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Yield and nutritive value of sainfoin (*Onobrychis vicifolia* Scop.) populations in different cuttings

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Received: 11 Oct 2019; Accepted: 13 Apr 2020.
DOI: 10.30479/ijgpb.2020.11725.1251

Abstract

Sainfoin (*Onobrychis vicifolia* Scop.) is one of the most important forage legumes cultivated as high quality forage. Fifty three species have been identified in this genus in Iran. A field experiment was conducted using randomized complete block design to compare yield and forage quality of 20 populations of sainfoin in Isfahan Agricultural and Natural Resources Center, Iran during 2013 – 2014. Populations were evaluated at three cutting dates (29 April, 20 July and 26 October, 2014). Dry matter yield and forage quality indices such as crude protein (CP), water-soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter digestibility (DMD), and ash content were determined. Results showed that populations were different in the majority of traits. Means of interaction effects indicated that the maximum and minimum dry matter yield were 5612.7 kg ha^{-1} at the third cutting and 118.5 kg ha^{-1} at the first cutting in 8199 and 9263, respectively. According to the results of quality analysis in the majority of populations, maximum DMD, CP and WSC were obtained at the third cutting and maximum ADF and NDF were obtained at the first cutting. In terms of forage yield some populations, such as 8199, 3800, Oshnavieh, 9147, 8799 and Isfahan camposit were superior to other populations. High digestibility populations (2759, 19402, PLC, 12542, 3001 and Oshnavieh) and high protein populations (8199, 15353, 2759, 3062, 15364 and 19402) had the best forage quality among populations. Regarding quantitative and qualitative characteristics of forage, these populations could be considered as desired parents for developing synthetic varieties.

***Key words*:** Crude protein, Dry matter digestibility, *Onobrychis vicifolia* Scop.*,* Water-soluble carbohydrates, Yield.

INTRODUCTION

Sainfoin (*Onobrychis vicifolia* Scop.) is a perennial forage plant from Fabaceae family cultivated in many parts of the world, including Asia, Europe and North America (Bhattarai *et al.,* 2016). *Onobrychis vicifolia Scop.* is widely grown in a wide range of climatic and soil conditions because of its tolerance to environmental stresses such as drought, salinity, cold and low nutrient conditions (Soares *et al.,* 2000; Carbonero *et al.,* 2011; Cicek *et al.,* 2020). This forage crop has a high nutritive value, high voluntary intake and palatability for hay, pasture and silage production (Delgado *et al.,* 2008; Aygün *et al.,* 2018). Sainfoin has high protein content and adequate mineral substances such as calcium and phosphorus that are vital for animal health (Aygün *et al.,* 2018). Unlike other legumes, *Onobrychis* species do not cause bloat in animals due to the presence of moderate concentrations of condensed tannins (Carbonero *et al.,* 2011; Hoste *et al.,* 2012; Sottie *et al.,* 2014; Wang *et al.,* 2015) and Iranian farmers commonly grow sainfoin mixed with alfalfa (Naseri and Alizadeh, 2017). Another desirable characteristic of sainfoin is resistance to alfalfa weevil (*Hypera postica* Gyll.), which has become a major

Table 1. Some physical and chemical characteristics of the experimental soil.

Gravel (%)	Clay (%)	Silt (%)	Sand (%)	OC (%)	TN (%)	ECe (dS/m)	pH
20-25	20	22	58	1.44	0.14	1.75	7.64
Na^+ (meq/l)	$SO4^{2-}$ (meq/l)	Cl^- (meq/l)	HCO_3^- (meq/l)	$Ca^{+2}+Mg^{+2}$ (meq/l)	Total cations	Total anions	
25	55.2	36	10.8	85	110	102	

pest in alfalfa fields (Achata Bottger *et al.,* 2013). In addition to animal feeding, it can also be used for soil organic matter improvement in vineyards and orchards (Porqueddu *et al.,* 2000), control of wind and water erosion (Xu *et al.,* 2006; Turk and Celik, 2006), as ornamental plant in arid area (Adel, 2014), as well as food source for bees and other pollinators (Potts *et al.,* 2010; Rozen *et al.,* 2010; Carbonero *et al.,* 2011).

Plant species are considered as one of the most important factors that affect forage quality (Leen and Martin, 2004). So far, 162 species have been identified around the world in the genus *Onobrychis* and 53 species have been found in Iran (Çelik *et al.,* 2011). Sainfoin germplasm has high variation within populations which provides an opportunity for breeders to develop new and improved cultivars with desirable traits (Majidi *et al.,* 2009). On the other hand, production of synthetic varieties for improving agronomic and quality traits is possible by crossing genetically diverse accessions (Hosainianejad *et al.,* 2011). Many researchers (Delgado *et al.,* 2008; Demdoum *et al.,* 2012; Mohajer *et al.,* 2013) found a wide range of variation among sainfoin accessions based on agro-morphological and quality traits. Plant breeders have focused on the improvement of forage yield, winter survival, stand persistence, grazing tolerance, resistant to diseases and increment of nutritional value in sainfoin crop (Mohajer *et al.,* 2013; Bhattarai *et al.,* 2016).

Sainfoin has an important role in forage production and cultivated as high - quality forage in Iran. So the present research was conducted to identify high quality and high-yield populations in order to use in breeding programs and improved seed production.

MATERIALS AND METHODS

In order to evaluate yield and forage quality in sainfoin populations, a field experiment was conducted using complete randomized block design with three replications in Isfahan Agricultural and Natural Resources Center (32° 37′ N, 51° 28′ E and an altitude of 1612 m), Iran during 2013-2014. The climate in the study area is semi-arid with an average annual precipitation of 140 mm (Yaghmaei *et al.,* 2009). The physical and chemical properties of the farm soil have been shown in Table 1.

Table 2. The origin of studied populations of sainfoin (*Onobrychis vicifolia* Scop.).

Populations	Origin
3001	Karaj
9147	Karaj
19402	Hamedan
15364	Karaj
Isfahan composite	Isfahan
PLC	Karaj
4083	Semirom
1601	Gorgan
3062	Bojnourd
15353	Karaj
334	Karaj
Oshnavieh	West Azarbaijan
8799	Kermanshah
2759	Hamedan
12542	-
3800	Garmsar
9262	Karaj
2399	Tehran
9263	Karaj
8199	Tehran

Twenty populations of sainfoin were provided from Natural Resource Gene Bank of Forest and Rangeland Institute of Iran. The populations originated from various regions of Iran (Table 2). Seeds were sown (5 October 2013) in rows in 40 cm apart and with 40 cm spacing intra rows. Each plot size was 1.5 m (length)×1 m (width). No chemical fertilizer was used before sowing and during the experiment. First irrigation was applied after cultivation and next irrigations were scheduled every 7 days. Weeds were controlled by hand. Plants were harvested on 29 April, 20 July and 26 October, 2014 (when approximately 50% of the plants per plot had flowered). After harvesting, forage samples were weighed in the field to get the fresh weight and final fresh yield was calculated in kg per hectare. Freshly harvested hay samples were dried in an oven at 70 °C for 48 h to constant weight then final dry matter yield was calculated in kg per hectare. In order to determine forage quality traits in three cuttings, samples were ground to pass through a 2 mm sieve. Then, the ground samples from each population were mixed and one sample transferred to the laboratory. Crude protein (CP), water-soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter digestibility (DMD), and ash content were estimated by NIR technology, using Inframatic 8620, 20 fixed–filter NIR instrument (Perten Instruments AB, Sweden), details of the methodology and calibrations have been explained by Jafari *et al.* (2003).

The obtained data were statistically analyzed by SAS (Ver 9.1) and means of treatments were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Dry matter yield

Results of analysis of variance (Table 3) showed that the effect of cutting, population and cutting×population on dry matter yield were significant (p≤0.01). According to the significant effects of the population×cutting on dry matter yield, the highest and the lowest values (5612.7 kg ha^{-1} and 118.5 kg ha^{-1}) were observed in 8199 at the third cutting and 9263 at the first cutting, respectively (Table 4).

Increasing dry matter yield is the main goal in sainfoin breeding programs (Jafari *et al.,* 2014). It is a complex trait highly influenced by genetic and environmental factors. Thus, evaluation of genotypic potential in sainfoin breeding is necessary before selecting desirable ones for commercial cultivation. Doyle *et al*. (1984) stated that the dry forage yield of sainfoin should increase by about 35% to 11.5 ton ha^{-1} for economic exploitation. Many authors reported that

Table 3. Analysis of variance for three cuttings in 20 sainfoin populations.

Source of variation	df	Mean of square (MS)
		Dry matter yield
Replication	3	4530^{ns}
Populations	19	20495**
Error 1	38	3118
Cutting	2	21739**
Cutting×Population	38	14586**
Error 2	80	1941
Coefficient of variation (%)		17.8

^{ns}: non significant and **: significant at p<0.01, respectively.

Table 4. Means of interaction effect of populations and cutting on dry matter yield of sainfoin.

Populations	First cutting		Second cutting		Third cutting	
3001	1528.3	rx	2358.7	ht	3454.3	cf
9147	2964.7	dl	3184.3	cj	2398	hr
19402	1464.6	tx	1740	px	2681.3	eo
15364	1345	vx	2290	jv	3123	cj
Isfahan composite	1948.3	nx	2377.7	hs	3940	bc
PLC	3179.3	cj	1133	x	3779.7	cd
4083	2053.7	mw	2902	dm	2158.3	lv
1601	1704	qx	2860.7	em	2931.3	dm
3062	2482	gq	2314.7	ju	3231	ci
15353	1534.7	rx	3094	ck	2792	en
3340	2157	lv	2099	lv	3553.3	ce
Oshnavieh	1842	ox	2192.7	kv	4635.7	b
8799	2416.3	gr	2625	fp	3255.7	ch
2759	1198.3	wx	1452.3	ux	2825.7	en
12542	1494	sx	1341.3	vx	1314	vx
3800	2613.7	fp	3455	cf	2872	em
9262	1157	x	2322.7	jv	3096	cj
2399	2340.7	iv	2340.7	iv	3302.3	cg
9263	118.5	wx	2423	gr	1491	sx
8199	2160	lv	2397	hr	5612.7	a

Means in each column followed by similar letter (s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test (p≤0.05).

sainfoin populations were different in terms of dry matter yield. Chemical composition and nutritional value of sainfoin hay were reported by Bal *et al*. (2006), Aufrère *et al*. (2008) and Scharenberg *et al*. (2008) as dry matter (89.7%), crude protein (15.2%), crude fibre (26.6%), NDF (47.7%), ADF (35.7%), lignin (9%), ether extract (2.1%) and ash (7.9%).

Mohajer *et al*. (2013) in the evaluation of 12 genotypes of sainfoin obtained the average values of 6.47 and 10.31 ton ha^{-1}for DM yield. In another experiment, total DM yield was reported 7.3 and 6.2 tons ha^{-1} under spaced plant and sward conditions, respectively (Mohajer *et al.,* 2011). Jafari *et al*. (2014) found DM yield of 5 to 5.75 ton ha^{-1} among populations in the assessment of the sainfoin production. Alizadeh *et al*. (2019) evaluated 17 sainfoin populations in Zanjan climatic condition and reported that two populations 15364 and 1601 with fresh forage yield of 9240 and 8608 kg ha^{-1} and dry forage yield of 3048 and 3057 kg ha^{-1} showed higher yields than other populations.

Various studies indicated that growth stage and harvest time influenced sainfoin yield. Toorchi *et al*. (2007) reported that the third and first cuttings had the highest and lowest yields, respectively. They explained that the development of the plant rooting system and cooling of the air resulted in increased yield at the third cutting in sainfoin accessions. Razmju *et al*. (2006) investigated the forge yield of 12 local populations of sainfoin and recorded that the first and sixth cuttings had the highest and lowest yield, respectively. Rezaee *et al*. (2008) studied the effect of different growth stages and cuttings on agronomic traits of sainfoin and showed that the fresh and dry weight of forage increased with the advancement of plant age.

Yield variations in sainfoin depend on leaf percentage at the first cutting, but it also affected by stem percentage at the next cutting stages (Mohammad Abadi and Kochaki, 1997). The production of populations with high-yield and high leaf to stem ratios is a major goal for sainfoin breeding, and it is possible by selecting plants with more and larger leaves (Sharifnabi and Nekouee, 1996). The first cutting has the highest percentage of leaves and the lowest percentage of stems. Forage yield increases at next cutting due to better plant establishment, increased underground storage, increased tillering ability and the number of stems per m^2, reduced leaf percentage and leaf to stem ratio. The percentage of foliage is inversely correlated with yield and height. So, selection for high yields, in spite of increasing height, results in a decrease in leaf percentage and forage quality (Gerami, 1990).

Forage quality

In the present study, sainfoin populations were harvested three times. The chemical composition of the forage is presented in Table 5. Forage quality traits varied among accessions and different cuttings. Most populations had the highest amount of protein (18.12-26.23%) at the third harvest. Crude protein has a key role in increasing nutritional value in forage plants. The high protein content is one of the most important qualitative characteristics of forage plants in the choice of forage for animal nutrition, and it is often considered as an indicator of digestibility. The populations had a good dry matter digestibility (65.32 -79.62%) at the third cutting. The WSC ranged from 19.34 to 24.38% among populations at the third cutting. Except for the population 9147, the rest of the populations had the highest values of WSC at the third harvest. Fresh sainfoin is suitable for ensiling with good silage characteristics and the water-soluble carbohydrates are important factors for its silage making. A decrease in WSC percent before ensiling will increase the silage pH and silage quality will decrease (Van Soest, 1991). Populations showed a suitable amount of ash. The first and third cuts with values of 5.82 -8.35% and 5.71- 8.26% had the highest ash contents in most populations. The ash percentage indicates the content of total nutrients in the plant and influences forage quality. The lowest and the highest amounts of NDF (20.72 and 38.29%) belonged to PLC and 9147 at the third cutting, respectively. Also, the populations 9147 and 2759 had the maximum and minimum ADF contents with 36.8 and 23.08%, respectively. The quality of forage improved at the third harvest, because the NDF and ADF percentages were less than 38.29% and 36.8%, respectively. ADF affects energy or total digestible nutritious material of forage (Hackmann *et al.,* 2008). NDF shows the intake potential in forage and increasing its ratios make the digestion difficult in livestock (Hackmann *et al.,* 2008; Kamalak *et al.,*2011) and the high values of ADF and NDF have a negative effect on forage quality (Jafari *et al.,* 2014).

In the majority of populations, the maximum values for DMD, CP, and WSC were obtained at the third cutting and the maximum ADF and NDF were obtained at the first cutting. The third cutting provided adequate forage quality among different harvesting times. The higher amounts of CP, DMD, WSC and the lower concentrations of NDF and ADF lead to improving feed quality. In addition to dry matter yield, forage quality has also great importance in the production of forage crops and it is necessary to supply high-quality forage for efficient animal production (Arzani *et al.,* 2006). Forage quality is the amount of nutrient material

Table 5. The chemical composition of sainfon populations in different cuttings.

Populations	CP (%)				DMD (%)				WSC (%)			
	First cutting	Second cutting	Third cutting	Means	First cutting	Second cutting	Third cutting	Means	First cutting	Second cutting	Third cutting	Means
3001	16.59	21.51	25.75	21.28	63.29	72.84	76.72	70.95	17.49	19.88	22.37	19.91
9147	19.27	18.84	19.80	19.30	63.43	69.72	65.32	66.15	15.93	21.10	19.34	18.79
19402	16.29	24.82	25.17	22.09	66.21	74.77	78.49	73.15	18.12	20.21	22.58	20.30
15364	19.55	24.09	23.23	22.29	64.89	70.15	75.76	70.26	16.66	17.91	19.67	18.08
Isfahan composite	20.65	21.30	23.03	21.66	65.99	67.47	69.09	67.51	17.48	18.53	19.66	18.55
PLC	14.16	22.56	24.79	20.50	59.99	78.38	77.04	71.80	17.70	22.50	24.38	21.52
4083	18.09	19.95	24.21	20.75	61.38	71.77	72.10	68.41	15.54	21.77	23.21	20.17
1601	14.41	21.49	19.84	18.58	62.68	70.28	68.96	67.30	18.56	17.23	21.68	19.15
3062	21.96	22.09	23.74	22.59	61.86	68.43	68.63	66.30	15.68	17.34	19.53	17.51
15353	22.46	22.29	24.02	22.92	65.82	71.86	73.76	70.48	15.92	18.33	22.10	18.78
3340	16.90	19.81	23.72	20.14	65.51	68.67	73.70	69.29	17.69	17.77	20.75	18.73
Oshnavieh	17.54	25.22	21.97	21.57	67.03	77.81	67.36	70.73	18.47	20.88	22.58	20.64
8799	16.42	17.80	21.51	18.57	67.73	65.89	74.10	69.24	17.88	18.53	20.36	18.92
2759	15.92	26.69	25.38	22.66	69.50	82.74	79.62	77.28	18.77	21.00	23.81	21.19
12542	19.18	21.06	23.98	21.40	66.22	71.12	76.98	71.44	17.14	20.12	22.29	19.85
3800	16.84	19.34	26.23	20.80	62.77	67.74	74.12	68.21	16.98	20.66	20.98	19.54
9262	22.84	23.60	18.12	21.52	64.71	68.68	68.57	67.32	16.02	20.46	22.33	19.60
2399	17.95	18.77	22.11	19.61	58.60	66.33	75.48	66.80	15.24	19.32	21.37	18.64
9263	16.14	21.58	21.01	19.57	64.21	68.26	71.21	67.89	16.03	20.70	21.77	19.50
8199	22.26	24.27	24.79	23.77	61.66	70.33	77.31	69.76	15.76	17.25	20.77	17.92

Table 5 (Continued). The chemical composition of sainfion populations in different cuttings.

Populations	NDF (%)				ADF (%)				Ash (%)			
	First cutting	Second cutting	Third cutting	Means	First cutting	Second cutting	Third cutting	Means	First cutting	Second cutting	Third cutting	Means
3001	50.96	39.28	24.44	38.22	37.24	29.35	26.90	31.16	6.59	6.55	7.41	6.85
9147	54.60	37.81	38.29	43.56	38.35	30.56	36.80	35.23	7.11	5.57	7.63	6.77
19402	48.65	35.32	29.86	37.94	34.31	28.46	24.90	29.22	6.89	6.22	6.66	6.59
15364	53.85	40.93	38.07	44.28	36.99	32.04	27.62	32.21	7.21	7.10	7.61	7.30
Isfahan composite	49.10	41.48	38.06	42.88	36.13	33.77	34.30	34.73	7.44	6.635	8.26	7.44
PLC	50.30	32.81	20.72	34.61	39.44	22.74	25.97	29.38	6.94	6.38	7.12	6.81
4083	55.52	34.93	28.45	39.63	39.95	29.08	30.40	33.14	7.57	5.52	6.81	6.63
1601	48.64	41.90	27.49	39.34	34.70	31.32	32.27	32.76	5.82	6.72	6.80	6.44
3062	57.52	40.75	36.87	45.04	40.96	33.60	34.50	36.35	8.35	6.90	8.02	7.75
15353	55.70	45.47	32.77	44.64	38.28	30.38	29.75	32.80	7.79	6.46	7.71	7.32
3340	47.92	44.20	35.90	42.67	35.04	32.27	29.34	32.21	7.17	6.63	6.73	6.84
Oshnavieh	45.16	34.60	33.22	37.66	32.15	25.93	34.96	31.01	6.03	6.23	6.05	6.10
8799	50.90	40.39	28.09	39.79	33.16	33.74	28.95	31.95	7.19	6.95	7.86	7.33
2759	45.34	36.19	24.20	35.24	28.84	21.20	23.08	24.37	6.43	6.55	6.86	6.61
12542	48.12	39.59	24.40	37.37	36.32	29.04	24.86	30.07	7.64	5.83	6.55	6.67
3800	53.65	35.31	35.58	41.51	38.25	33.13	29.39	33.59	6.97	7.40	7.00	7.12
9262	54.28	37.56	34.40	42.08	38.41	33.67	32.77	34.95	7.38	6.29	5.71	6.46
2399	56.85	40.76	26.03	41.21	43.32	34.24	26.64	34.73	7.69	6.88	7.08	7.21
9263	54.89	36.13	27.99	39.67	39.88	32.72	30.38	34.32	7.40	6.54	5.73	6.55
8199	56.85	44.28	29.32	43.48	41.18	32.04	25.38	32.86	7.63	7.17	7.78	7.52

that an animal can obtain from a forage in the shortest possible time (Walton, 1983) and this character may differ among forage crops because of several factors such as plant species, leaf-to-stem ratio, stage of growth, soil agents, climate, harvest time, disease and pests (Arzani *et al.,* 2001; Leen and Martin, 2004).

The nutritive value of sainfoin is determined by cultivar, growth stage, and environmental conditions (Bhattarai *et al.,* 2016). A number of studies indicated that the forage quality of sainfoin varied not only among the growth stages, it also varied within the same growth stages in different populations (Khalilvandi-Behroozyar *et al.,* 2010; Turk *et al.,* 2011; Kaplan, 2011). Alizadeh *et al*. (2019) reported that the crude fiber and neutral detergent fiber of two populations 8799 and 4083 (36.48, 36.9) were lower than other populations. Therefore, they have the high quality of forage. Also, three populations 3001, 15353 and Oshnavieh have high-quality forage due to high dry matter digestibility. There are different results regarding the forage quality characteristics in various growth stages and cuttings in sainfoin. Rezaee *et al*. (2008) reported that sainfoin had more desirable qualitative and quantitative yield at the early flowering stage and the third cutting. Also, they recorded that NDF increased with the advancement of plant age, while the CP content, decreased. The results of Alizadeh *et al*. (2018) showed that populations such as 15353, 3001, Oshnavieh and Polycross had higher values of CP, WSC and ash content. Tadayyon and Rafieiolhossaini (2013) evaluated the qualitative forage characteristics of different sainfoin ecotypes at three stages of pre-flowering, initiation of flowering and full flowering. They showed that the 2nd and 4th cuttings produced the maximum protein content and the 5th cutting produced the maximum fiber content. Razmju *et al*. (2006) noted that different cuttings significantly affected the chemical composition of sainfoin and the highest percentage of crude protein (28%) was obtained at the second cutting, while the third cutting had the maximum percentage of crude fiber. Mohajer *et al*. (2013) in the assessment of 12 accessions stated that there was a wide range of variation in all traits and the adequate forage quality was obtained from the second harvesting year. Abbasi (2012) showed that the number of leaves per stem and the number of paired leaflets remarkably affected the forage quality due to the increased protein to fiber ratio.

CONCLUSION

Twenty accessions of sainfoin were evaluated for their forage yield and nutritional differences at three cuttings (flowering stage). This assessment revealed a range of variation within populations in most traits. Remarkable levels of dry matter yield and forage quality were obtained at the third harvesting. In order to improve a synthetic variety, forage yield and palatability must be considered simultaneously. So, populations with high forage yield, protein and digestibility were identified as superior populations. In terms of forage yield some populations, such as 8199, 3800, Oshnavieh, 9147, 8799 and Isfahan composites were superior to other populations. High digestibility populations (2759, 19402, PLC, 12542, 3001 and Oshnavieh) and high protein populations (8199, 15353, 2759, 3062, 15364 and 19402) had the best forage quality among populations. Results implied that the last-mentioned populations could be considered as desired parents for developing synthetic varieties. Besides, other observed traits such as rejuvenation of plants after cutting, soil wrapping, various phenological behaviors, etc, in these genotypes can be used in rangeland management (control of erosion in step lands), pest management (Vs. powdery mildew) and field management (dispense of worker and mashine).

ACKNOWLEDGEMENTS

The authors acknowledge the support of Mr Aliakbar Mokhtarzade, researcher of fodder crops in Isfahan Agricultural and Natural Resources Research Center.

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