn. *O. sativa* Lam.), is a most important perennial forage legume, which is highly regarded by farmers due to high levels of palatability and nutrient concentrations (Delgado et al. 2008). Sainfoin was introduced to agriculture as a drought- and salinity-tolerant plant, can produce yields comparable with those of alfalfa and due to its deep roots is well adapted to dry and desert ecosystems (Soares et al. 2000). It contains condensed tannins which lower bloat incidence in grazing animals and improve protein digestion in the intestines (Rumball and Claydon 2005). Sainfoin, also known as holy clover, is often intercropped with forage grasses to improve soil fertility and pasture quality via nitrogen (N) fixation (Lu et al. 2000). The ability of this forage crop to fix N can reduce applications of chemical fertilizers (Greub et al. 1984). The honey obtained from nectar of sainfoin flowers is very bright and sweet, with a distinct flavor, and displays concentrated crystallization at any temperature with yields in the range of 20–51 kg/ha (Pérez-Arquillue et al. 1995). Sainfoin is also tolerant of *Hypera postica* (Gyllenhal) (Curculionidae), a common pest of alfalfa crops (Allen and Allen 1981).

The *Onobrychis* genus comprises 69 species (both annual and perennial) in Iran (Mabberley 1997). The species are concentrated in the Zagros mountains of Iran (60 species) and Turkey (52 species) (Çelik et al. 2011), and these areas appear to be the major origins of genetic diversity in sainfoin populations (Mohajer et al. 2013). The plant is commonly grown in both irrigated and dry lands of Iran, including Charmohal Bakhtiary, Lorestan, Fars, Kerman, Kordestan, Kermanshah, Zanjan and Mazandaran provinces (Hidarian and Mollaei 2001) and is capable of acceptable growth in cropping systems, which are inappropriate for clover and alfalfa cultivation.

However, more widespread cultivation of sainfoin in the main growing regions of Iran is limited by the incidence of powdery mildew, which reduces forage yield during the second harvest (Majidi 2010). Severe infections of powdery mildew occur in Azarbayjan, Charmohal Bakhtiary, Esfahan, Fars, Kerman, Kermanshah, Kordestan, Lorestan, Mazandaran and Zanjan provinces (Bamdadian 1991; Behdad 1996). Naseri and Alizadeh (2017) reported a number of climatic indicators of development of powdery mildew infections in Zanjan province. This disease can appear at the end of the growing season and causes noticeable yield losses at the last cutting. The causal agent of this disease is a fungus known as *Leveillula taurica*, with *Oidiopsis* sp. as the asexual form. This pathogen also infects other plant species such as alfalfa, sunflower, safflower and hemp. The aerial plant parts severely infected by this pathogen develop spots, become dehydrated and are shed. Depending on the region, the disease appears in August–September in Iran (Sharifnabi and Banihashemi 1990; Ershad 1995; Naseri and Marefat 2008). Alizadeh and Jafari (2014) evaluated the susceptibility of 56 accessions of sainfoin to powdery mildew in Alborz province during 2010–2012. Their results showed that 4 accessions (Polycross, Oshnavieh, 3001 and 15353) were more resistant than the remaining accessions and were considered desirable parents for breeding superior sainfoin cultivars.

The objectives of this study were: (i) to test the resistance to powdery mildew disease of these promising accessions across 4 geographical regions of Iran in comparison with a set of 15 inbred accessions collected in different parts of the country; and (ii) to identify accessions with superior dry matter yield and forage quality.

Materials and Methods

The study was conducted at 4 locations (Khoramabad, Kheirabad, Semirom and Tabriz) in Iran as described in Table 1. These 4 locations (Figure 1) were included in the current research to cover a range of agro-ecological conditions for powdery mildew development and sainfoin productivity. In this experiment, reactions of tolerant and semi-tolerant accessions in comparison with susceptible accessions were evaluated during 4 growing seasons under irrigated conditions using a randomized complete block design with 3 replications. The seeds of 19 accessions (Table 2) were collected from plants grown in the earlier research project. For each accession, seeds were sown in 4 drilled rows (2 m long and 0.25 m apart) in sward conditions in April 2014. Irrigation was applied according to the plant requirements. Weeds were controlled mechanically and fertilizers (75 kg N/ha as ammonium nitrate and 150 kg P/ha as superphosphate) were applied based on scientific advice and recommendations. In the establishment year (2014), plots were cut once and no data were collected.

Table 1. Ecological and geographical characteristics of the 4 test locations in Iran.

Location	Province	Climate	Elevation (masl)	Temperature (°C)			Lat (N)	Long (E)	Annual rainfall (mm)	Soil type
				Maximum	Minimum	Mean				
Koramabad	Lorestan	Semi-arid	1,148	47.8	-1	22.9	33°47'	48°36'	406	Loamy clay
Tabriz	Azarbayjan	Cold, semi-arid	1,359	39	-22.5	14.0	38°08'	46°27'	322	Sandy loam
Semirom	Esfahan	Cold, arid	1,612	47.8	-1	22.5	32°65'	51°28'	300	Clay
Kheirabad	Zanjan	Cold, arid	1,763	18.4	-29	10.7	36°41'	48°29'	267	Loamy clay

Figure 1. Map of Iran showing the 4 study locations in their respective provinces. Source: Encyclopædia Britannica.

Table 2. Identification and origin of 19 sainfoin (*Onobrychis viciifolia*) accessions studied at 4 locations in Iran.

Gene bank code	Collection area	
	Province	District
334	Alborz	Karaj
1601	Gorgan	Gorgan
2399	Tehran	Tehran
2759	Hamadan	Hamadan
3062	North Khorasan	North Khorasan
3800	Semnan	Garmsar
4083	Esfahan	Semirom
8199	Tehran	Tehran
8799	Kermanshah	Kermanshah
9147	Alborz	Karaj
9262	Alborz	Karaj
9263	Alborz	Karaj
12542	Unknown	Unknown
15364	Alborz	Karaj
19402	Hamadan	Hamadan
3001	Alborz	Karaj
15353	Alborz	Karaj
Oshnavieh	Urumia	Oshnavieh
Plc (Polycross)	Alborz	Karaj

In the second year, assessments of powdery mildew were made under natural infections in which all accessions (tolerant and susceptible) were cultivated adjacent to each other. Thus, disease severity was evaluated when plants were exposed to airborne contamination from natural infections under environmental conditions specific to the 4 study regions. A disease severity index (DSI) was applied to each accession according to the percentage of aerial parts covered with fungal mycelium. This assessment was performed a few days before the second and third harvests, when powdery mildew infections on plants were rated according to a 0–4 scale (Horsfall and Cowling 1978), with a score of 0–2 (resistant) allocated when fungal mycelium covered 0–25% of aerial parts, a score of >2–3 (semi-resistant) when mycelium covered 25–50% of aerial parts and >3–4 (susceptible) when mycelium covered 51–100% of aerial parts as an indicator of plant susceptibility to powdery mildew.

At the 50% flowering stage plants were harvested by hand and forage produced by each plot was weighed immediately. Three harvests were performed at each site, i.e. early May (first harvest), early June (second harvest) and early September (third harvest). A 300 g representative subsample of fresh forage from each plot was collected, placed in a bag and transferred to the laboratory, where it was oven-dried at 75 °C for 48 hours and then weighed to determine DM percentage. DM yields were calculated for each experimental plot. In addition, subsamples were milled and dry matter digestibility (DMD) plus crude protein (CP) and water-soluble carbohydrate (WSC) concentrations were determined in the laboratory of Research Institute of Forests and Rangeland, Tehran, Iran, based on the method of Jafari et al. (2003).

Statistical analysis

To simplify interpretation of statistical analysis of quality-severity-yield data obtained from 19 sainfoin accessions examined over 4 growing seasons at 4 different geographical locations, low variable data over years were pooled and analyzed to estimate the extent of variability among genotypes and locations. Thus, due to the lack of significant effects of year, annual DM yields, disease severity ratings and quality traits (DMD, WSC and CP) were averaged over the study years to be used for combined analysis over 4 locations. Mean comparisons were conducted based on Duncan's method. Although several stability parameters have been proposed, Eberhart and Russell (1966) considered a stable genotype should have a slope (b value) equal to unity and deviation from regression (S^2_d) equal to zero. Stable genotypes would be those having mean yield higher than the average yield of all genotypes under test. This method has been used widely for evaluating yield stability in both annual and perennial plants. Based on the Eberhart/Russell stability regression model, regression coefficient values (bi) and deviation from regression (S^2_{di}) were calculated for each of the 19 genotypes. Stable genotypes with high mean yields were identified if the regression coefficient equated to one (bi = 1) and deviation from regression equated to zero ($S^2_{di} = 0$; Eberhart and Russell 1966). The stability tests were performed for both DM yield and disease severity index (DSI) using AGROBASE (Agronomix Software Inc., Winnipeg, Canada). Minitab 16 was used to illustrate relationships among genotypes and environments.

Results

Considering the lack of significant effects of study year, the combined analysis of variance across 4 environments showed significant differences between locations (except for DMD; P>0.01), accessions and G × E interactions for all traits (P<0.01; Table 3). Mean values for DM yield, CP and WSC concentrations, DMD and DSI at each location and averages over 4 locations for sainfoin accessions are presented in Tables 4–8.

While some accessions performed consistently in terms of DM yield across all locations, others performed

Table 3. Combined analysis of variance of DM yield (DMY), disease severity index (DSI), dry matter digestibility (DMD) and crude protein (CP) and water soluble carbohydrate (WSC) concentrations for sainfoin accessions (G) over 4 locations (E) in Iran.

Source	df	Mean Square				
		DMY	DSI	DMD	CP	WSC
E	3	565484**	9819.49**	128.25	355.47*	11.50**
Rep (E)	8	20838	345.29	93.28	73.24	6.42
G	18	16073**	1794.09**	20.81**	5.03**	2.92**
G × E	54	17776**	471.34**	11.32**	2.77**	1.44**
Error	144	8787	40.67	8.10	0.89	0.60
CV (%)		2.92	15.52	3.87	4.39	4.18

well at some locations but relatively poorly at others. Accession 8199 was a consistent performer producing higher DM yield at Esfahan (3,390 kg/ha) and Khoramabad (5,653 kg DM/ha) than most other accessions, and had equal highest mean yield over all locations (3,897 kg DM/ha), along with accession 9263 (3,614 kg DM/ha) (Table 4). While accession 9263 performed very well at 3 locations (mean 4,253 kg DM/ha), DM yield at Esfahan was only half that of the highest yield (1,700 vs. 3,390 kg DM/ha) at that location. All accessions grew well in at least one location but often grew poorly at others, e.g. Polycross at Tabriz (4,470 kg DM/ha) and Koramabad (2,600 kg DM/ha) and accession 19402 at Koramabad (5,554 kg DM/ha) and Zanjan (1,681 kg DM/ha).

Crude protein concentrations at Tabriz were consistently lower for all accessions than at the remaining locations (Table 5) and there was much less variation between accessions in terms of CP% than there was for DM yield. Overall CP means for individual accessions across the 4 locations ranged from 18.1 to 20.3%. As for CP%, dry matter digestibility of accessions at Tabriz was consistently lower than at the other 3 locations (Table 6). While accessions 2759, 3001 and 19402 had consistently high DMD values at all locations, almost all accessions had overall mean DMD greater than 68%. As for CP% and DMD, WSC concentrations at Tabriz were much lower than at the other 3 locations (Table 7). While significant differences between accessions for WSC concentration were observed, overall mean values for different accessions varied from 17.3 to 19.0%, with only 4 accessions below 18.0%.

Table 4. Mean forage dry matter yield (kg DM/ha) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	2,603f	4,080d	3,881c	1,601e	3,041ef
1601	Gorgan	2,499g	2,865e	3,084d	3,048b	2,874g
2399	Tehran	2,661ef	4,305c	3,443cd	2,207d	3,154e
2759	Hamadan	1,826k	4,298c	4,444a	1,310f	2,969f
3062	North Khorasan	2,676ef	3,831d	3,728c	2,227d	3,115e
3800	Garmsar	2,980b	4,355c	3,286d	2,748c	3,342dc
4083	Semirom	2,371h	2,945e	3,701c	1,789e	2,701h
8199	Tehran	3,390a	5,653a	3,983c	2,563c	3,897a
8799	Kermanshah	2,766d	2,864e	3,683c	2,184d	2,874g
9147	Karaj	2,849c	4,619b	3,595cd	2,328d	3,347dc
9262	Karaj	2,192i	4,537bc	3,482cd	2,222d	3,108e
9263	Karaj	1,700l	4,847b	4,571a	3,340a	3,614ab
12542	Unknown	1,383m	4,772b	3,606cd	1,876e	2,909fg
15364	Karaj	2,253i	4,779b	3,572cd	3,057b	3,415b
19402	Hamadan	1,962j	5,554a	4,261b	1,681e	3,364c
3001	Karaj	2,447hg	5,425a	3,736c	1,611e	3,304d
15353	Karaj	2,474g	4,532bc	4,122b	2,238d	3,341dc
Oshnavieh	Oshnavieh	2,890c	4,264c	3,826c	2,503c	3,370c
Plc	Karaj	2,697ed	2,600e	4,470a	2,272d	3,009f
Mean		2,454	4,270	3,814	2,253	3,198

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 5. Mean crude protein concentration (%) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	20.1b	19.1b	17.2a	22.0a	19.6c
1601	Gorgan	18.6c	17.2c	15.6b	21.0ab	18.1e
2399	Tehran	19.6bc	19.0b	16.4ab	22.1a	19.3d
2759	Hamadan	22.7a	19.9b	16.0b	20.6b	19.8c
3062	North Khorasan	22.6a	20.2b	15.6b	21.4a	19.9b
3800	Garmsar	20.8b	18.9b	15.6b	22.2a	19.4cd
4083	Semirom	20.8b	20.8ab	16.3ab	22.8a	20.1a
8199	Tehran	23.8a	19.7b	14.7b	21.1ab	19.8bc
8799	Kermanshah	18.6c	18.1bc	15.3b	20.8b	18.2e
9147	Karaj	19.3bc	19.2b	15.4b	22.0a	19.0e
9262	Karaj	21.5ab	19.3b	16.5ab	21.4a	19.7c
9263	Karaj	19.6bc	18.5b	15.1b	20.8b	18.5e
12542	Unknown	21.4ab	20.7ab	15.3b	21.3ab	19.7c
15364	Karaj	22.3a	19.9b	17.2a	21.7a	20.3a
19402	Hamadan	22.1a	21.4a	15.8b	20.5b	19.9b
3001	Karaj	21.3ab	20.0b	17.8a	21.4a	20.1a
15353	Karaj	22.9a	19.7b	17.1a	20.2b	20.0b
Oshnavieh	Oshnavieh	21.6ab	20.1b	15.9b	22.4a	20.0b
Plc	Karaj	20.5b	19.6b	14.9b	22.4a	19.4d
Mean		21.1	19.5	16.0	21.48	19.5

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 6. Mean dry matter digestibility (%) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	69.3c	72.1b	62.0c	74.1ab	69.4bc
1601	Gorgan	67.3cd	71.4b	66.1a	72.2ab	69.3bc
2399	Tehran	66.8d	74.3ab	61.8c	74.1ab	69.3bc
2759	Hamadan	75.1a	75.9a	64.7b	75.4a	72.0a
3062	North Khorasan	66.3d	73.9ab	57.2d	70.8b	67.1d
3800	Garmsar	68.2cd	74.0ab	61.6c	74.6ab	69.6bc
4083	Semirom	68.4cd	74.0ab	62.7bc	74.7ab	70.0bc
8199	Tehran	69.8c	72.5b	64.0b	73.7ab	70.0b
8799	Kermanshah	69.2c	70.0b	67.7a	74.0ab	70.2b
9147	Karaj	66.2d	72.6b	61.5c	72.3ab	68.1c
9262	Karaj	67.3cd	73.8ab	68.7a	73.3ab	70.8b
9263	Karaj	66.9d	73.4ab	6119c	73.6ab	68.7c
12542	Unknown	71.4c	69.8b	61.6c	71.7b	68.6c
15364	Karaj	70.3c	72.7b	60.4c	71.6b	68.8 c
19402	Hamadan	73.2b	75.5ab	64.7b	73.2ab	71.6a
3001	Karaj	71.0c	73.6ab	68.6a	76.3a	72.4a
15353	Karaj	70.5c	73.1b	61.7c	75.1a	70.1b
Oshnavieh	Oshnavieh	70.7c	74.0ab	61.3c	73.4ab	69.9bc
Plc	Karaj	71.8c	74.7ab	67.1a	75.1a	72.2a
Mean		69.5	73.2	63.4	73.6	69.9

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 7. Mean water soluble carbohydrate concentration (%) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	18.7ab	18.5c	16.3ab	18.6ab	18.0d
1601	Gorgan	19.2ab	19.3b	15.9b	17.8b	18.0d
2399	Tehran	18.6ab	19.5b	16.2ab	18.1ab	18.1d
2759	Hamadan	21.2a	19.6b	16.2ab	18.8ab	19.0a
3062	North Khorasan	17.5b	18.3c	15.6b	17.9b	17.3e
3800	Garmsar	19.5ab	19.8b	16.5ab	18.9ab	18.7b
4083	Semirom	20.2ab	19.2b	16.6ab	18.9ab	18.7b
8199	Tehran	17.9b	19.2b	17.0ab	18.5ab	18.1cd
8799	Kermanshah	18.9ab	18.1c	15.1b	18.7ab	17.7de
9147	Karaj	18.8ab	19.1b	15.9b	18.1ab	18.0d
9262	Karaj	19.6ab	20.3a	15.5b	18.4ab	18.4c
9263	Karaj	19.5ab	18.5c	16.7ab	18.4ab	18.3c
12542	Unknown	19.9ab	18.1c	16.9ab	18.7ab	18.3c
15364	Karaj	18.1b	19.1b	15.5b	17.7b	17.6e
19402	Hamadan	21.7a	19.1b	15.7b	19.2 a	18.9a
3001	Karaj	19.9ab	19.6b	17.2a	18.6ab	18.8a
15353	Karaj	18.8ab	19.2b	17.6a	18.6ab	18.5bc
Oshnavieh	Oshnavieh	20.6ab	19.2b	17.4a	18.6ab	19.0a
Plc	Karaj	21.5a	19.3b	15.9b	18.9ab	18.9ab
Mean		19.5	19.1	16.3	18.5	18.3

Values within columns followed by the same letter are not significantly different (P>0.05).

Overall mean disease severity index (DSI) values for the 4 locations were much higher at Zanjan (56.1) and Tabriz (48.0) than at Esfahan (31.6) and Koramabad (28.6), indicating a greater incidence of powdery mildew at the former 2 locations (Table 8). However, at all locations accession 3001 consistently displayed the greatest resistance to powdery mildew infection (DSI 5.0–22.3). Over all locations this accession displayed a mean DSI of 12.9, compared with an overall mean for all accessions of 41.1. While accessions 15353, 2759, 9263 and Polycross performed well at Esfahan and Koramabad, they were severely infected at Tabriz and Zanjan.

Correlation analysis

The results of correlation analysis demonstrated that the only associations between DSI and other parameters were a positive correlation (P<0.01) between DM yield and DSI at Esfahan (Table 9) and negative correlations between DSI and both CP% and WSC% at Tabriz and overall, as well as between DSI and DMD overall. There was a significant negative correlation (P<0.05) between DM yield and WSC concentration at Esfahan and Zanjan locations. The strongest relationships from the data were strong positive correlations between DMD and WSC concentration for 3 of the 4 locations and overall (Table 9).

Stability analysis

Results of Eberhart/Russell regression response indices (b), deviation from regression (S^2_d) and mean DM yield and DSI for 19 sainfoin accessions over 4 locations are presented in Table 10. Plots of the relationships between regression coefficients (bi) and mean DM yield for 19 accessions (Figure 2) showed that accessions 8199, 9263, 15353, 15364, 9147 and Oshnavieh had DM yields higher than the average value and b values were near unity, indicating their DM yield was stable across environments. Accessions 19402 and 3001 with bi >1 coupled with high DM yield had above-average stability for high performing environments, Khoramabad and Tabriz. A higher deviation from regression indicated sensitivity to environmental changes for DM yield (Table 10).

Similarly, the regression coefficients plotted against the test accessions for DSI are presented in Figure 3. For DSI, accessions 15353, 3001 and 9262 with b values near unity were considered stable over the 4 locations. Oshnavieh in Khoramabad and accessions 2759, 9263 and Plc (Polycross) in Esfahan with a greater resistance to powdery mildew were stable only for those locations. In general, accessions 15353, 9262 and 3001, located close to the regression line, appeared to be more stable in terms of higher production and lower disease severity index across locations.

Table 8. Mean disease severity index for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	50.0c	37.2c	46.9b	62.1b	49.1cd
1601	Gorgan	50.0	30.7c	55.8a	76.1a	53.2bc
2399	Tehran	35.0de	51.2a	55.6a	80.9a	55.7b
2759	Hamadan	10.0g	2.7e	50.8b	50.4c	28.5h
3062	North Khorasan	25.0e	27.5c	57.5a	50.7c	40.2f
3800	Garmsar	70.0a	49.9a	48.1b	79.4a	61.8a
4083	Semirom	20.0f	44.9ab	58.3a	66.5b	47.4d
8199	Tehran	63.3b	41.1b	39.7c	63.4b	51.9c
8799	Kermanshah	56.6bc	42.6b	59.7a	56.8c	54.0b
9147	Karaj	51.6c	13.0e	54.7a	62.3b	45.4e
9262	Karaj	28.3e	18.5d	48.1b	45.6d	35.1g
9263	Karaj	6.6g	26.0c	32.8d	67.0b	33.1g
12542	Unknown	11.6g	40.2b	51.1b	62.2b	41.3f
15364	Karaj	26.6e	45.5ab	39.7c	46.6d	39.6f
19402	Hamadan	30.0e	26.8c	49.8b	60.9b	41.9f
3001	Karaj	5.0g	6.7e	17.6e	22.4e	12.9h
15353	Karaj	6.6g	11.4e	46.4b	28.6e	23.3h
Oshnavieh	Oshnavieh	48.3d	8.0e	45.3b	29.5e	32.8g
Plc	Karaj	5.9g	20.5d	54.2a	54.2c	33.7g
Mean		31.6	28.6	48.0	56.1	41.1

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 9. Correlations between traits for 19 sainfoin accessions at 4 locations in Iran.

Trait	Location	DMY	DSI	CP	WSC
DSI	Esfahan	0.68**			
	Koramabad	-0.16			
	Tabriz	-0.22			
	Zanjan	0.25			
	Mean	-0.12			
CP	Esfahan	-0.02	-0.24		
	Koramabad	0.35	-0.15		
	Tabriz	-0.19	-0.42*		
	Zanjan	0.03	0.19		
	Mean	0.17	-0.43*		
WSC	Esfahan	-0.42*	-0.36	-0.01	
	Koramabad	0.16	-0.27	-0.01	
	Tabriz	0.19	-0.49*	0.18	
	Zanjan	-0.52*	-0.04	-0.03	
	Mean	-0.01	-0.40*	0.30	
DMD	Esfahan	-0.33	-0.36	0.48*	0.65**
	Koramabad	0.16	-0.37	0.40*	0.60**
	Tabriz	0.02	-0.16	0.07	-0.20
	Zanjan	-0.39	-0.24	0.02	0.61**
	Mean	-0.10	-0.44*	0.20	0.75**

CP = crude protein concentration; DMD = dry matter digestibility; DMY = dry matter yield: DSI = disease severity index; WSC = water soluble carbohydrate concentration.

Table 10. Regression response indices (b), deviation from regression (S^2_d) and mean DM yield and Disease Severity Index determined for 19 sainfoin accessions across 4 locations in Iran.

Accession	Origin	DM yield (kg/ha)			Disease severity index (DSI)		
		Mean	b	S^2_d	Mean	b	S^2_d
334	Karaj	3,041	1.12**	174085	49.1	0.62**	55.1
1601	Gorgan	2,874	0.08**	97248	53.2	1.30**	83.2
2399	Tehran	3,154	0.90**	71732	55.7	1.21**	155.2
2759	Hamadan	2,969	1.60**	204442	28.5	1.90**	62.0
3062	North Khorasan	3,115	0.78**	36073	40.2	1.12**	72.8
3800	Garmsar	3,342	0.63**	160196	61.8	0.46**	294.5
4083	Semirom	2,702	0.67**	336430	47.4	1.26**	204.3
8199	Tehran	3,897	1.20**	417212	51.9	0.22**	246.9
8799	Kermanshah	2,874	0.41**	319531	54.1	0.38**	46.8
9147	Karaj	3,348	0.96**	117272	45.4	1.28**	302.2
9262	Karaj	3,108	1.10**	92653	35.1	1.00**	36.1
9263	Karaj	3,614	1.21**	914955	33.1	1.63**	255.1
12542	Unknown	2,909	1.53**	214034	41.3	1.31**	261.1
15364	Karaj	3,415	0.93**	399054	39.6	0.30**	98.3
19402	Hamadan	3,365	1.86**	52218	41.9	1.23ns	-3.8
3001	Karaj	3,305	1.61**	307139	12.9	0.63ns	-0.9
15353	Karaj	3,341	1.15*	2325	23.3	1.05**	199.1
Oshnavieh	Oshnavieh	3,371	0.81**	12757	32.8	0.38**	470.4
Plc	Karaj	3,010	0.47**	144876	33.7	1.69**	146.3

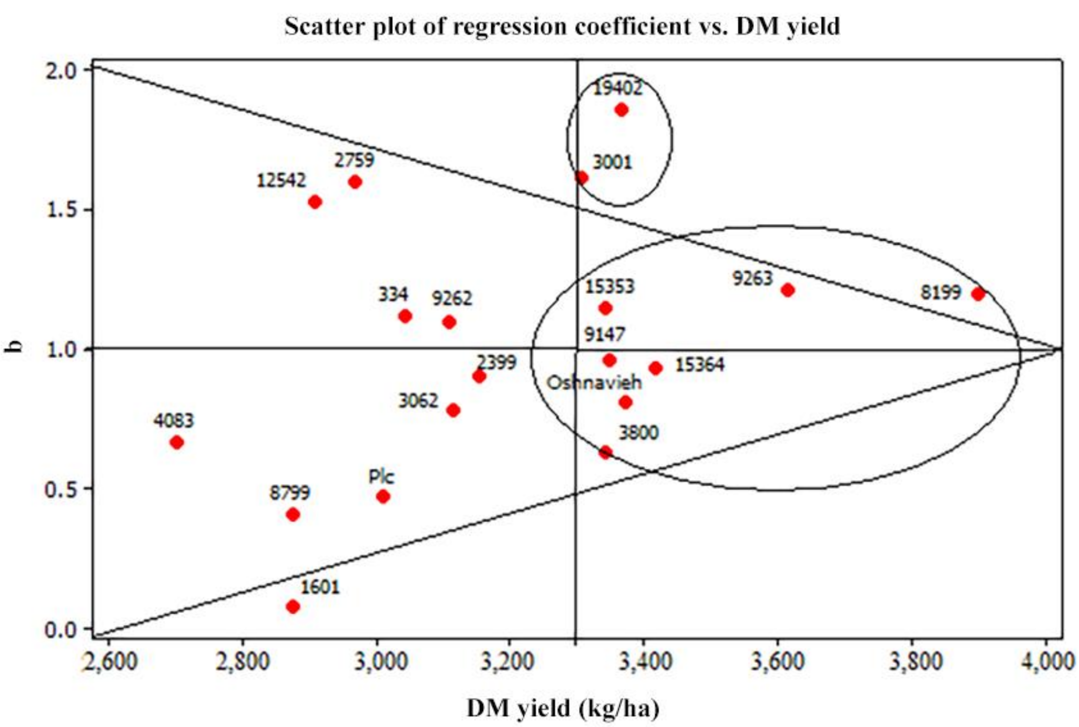

Figure 2. Regression coefficient b plotted against DM yield for 19 sainfoin accessions in Iran.

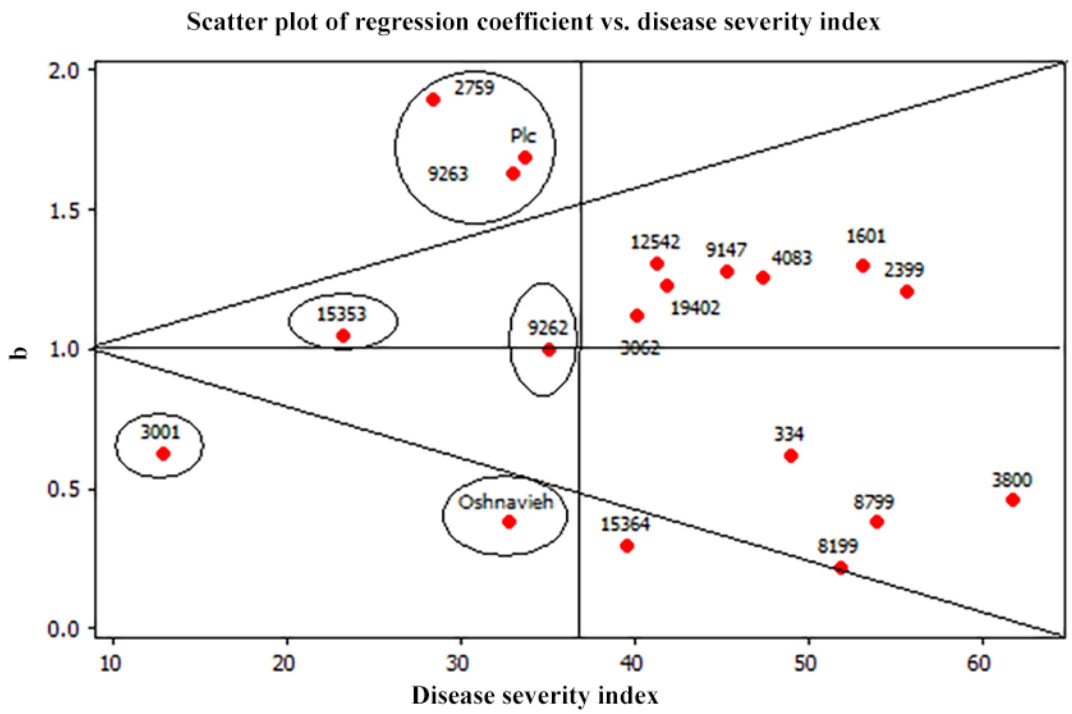

Figure 3. Regression coefficient b plotted against disease severity index for 19 sainfoin accessions in Iran.

Discussion

In order to improve sainfoin as bioactive forage, improvements in resistance to powdery mildew, yield and quality traits are required. As an initial step, quantifying the variability in existing sainfoin germplasm would allow the identification of superior genotypes with disease resistance and yield stability across various environments. According to earlier evidence, Iran is recognized as one of the most important sources of genetic diversity in populations of sainfoin worldwide (Mohajer et al. 2013). From Iranian germplasm the present research has identified 2 promising sainfoin accessions not only resistant to powdery mildew, but also with good forage quality and high DM yield, which was stable across a range of different environments and growing seasons. Since the arid and warm climate in Iran provides a high genetic diversity in populations of *Leveillula* powdery mildew (Khodaparast et al. 2012), testing under natural conditions in Iran provided an ideal environment for comparing accessions. It would seem reasonable to conclude that differences in climatic conditions across the diverse geographical areas would result in genetically diversified populations of the pathogen providing an ideal challenge to accessions. Moreover, this variability in environmental conditions across different Iranian regions resulted in significant differences in powdery mildew severity, DM yield and quality traits among the same accessions grown at 4 study locations. Although Naseri and Alizadeh (2017) reported the association of climate with sainfoin powdery mildew resistance and yield in Zanjan, the interaction of environmental parameters from different regions with the disease, resistance, quality and yield of this valuable forage crop merits further investigation in future.

Our findings demonstrated low disease severity index (<25%) in accessions 15353 and 3001 across 4 different environments, identifying them as powdery mildew-resistant sainfoin genotypes. Furthermore, accessions 9262 and Oshnavieh were considered as semi-resistant or semi-susceptible to powdery mildew. While various levels of sainfoin resistance to powdery mildew based on data from a single location or year have been reported previously (Alizadeh et al. 2014; Alizadeh and Jafari 2014), to the best of our knowledge this is the first report of stability of disease resistance in 2 sainfoin genotypes over a 4-year study conducted across different geographical areas. This information is a valuable first step in global breeding programs to increase powdery mildew resistance in sainfoin populations.

It has been reported that forage WSC provides efficient energy needed for plant growth following reduction in photosynthesis, rejuvenation after leaf loss and recovery after drought and freezing stresses (Humphreys and Eagles 1988; Humphreys 1989). According to Wilkins and Lovatt (1989), it is desirable to maintain CP concentration of forage above 12%, to ensure rumen microflora have adequate nitrogen for both milk and meat production by livestock. According to our findings, there were significant negative correlations between DSI and forage quality traits tested, indicating that lower powdery mildew levels on accessions corresponded with higher DMD plus CP and WSC concentrations. There are a number of earlier Iranian reports on the linkage of sainfoin powdery mildew severity with forage quality (Mohajer et al. 2013; Alizadeh et al. 2013); however, none of them explored the stability of these relationships over diverse environments and seasons. Moreover, the present research identified a positive correlation between DMD and WSC and CP concentrations in the accessions of sainfoin tested. This result was in agreement with an earlier report of Alizadeh et al. (2013). Therefore, one of the important benefits of reducing powdery mildew infections via breeding of resistant lines may be maintenance of sufficient CP, DMD and WSC to improve sainfoin revival and produce high quality forage. It seems that accessions 3001 and 15353 are potential candidates for both commercial production and use in breeding programs. Further testing of genetic material to identify other accessions for use in breeding programs seems warranted.

Acknowledgments

We thank the director and researcher deputy of Research Institute of Forest and Rangeland in Iran for providing financial support for this study (Projects 014-09-09-8704-87005 and 0-09-09-92-120) and also our colleagues in Natural Resources Gene Bank of Iran.

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(Note of the editors: All hyperlinks were verified 15 December 2020.)

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(Received for publication 20 January 2020; accepted 11 October 2020; published 31 January 2021)