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# Prediction of quality parameters in *Onobrychis sativa* L. by near infrared reflectance spectroscopy

M. Mohammadi Sarab Badieh<sup>1\*</sup>, S. M. Hesam Zadeh Hejazi<sup>2</sup>, S. R. Tabaei Aghdaei<sup>2</sup>, M. R. Naghavi<sup>3</sup> and A. A. Jafari<sup>2</sup>

<sup>1</sup>Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran <sup>2</sup>Research Institute of Forests and Rangelands, Tehran, Iran <sup>3</sup>Tehran University, Iran

### ABSTRACT

In this paper we calculate Sainfoin quality parameters of nineteen populations by means of NIRs method. Based on the obtained results, different populations have significant effects on forage quality. We found a negative and significant correlation between water soluble carbohydrates, total ash, crude fiber and Neutral Detergent Fiber. This implies that digestibility can improve Sainfoin forage quality. Principal component analysis is a cluster analysis complementary and hence for all populations principal component analysis were done. The first and the second main components justify 71.310 % of the total variance. The first component has a high positive correlation with the traits NDF, CF and ASH and high negative correlation with the trait WSC. The second component has a high positive correlation with DDM and so with CP. Therefore, the selection based on these two components will have a positive impact in improvement of the traits. The populations based on the cluster analysis method are divided into two main groups. The scattering plot obtained from principal analysis of components verifies the results of the cluster analysis and partially could distinguish the populations. The calculations show with different combinations of the traits, it is possible to improve the quality of Sainfoin.

Keyword: Sainfoin, NIRs method, Forage Quality, Correlation Coefficient, Principal component analysis

## INTRODUCTION

In order to calculate the grazing capacity of a pasture, in addition to product estimation, the quality of forage in a variety of factors can be considered as the most important one. Recent studies on animal nutrition have shown that forage legumes such as tannins and flavonoids with moderate levels of secondary compounds are very useful. In addition to increasing the efficiency of nitrogen use in the gastrointestinal tract, they can reduce the risk of bloating and parasitic diseases; therefore, we might convince farmers to grow forage crops such as sainfoin with mentioned properties. Sainfoin is a perennial forage legume which can be grouped with other products such as alfalfa, white clover, red clover and so on [3].

Legumes in a symbiotic association with bacteria are capable of converting atmospheric nitrogen gas into nitrogen compounds of biological inorganic (ammonium) which directly can be used to produce proteins for plants. Identify the quality and nutritional value of plants due to their importance in animal nutrition can be an effective help to describe the animal's diet. These species are very palatable and important in rangelands of Iran. Various factors such

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as crude protein, total ash, and crude fiber and so on affect the nutritional value of plants.

Developments in NIRs method has made it possible to estimate components of a wide range of important agricultural products including forage. The aim of this study is the calculation of Sainfoin quality parameters of nineteen populations by means of NIRs method because of its speed and reliable outcomes. One of the limitations of this method is need for calibration for different species of different areas. This can be achieved by using comprehensive techniques for example laboratory tests. The results show that this method is fast, efficient and effective for estimating character traits and qualities of the vast range of species.

#### MATERIALS AND METHODS

In this study, populations of nineteen Sainfoin located in the gene bank of the Research Institute of Forests and Rangelands of Iran were selected and investigated. Samples were taken from populations of sainfoin at the vegetative growth stage. The studied Germplasm includes O.sativa. The complete collection of germplasm and their origins are presented in Table 1.

Five samples from each plant populations have been prepared and were dried in the open air for two weeks and then were passed through a 1mm sieve. Samples were analyzed using NIRs method. Seven quality traits (Digestible Dry Matter (DDM), Crude Protein (CP), Water Soluble Carbohydrates (WSC), Acid Detergent Fiber (ADF), Total Ash (ASH), crude fiber (CF) and Neutral Detergent Fiber (NDF)) were estimated using near infrared spectroscopy (NIR), An *Inframatic 8620*, 20 fixed-filter NIR instrument (*Perten* Instruments AB, Sweden), Details of the methodology and calibrations of NIR are given by Jafari [6].

No	Genbank Code	Source
1	3026	North Khorasan
2	9262	Karaj
3	1601	Golestan (Gorgan)
4	232	Qazvin
5	3800	Semnan (Garmsar)
6	1586	Golestan (Gorgan)
7	182	Karaj
8	3981	Karaj
9	3002	North Khorasan
10	17703	Qom
11	2985	Eastern Azerbaijan (Tabriz)
12	281	Hamedan (village HameKasi)
13	3013	North Khorasan
14	3001	North Khorasan
15	329	Karaj
16	2979	Eastern Azerbaijan (village Zenouz)
17	15353	Karaj
18	1763	West Azerbaijan (Urmia)
19	6014	Eastern Azerbaijan (village Sivan)

#### Table 1 Source and code of Sainfoin populations studied

**RESULTS AND DISCUSSION** 

In order to clarify the traits several statistical analysis was performed. The main objective of the experimental projects is finding the differences between the treatments. We examined plants collected in different parts of the country. The dried and powdered samples in the laboratory for qualitative traits such as DMD, WSC, CP, ADF, CF, NDF and ASH by NIR method were investigated. The statistical analysis was done using SPSS 16 software. The data were analyzed using a completely randomized design (see Table 2).

S.O.V	df	mean square factors were measured						
3.0.V		DDM	CP	WSC	ADF	ASH	CF	NDF
Population	18	30.16 <sup>ns</sup>	6.91 <sup>ns</sup>	11.28 **	27.25 <sup>ns</sup>	1.94 **	20.33 **	74.42 **
Error	77	18.04	4.29	2.26	16.19	0.48	6.69	18.13
Mean	-	71.57	27.55	22.31	22.20	4.03	19.74	34.15
Min	-	59.86	22.12	17.26	10.42	2.24	12.76	20.63
Max	-	83.53	32.81	27.00	31.69	5.84	25.52	45.97
CV	-	5.93	7.51	6.73	18.12	17.19	13.10	12.46

Table 2. Analysis of variance in quality of forage

ns, \*\* and \* mean respectively non significant and significant at 1% and 5%

Statistical analysis showed that among populations there was significant difference in terms of WSC, CF, NDF and ASH traits.Based on the obtained results, different populations have significant effects on forage quality. For the most of the considered traits, considerable variation in germplasm collection was observed. This diversity was expected due to wide range of geographical origins and the status of grow of germplasm. Data were compared by Duncan test (see Table 3).

Table 3. Comparison of forage quality traits according to Duncan's multiple range test

Pop	DDM	СР	WSC	ADF	ASH	CF	NDF
1	73.59 bc	27.14 abc	fgh23.80	a18.91	bcde3.91	abcdef18.90	a25.91
2	67.85 ab	27.16 abc	cdefg22.58	bc26.18	bcde4.02	cdef20.89	cde36.92
3	70.49 abc	27.26 abc	bcdef21.98	abc22.21	de4.51	ab16.81	bcd32.50
4	74.33 c	27.21 abc	bcde21.63	a20.02	cde4.34	def21.67	de38.14
5	69.87 abc	28.44 bc	abcd21.18	abc24.62	cde4.20	bcdef20.30	de37.48
6	70.85 abc	27.81 abc	cdefg22.57	abc22.16	abc3.48	bcdef19.89	bcd33.45
7	66.03 a	27.16 abc	defgh22.21	c27.00	abcd3.58	cdef20.50	bcd32.07
8	74.08 c	27.35 abc	cdefg22.37	ab20.51	cde4.30	def21.97	cde34.25
9	72.93 bc	28.02 abc	ab20.05	abc23.08	f5.39	ef22.44	e40.06
10	71.43 abc	25.44 a	h24.74	abc22.01	ab3.09	bcdef19.72	bcd32.06
11	73.77 bc	29.13 c	a19.29	a20.36	cde4.28	f22.57	de37.68
12	69.09 abc	26.57 abc	abc20.89	abc23.90	bcde4.00	cdef20.69	de37.54
13	70.68 abc	25.61 ab	bcdef21.68	abc22.24	cde4.07	bcdef19.52	cde35.51
14	71.28 abc	28.09 abc	cdefg22.36	abc23.52	ef4.73	abcd18.32	cde35.05
15	74.01 c	28.08 abc	efgh23.46	a19.84	cde4.08	a15.65	bc31.30
16	72.03 bc	29.17 c	gh24.35	ab21.17	a2.85	abcd18.49	ab28.01
17	73.05 bc	29.12 c	cdefg22.59	ab21.13	abcd3.74	abc17.68	bcd33.86
18	72.46 bc	28.66 c	bcdef22.07	ab20.85	abcd3.64	abcde18.87	bcd33.50
19	72.92 bc	26.20 abc	efgh23.33	ab21.03	cde4.26	bcdef19.32	bcd32.85

no significant difference with the same letters are not together

In fact, this variation in traits represents an interesting source for the future of the traditional growing programs and undoubtedly reflects the influence of climate, landscape, and the incorporation of farming on the phenotype.

**Correlation between the sainfoin forage quality characteristics:** The correlation coefficient between digestibility and aAcid Detergent Fiber equals -0.921. Marten [7] and Hacker [4] reported a negative correlation coefficient between ADF and digestibility. The decreasing of ADF means the increasing of digestibility [9]. As we can see in Table 3, the population 7 with the lowest digestibility (66.03) has the greatest ADF (27.00). The results of Schroeder [9] verify these results. The populations 4 and 15 have high DDM and low ADF. In fact, all done calculations for the existing energy in forage are obtained from ADF [5].

Table 4. Correlation between forage quality traits in sainfoin

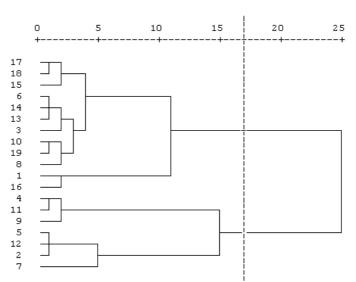
	DDM	CP	WSC	ADF	ASH	CF	NDF
DDM	1						
CP	0.263	1					
WSC	-0.059	-0.267	1				
ADF	-0.921 **	-0.151	-0.115	1			
ASH	0.209	0.019	-0.663 **	0.046	1		
CF	-0.052	-0.091	-0.545 **	0.223	0.264	1	
NDF	-0.096	0.021	-0.814 **	0.361	0.603 **	0.610 **	1
<b>**</b> and <b>*</b> mean respectively significant at 1% and 5%							

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The correlation between these traits supports this theory that digestibility can improve sainfoin forage quality. The correlation coefficient between crude protein and ash is positive. Nitrogen is one of the minerals, so, this is normal and expected. There is a negative and significant correlation between water soluble carbohydrates, total ash, crude fiber and Neutral Detergent Fiber. It is seen a close and positive association between Neutral Detergent Fiber and total ash and crude fiber traits in many studies.

**Cluster analysis of forage quality:**The sainfoin populations based on the cluster analysis method are divided into two main groups (see Figure 1). The first group includes the populations 1,3,6,8,10,13,14,15,16,17,18 and 19; the second group includes the populations 2,4,5,7,9,11 and 12.



Dendrogram using Ward Method

Figure 1. Dendrogram of cluster analysis with the Ward method

**Principal Component Analysis (PCA):**Principal component analysis reduces data volume. In fact, principal component analysis is a cluster analysis complementary and hence for all populations principal component analysis were done. The first and the second main components justify 71.310 % of the total variance. Principal components analysis shows that most of the changes occur in the first component belong to the traits NDF, WSC, CF and ASH (See Table 5).

#### Table 5. Eigen values of first and second components

Factor	First Component	Second Component
DDM	-0.153	0.955
CP	0.057	0.455
WSC	-0.893	-0.277
ADF1	0.407	-0.864
ASH	0.711	0.334
CF	0.713	-0.034
NDF	0.934	0.012

The first component has a high positive correlation with the traits NDF, CF and ASH and high negative correlation with the trait WSC, thus, this component is said to be NDF component. The second component has a high positive correlation with DDM and so with CP. On the other hand its correlation with the trait ADF is negative so the second component is said to be dry matter digestibility component.Due to close correlation between NDF and the traits ASH and CF, the first component can be introduced as a good indicator for forage quality evaluation because with the increase of this substance, forage becomes less edible. On the other hand increasing the second component i.e., digestibility improves the quality of forage in sainfoin. Therefore, the selection based on these two components will have a positive impact in improvement of the traits. In order to group the populations we use scattering plot which groups them in coordinate axes based on the first and the second components. The populations will be located

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together in a way that their distance from each other is minimum and the distance between groups is maximum.

In order to study the relationship between variables, the biplot graphic display of multivariate data is useful. As it shown in the table of values and eigenvectors, 71.31% of the total variation can be determined by the first two components. Since these components change independently form each other, we can use them as the axes of a Cartesian coordinate system and then graph the populations as a function of these variables. We should mention that although drawing such a graph is very useful for grouping the populations but it seems that it is not as accurate as cluster analysis because it does not use all information about dollops. According to the above results and the way of naming the considered components, the distinct coefficients of these independent vectors showed that with different combinations of the traits, it is possible to improve the quality of sainfoin. In the biplot (see Figure 2) the populations with low amount of the first component and high amount of the second component are important. Among them:

The populations 1,15,16.17,18 and 19 have low NDF and high DDM The populations 4,8,9 and 11 have both high NDF and DDM The populations 3,6,7 and 10 have both low NDF and DDM The populations 2,5,12 and 13 have high NDF and low DDM

Therefore, the populations 1, 15,16.17,18 and 19 are located in the desired area of the biplot and have the highest forage quality respect to the measured parameters.

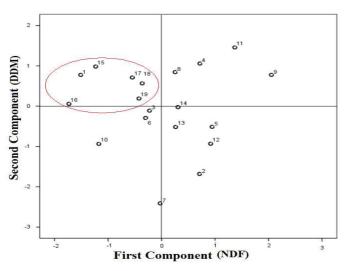


Figure 2. Biplot of populations based onfirstandsecondcomponents

The scattering plot obtained from principal analysis of components verifies the results of the cluster analysis and partially could distinguish the populations. In the study, except for DDM, CP and ADF in the forage quality parameters, there were statistically significant differences between the 19 populations. The germplasm are highly variable in terms of forage quality, this is the result of the pollination of sainfoin. High variability for the selection of these traits can be useful. With decreasing the percentage of CP and increasing the percentage of ADP the quality of forage and metabolic energy decreases. Studies showed that increase in fiber content in forage plants is associated with decrease in CP and DDM [2].

Positive and significant correlation between the percentage of crude protein and total ash shows that increase in crude protein in plants helps for their better digestion. The correlation coefficient between soluble sugars and ADF is negative and significant.Gradually, with the increase in fiber composition, structural carbohydrates increase but non-structural carbohydrates decrease[1, 8].

According to the results of cluster analysis and principal components analysis, it was observed that there is an

acceptable correlation between geographical diversity and forage quality. This could be due to fact that some samples were moved geographically but had a common origin. Therefore, categorizing the populations based on traits is more efficient than their geographical origin. The results indicate the potential of NIR as a rapid and reliable method to determine the forage quality in the vast range of plants [10]. This method is suitable for determining grazing capacity and appropriate time for grazing in rangelands. Due to the high quality and performance of sainfoin, the plant can be used in fodder production. Sainfoin is a suitable plant for farmers willing to become more environmentally sustainable and want to use environmentally friendly products. In summary, the populations 1, 15,16,17,18 and 19 have the highest forage quality respect to measured parameters. Thus, it can be expected that modern growing programs will develop high performance varieties and can be a stable alternative for forage crops which cultivated dramatically at present.

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