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Nutritional evaluation of Sulla (*Hedysarum flexuosum* L.) ecotypes grown in Northwest region of Morocco

Anass El Yemlahi[1], Abdelhay Arakrak[1], Amin Laglaoui[1], Mohamed Ayadi[2], Mohammed Bakkali[1]

[1]*Biotechnology Research and Engineering of Biomolecules Team (ERBGB), Faculty of Science and Technology, University Abdelmalek Essaâdi, Tangier, Morocco.*
[2]*Animal production research unit, National Institute of Agricultural Research, 78 Av. Sidi Mohamed ben Abdellah,Tangier, Morocco.*

Abstract
The use of legume forages species as fodder for ruminant is increasingly becoming important in livestock production. In order to evaluate natural and autochthonous forage species in Northwest of Morocco, *Hedysarum flexuosum* L. known as Sulla was collected at late vegetative stage in five sites in order to determine chemical composition, mineral content and *in vitro* enzymatic digestibility of the whole plant, along with pedological characteristics at each harvested site. Results shows significant differences were recorded among tested ecotypes between all estimated variables in relation to their soil origin. Thus, *Hedysarum flexuosum* L. is capable of providing high aerial biomass dry weight (18.90%FM), satisfactory levels of crude protein (21.7%DM), and low neutral detergent fiber (22.51%DM)in heavy clay soil contain high level of calcium (Ca),magnesium (Mg),manganese(Mn), iron (Fe) and low level of potassium (K).Considering the percentage of organic matter digestibility up to 76%OM, *Hedysarum flexuosum* L. have high potential grazing value compared to mostly legume forages used as fodder or grazed pasture for ruminants. The results of the present study could contribute to the development of pastoral improvement programs through the domestication of natural forage plants.

Keywords: *Hedysarum flexuosum* L., chemical composition, *in vitro* enzymatic digestibility and pedological characteristics.

Introduction
Spontaneous forage legumes from natural pastures are important components in the diets of ruminant animals in different country of Africa including Morocco. One of the interesting groups of temperate forages in Mediterranean regions we found *Hedysarum* spp. Different species of this genus were found over a remarkable range of bioclimatic and soil conditions (Abdelguerfi-Berrekia *et al*., 1988; Abdelguerfi-Berrekia *et al*., 1991). The interest in the genus turn up from the good agronomical traits of some species, among others, remarkable productivity, drought resistance, large adaptability to poor soils (Gutierrez-Mas 1983; Lupi *et al*., 1988; Flores *et al*., 1997; Douglas *et al*., 1999; Borreani *et al*., 2003; Moore *et al*., 2006; Dhane Fitouri S. 2012) aside from being self sufficient in nitrogen nutrition thanks to their ability to establish N_2-fixing symbiotic associations with rhizobia. Among this species, *Hedysarum flexuosum* L. known as Sulla is an important forage legume in North of Morocco with a wide natural distribution. In general, it is adapted to well drained, loam to clay soils and neutral to alkaline soil pH, making them a good potential fodder for local livestock nutrition. Although, their nutritive value could depends enormously on pedo-climatic conditions, the type of pastures and environmental factors, therefore, an awareness of quality changes of *Hedysarum flexuosum* L. in different environments is necessary to optimize its

CONTACT M. Bakkali ✉ medbakhas@yahoo.fr ERBGB, Faculty of Science and Technology, Tangier, Morocco

potential. Since previous studies focused mainly on impact of species, cultivar, maturity and season effects on fodder and pastoral quality, there is little or remain scattered regarding studies assessing variation of forage quality toward soil properties. Thereupon, it is seem primary to acquire a better understanding of the quality of *Hedysarum flexuosum* L. in a particular soil.

Hence, the aim of this study is to analyze nutritive value of five ecotypes of Sulla growing spontaneously in North of Morocco based on chemical composition and enzymatic digestibility as well as physical and chemical soil composition in which the plant grown naturally in order to establish relationship between nutritional compounds of Sulla and soil properties.

Materials and methods

Forage sampling

Five ecotypes of *Hedysarum flexuosum* L. named according to their harvesting sites were collected at late vegetative stage in March 2012 in the North-West of Morocco. The aerial biomass of each samples were cut from three replicate plots ($1m^2$) established in the experimental field. These plants were immediately weighed to determine fresh weight and transported to laboratory for chemical analysis. Moreover, soil samples from each pasture site were taken from the top (20 cm depth) to analyze.

Chemical analysis

The analysis concerned the determination of the nutritional compounds of different samples after they were oven-dried at 70°C until reaching a constant weight to determine dry matter (DM) content. Subsamples were systematically oven-dried at 50°C for phenolic compounds analysis. Finally, all samples were ground and screened through a 1 mm mesh, homogenized and analyzed.

The ash content was estimated by incineration in a muffle furnace at 550°C and the organic matter (OM) was calculated by subtracting the ash content from the total dried sample (100%). The nitrogen contents were determined by a conventional Kjeldahl method and crude protein (CP) content was calculated by multiplying N*6.25.

The crude fat fraction, also known as ether extract (EE), was determined by exhaustively extracting samples using Soxhlet extraction method with diethyl ether as a solvent. The crude fiber (CF) content was estimated by Weende method as described by AOAC (1990). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined using methods described by Van Soest *et al.*, (1991) with addition of sodium sulphite and heat-stable amylase. Hemicellulose (HEM) and cellulose (CEL) contents were calculated as NDF – ADF and ADF – ADL respectively and Non-fiber carbohydrates (NFC) were calculated as 1000 – (NDF + CP + EE + ASH) according to Van Soest *et al.*, (1991). All analysis were made using an Ankom Fiber Analyzer in trireplicate and expressed as % of dry matter (DM).

Chemical extraction for phenol components analysis was done following the procedures of (Makkar, 2000). Total extractible phenols (TEP) were assayed using the Folin-Ciocalteu's reagent based on the tannic acid standard as described by Makkar (2000).Extractible condensed tannins (ECT) were extracted by the HCl-butanol method according to Porter (1986) and their content was expressed as a leucocyanidin equivalent.

Moreover, forage samples were analyzed formacro-elements such as calcium (Ca),potassium (K),magnesium (Mg), phosphorus (P), sulphur (S) and sodium (Na) and micro-elements such as manganese (Mn), zinc (Zn), copper (Cu), iodine (I) and iron (Fe)using the « Fluorescence X » method at the National

Center of Scientific and Technical Research (CNRST) in Rabat, Morocco. All determinations were expressed on a dry matter basis.

In vitro DM and OM digestibility

The enzymatic method used was the one reported by Lila *et al.* (1986**)** which is a three-stage method: 500 mg ground dried forages sample (oven dried at 60 ºC and ground to pass a 1 mm screen) were first digested with 20 ml of 0.08 p. 100 (w/v) amylase «α-Amylase from aspergillus oryzyae » buffered solution and the samples were shaken and incubated at 39°C for 24 hours. In the second stage, the samples were constantly stirred and incubated at 39°C with 20 ml of 2% pepsin solution (from porcine gastric mucosa) diluted in 0.1 N hydrochloric acid and it was stirred constantly. In third stage, the samples were then further solubilised in 50 ml buffer solution containing 0.1 p. 100 (w/v) cellulase (from *Trichoderma viride*). Each sample was shaken and incubated at 39 ºC for 48 h. After the incubation was finished, the samples were flushed with warm distilled water and then returned to the incubator for next 48 h at 60°C. The remaining residue was incinerated in the muffle furnace at 550°C for 12 hours to determine organic matter digestibility (OMD).

In vitro digestion of crude protein

Crude protein digestibility was measured by enzymatic hydrolysis for 24 h by a protease extracted from *Streptomyces griseus* (protease from *S. griseus* types XIV. Japan) in a borate-phosphate buffer at pH 8 according to the method proposed by (Aufrère *et al*., 1989). Protein hydrolysis is the ratio between the quantities of nitrogen solubilised and the total nitrogen content measured using Kjeldahl method and reported to the initial dry matter of the sample.

Soil analysis

Soil samples were air-dried, ground and passed through a 2-mm sieve and homogenized prior to analysis for physicochemical parameters like particle size, pH, organic matter, calcium carbonate at National Institute of Agronomic Research (INRA)-Morocco-Rabat. Total nitrogen (N) was determined by the modified Kjeldahl method (NF ISO 11261, 1995), Phosphorus (P) was determined with the Olsen method and potassium (K)with sodium acetate method. Other mineral elements were analyzed using the method «Fluorescence X » at the National Center of Scientific and Technical Research (CNRST) in Rabat, Morocco. The results of the various physico-chemical analyzes of different samples of soil are shown in Table 1 and 2.

Numerical analysis

Values are means ± standard deviation (SD), compared by duncan's test. Analysis of variance was carried out using the general linear model procedure (PROC GLM). The results of the soil and forage tests will be used to determine correlations (matrix of correlations) between chemical composition, soil proprieties and forage quality of plants using PROC CORR. All Statistical analysis was performed using the statistical program of SAS (Statistical Analysis System. Version 9.1. 2002).

Results and discussion

The collection sites of the five Sulla ecotypes were fairly representative of various Mediterranean environments across North of Morocco as indicated by their variation in soil characteristics (Tables 1 and 2). The results shows that Sulla is very adapted to a variety of soil which grown naturally. The natural pastures of this plant are found predominantly in clays soils (Table 1), varying remarkably in their organic matter (OM) and calcium carbonate ($CaCO_3$) content (Table 2). Instead, soil pH did not vary so much across the study sites

Table 1. Physical soil analysis of the different sampling sites.

Sites	Clay (%)	Fine Silt (%)	Coarse Silt (%)	Fine Sand (%)	Coarse Sand (%)	Texture[a]
Khandak Lihoudi	47.12	26.18	12.87	2.20	1.88	silty clay
Ksar Sghir	69.52	18.72	0.11	2.67	0.80	clay
Melloussa	52.63	15.79	10.76	1.84	1.53	silty clay
Boukhalef	63.83	13.30	0.09	1.49	1.97	clay
Beni Guerfet	58.20	31.75	0.39	0.85	4.55	clay

(a) based on the use of the USDA Soil Textures.

(slightly alkaline) confirming the adaptation of this plant to alkaline soils (Moore *et al.*, 2006). While, nominal values of mineral soil composition among the five sites investigated vary considerably (Table 2), suggesting that some soil properties could cause differential responses of Sulla quality. In fact**,** Sulla quality is genetically determined and modified under the influence of individual or combined external factors such as soil texture and minerals availability (Kavut & Avcioglu, 2015). Spatial variations at higher scales such as the field level could introduce other factors that can be limiting to a different extend and lead to a more gradual relation. The complexity of the interrelationships between soil properties makes it difficult to distinguish which one has the most significant effect on Sulla quality. Therefore, an investigation into some of the individual factors may provide some insights into how soil physical and chemical indicators are related to variation in plant growth and quality. Thus, Sulla grown in soils with high clay and/low sand content as the case of Ksar Sghir ecotype accumulated the highest dry matter yield (18.90%; Table 3), suggesting that this ecotype could acquire more mineral elements (Table 4) probably due to the inherent characteristics of the ecotype to extract and accumulate nutrients from soil in relation to pivotal roots system(up to 2 m depth)witch providing better soil exploration but mostly in relation to soil properties since mineral concentrations in plants generally reflect the adequacy with which the soil can supply absorbable minerals to the roots (Underwood & Suttle. 1999).While, Sulla grown in soils with low clay and high sand as the case of ecotype Melloussa accumulated the lowest dry matter (12%). In fact, soil texture defining by “sand, silt and clay” fractions is one of the most important soil properties controlling water and nutrient permeability and holding capacity through a soil profile which may affect plant growth and nutrients uptake.

A significant ($p<0.05$) difference between ecotypes was observed for crude ash values (Table 4), and could be a function of soil mineral content, nature and type of soil on which forages are grown (Spears. 1994; Gagnon *et al*., 2003; Abolhassan Fajiri. 2006; Shi *et al*., 2004; Yin & Vyn. 2003; Macolino *et al*., 2013). The highest concentration was observed in ecotype Beni Guerfet (14.59%DM), while, the lowest value was noted in Boukhalef ecotype (10.43% DM), in concordance with mineral composition. In this regard, results (Table 4) show that the most of the ecotypes, particularly from Ksar Sghir and Beni Gorfet sites, contained high concentration of (mean gram per Kilogram dry Matter) calcium (17.18), potassium (10.79) and sodium (11.48). In fact, forages are generally satisfactory sources of mineral elements for grazing livestock particularly when they contain leguminous species (Underwood & Suttle 1999). Their variation (particularly Na and K) could be attributed to environmental factors (Wang et al., 2013), but mostly to intrinsic genetic characteristic of *Hedysarum* genus. Thus, highest concentration of Ca was registered in this study, similar to that of *Hedysarum coronarium* L. (Arab *et al*., 2009; Gasmi- Boubaker *et al*., 2012; Laamouri *et al*., 2015) and could be accounted for their capacity to accumulate

Table 2. Mean chemical soil analysis of the different sampling sites.

Sites	Water content (%)	pH 1:2.5 w/v soil:water	P_2O_5 (ppm)	K_2O (ppm)	N(%)	Ca(%)	Mg(%)	Na(%)	S(%)	Fe(%)	I(%)	Mn(%)	Al (%)	Si (%)	OM (%)	CaCO3 (%)
Khandak Lihoudi	5.63 ±0.12^{b}	7.9	17.17 ±0.21^{a}	98.94 ±0.03^{e}	0.097 ±0.003^{c}	3.01 ±0.11^{a}	1.04 ±0.12^{a}	0.402 ±0.05^{a}	0.046 ±0.003^{d}	3.73 ±0.28^{a}	0.016 ±0.001^{d}	0.054 ±0.001^{ab}	9.61 ±0.19^{b}	25.5 ±0.18^{e}	0.85 ±0.0^{4b}	9.29 ±0.035^{c}
Melloussa	5.66 ±0.01^{b}	7.8	8.13 ±0.03^{c}	138.37 ±0.32^{c}	0.147 ±0.002^{a}	0.25 ±0.01^{e}	0.438 ±0.05^{b}	0.224 ±0.01^{c}	0.064 ±0.004^{c}	1.78 ±0.02^{d}	0.024 ±0.001^{b}	ND^{1}	8.19 ±0.08^{c}	34.1 ±0.11^{a}	1.37 ±0.02^{a}	16.54 ±0.056^{b}
Boukhalef	5.24 ±0.04^{c}	7.9	13.2 ±0.02^{b}	147.65 ±0.05^{b}	0.146 ±0.004^{a}	0.64 ±0.04^{c}	0.618 ±0.03^{bc}	0.277 ±0.004^{bc}	0.080 ±0.001^{b}	3.27 ±0.12 ^{b}	0.018 ±0.001^{c}	0.043 ±0.004^{b}	10.7 ±0.21^{a}	29.1 ±0.04^{d}	1.38 ±0.02^{a}	18.19 ±0.010^{a}
Ksar Sghir	6.33 ±0.11^{a}	8.1	8.47 ±0.42^{c}	177.43 ±0.49^{a}	0.115 ±0.003^{b}	0.46 ±0.03^{d}	0.662 ±0.04^{b}	0.311 ±0.04^{b}	0.080 ±0.001^{b}	2.65 ±0.05^{c}	0.028 ±0.001^{a}	0.026 ±0.003^{c}	7.27 ±0.57^{c}	33.2 ±0.05^{b}	0.49 ±0.001^{c}	7.63 ±0.012^{d}
Beni Guerfet	5.31 ±0.16^{c}	8.0	13.33 ±0.49^{b}	108.3 ±0.26^{d}	0.113 ±0.001^{b}	1.75 ±0.06^{b}	0.957 ±0.06^{a}	0.307 ±0.04^{ab}	0.083 ±0.005^{a}	3.19 ±0.01^{b}	0.024 ±0.002^{b}	0.056 ±0.003^{a}	9.23 ±0.04^{b}	29.6 ±0.09^{c}	0.18 ±0.018^{d}	4.03 ±0.003^{e}

Means with different letters in column are significantly different ($p<0.05$). N, nitrogen; Ca, Calcium; Mg, Magnesium; Na, Sodium; Cl, Chlorine; S, Sulfur; Fe, iron; I, iodine; Mn, Manganese; Cu, Cooper; Zn, Zinc; Al, aluminum; Si, Silicon; OM, organic matter. (1)ND, Not detected.

Table 3. Composition of macro- and micro-minerals of different Sullaecotypes.

Ecotypes	Macro-elements (g/kg DM)							Micro-elements (mg/kg DM)			
	Ca	**P**	**Ca : P ratio**	**K**	**Mg**	**S**	**Na**	**Fe**	**I**	**Mn**	**Zn**
Khandak Lihoudi	10.10 ±0.05^{e}	1.25 ±0.00^{d}	8.08:1	3.71 ±0.02^{e}	4.61 ±0.02^{b}	5.29 ±0.02^{c}	15.10 ±0.07^{a}	990.29 ±0.29^{a}	62.60 ±0.29^{d}	ND	ND
Ksar Sghir	22.90 ±0.21^{b}	2.14 ±0.01^{a}	10.70:1	17.10 ±0.15^{a}	6.16 ±0.06^{a}	6.23 ±0.06^{b}	8.79 ± 0.08^{d}	799.86 ±0.20^{b}	112.25 ±0.25^{a}	359.54 ±0.47^{a}	89.60 ±0.82
Melloussa	11.80 ±0.12^{d}	1.71 ±0.02^{c}	6.90:1	13.60 ±0.14^{b}	3.11 ±0.03^{d}	4.27 ±0.04^{d}	10.20 ±0.10^{c}	700.15 ±0.21^{c}	ND^{1}	ND	ND
Boukhalef	16.20 ±0.50^{c}	1.65 ±0.05^{c}	9.82:1	8.52 ±0.26^{d}	2.61 ±0.08^{e}	6.58 ±0.20^{a}	11.80 ±0.37^{b}	216.42 ±0.42^{e}	76.50 ±0.30^{c}	ND	ND
Beni Gorfet	24.90 ±0.43^{a}	2.03 ±0.03^{b}	12.27:1	11.00 ±0.19^{c}	4.28 ±0.07^{c}	5.30 ±0.09^{c}	11.50 ±0.20^{b}	300.79 ±0.22^{d}	109.47 ±0.47^{b}	154.33 ±0.33^{b}	ND
S.E.M^{2}	1.570	0.084	0.50	1.213	0.332	0.218	0.563	89.91	6.409	45.887	0.471
Sig.	***	***	***	***	***	***	***	***	***	***	ND

Means with different letters within a column are significantly different (*p<0.05*). Ca, Calcium;P, phosphorus; K, potassium; Mg, Magnesium; S, Sulfur;Na, Sodium; Fe, iron; I, iodine; Mn, Manganese; Zn, zinc. (1)ND, Not detected. (2)S.E.M., standard error of the means. Sig.: *** Level of significance: *p<0.001*.

Ca^{2+} intracellularly from soil in a specific organ called “shovels” localized in their roots (Tola *et al*., 2009).In contrast, the results show low content in phosphorous (P) and magnesium (Mg) (Table 2) in comparison with other macro-elements. Their deficiency in plants (P and Mg) could be accounted to the antagonistic effect of some soil elements such as Al^{3+}forCa^{2+} (Farhat *et al*., 2015), as well as H^{+}, NH^{4+} and Na^{+} (Mengel & Kirkby 2001; Shaul 2002), or to their availability in the soil (especially for phosphors). In fact, studies have documented that a great proportion of phosphorus becomes unavailable to the plants due formation of strong bonds between phosphorous with calcium and magnesium in alkaline/calcareous soil (Arpana *et al*., 2002; Hopkins & Ellsworth. 2005) even the soil content high level of phosphorous. Otherwise, the presence of microorganisms in soil could improve mobility and rapid uptake of mineral elements. Comparatively, Labidi *et al*. (2012, 2015) showed that native Arbuscular mycorrhizal fungi (AMF) associated to wild Sulla (*Hedysarum coronarium* L.) significantly enhanced macronutrients uptake such as nitrogen (N) potassium (K) magnesium (Mg) and micronutrients such as zinc (Zn) copper (Cu) iron (Fe) and manganese (Mn) even on a calcareous soil.

Like the ach content, crude protein (CP) content varies from site to site (in mean18.18%DM) (Table 4) and could be ascribed to either soil nitrogen content or to the ability to fix atmospheric nitrogen. In this study, available soil nitrogen was very low ranges from 0.097-0.146% (Table 2), therefore it is possible that most N available in the forage would be provided by atmospheric fixation related to associated indigenous Rhizobia. According to Sulas (2009), 78.2 to 82.7% of the nitrogen requirements of this plant come from atmospheric fixation. This proportion can exceed 90% in the case of presence of efficient Rhizobia (Casella *et al*., 1984; Kishinevsky *et al*., 2003; Dhane Fitouri

Table 4. Mean chemical composition (% DM basis) of different Sullaecotypes.

Ecotypes	DM	Ash	OM	CP	EE	CF	NDF	ADF	ADL	HEM	CEL	NFC	TEP[1]	ECT[2]
Khandak Lihoudi	16.31 ±0.46^{b}	12.86 ±0.08^{b}	87.13 ±0.08^{c}	21.80 ±0.48^{a}	3.61 ±0.11^{a}	21.37 ±0.27^{d}	45.35 ±0.58^{b}	31.16 ±0.40^{b}	18.75 ±0.24^{b}	14.19 ±0.18^{c}	12.41 ±0.16^{b}	16.38 ±0.10^{c}	5.16 ±0.37^{d}	0.95 ±0.04^{d}
Ksar Sghir	18.90 ±0.47^{a}	12.61 ±0.16^{b}	87.39 ±0.16^{c}	14.71 ±0.79^{c}	2.00 ±0.18^{c}	28.98 ±0.38^{a}	47.82 ±0.63^{a}	32.27 ±0.42^{a}	20.04 ±0.26^{a}	15.55 ±0.20^{b}	12.23 ±0.16^{b}	23.42 ±0.79^{b}	6.46 ±0.19^{c}	4.38 ±0.13^{a}
Melloussa	12.69 ±0.18^{e}	11.71 ±0.17^{c}	88.29 ±0.17^{b}	15.97 ±0.48^{c}	2.29 ±0.11^{c}	23.14 ±0.29^{c}	45.59 ±0.57^{b}	31.16 ±0.39^{b}	17.64 ±0.22^{c}	14.42 ±0.18^{c}	13.52 ±0.17^{a}	24.45 ±1.34^{b}	13.65 ±0.12^{a}	3.49 ±0.07^{b}
Boukhalef	13.73 ±0.47^{d}	10.43 ±0.46^{d}	89.57 ±0.46^{a}	20.64 ±0.42^{a}	3.34 ±0.10^{a}	25.42 ±0.26^{b}	47.12 ±0.49^{a}	27.32 ±0.28^{c}	16.22 ±0.17^{d}	19.80 ±0.20^{a}	11.10 ±0.11^{c}	18.46 ±1.46^{c}	8.58 ±0.41^{b}	2.04 ±0.10^{c}
Beni Gorfet	14.59 ±0.13^{c}	14.68 ±0.36^{a}	85.31 ±0.36^{d}	17.77 ±0.60^{b}	2.69 ±0.14^{b}	14.90 ±0.10^{e}	36.01 ±0.24^{c}	22.51 ±0.15^{d}	15.94 ±0.10^{d}	13.49 ± 0.09^{d}	6.58 ± 0.05^{d}	28.47 ±0.40^{a}	3.28 ±0.14^{e}	0.11 ±0.00^{e}
min	12.510	10.107	85.062	14.252	1.897	14.831	35.841	22.409	15.870	13.431	6.539	16.31	3.187	0.108
max	19.370	14.937	89.892	22.141	3.686	29.253	48.265	32.570	20.226	19.941	13.642	28.76	13.734	4.473
S.E.M[3]	0.59	0.47	0.47	0.81	0.18	1.56	1.43	1.20	0.51	0.75	0.81	1.46	1.08	0.52
Sig.	***	***	***	***	***	***	***	***	***	***	***	***	***	***

Means in the same column with different superscript differ significantly (*P<0.05*); DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, and acid detergent lignin; CF, crud fiber; EE, ether extract; HEM, hemicellulose; CEL, cellulose; NFC, Non-fibre carbohydrates; TEP, total extractible phenols; ECT, extractable condensed tannins. [(1)]Expressed as g equivalent tannic acid /100g of DM. [(2)]Expressed as g equivalent of leucocyanidin /100g of DM. [(3)]S.E.M., standard error of the means. Sig., *** Level of significance, *p<0.001*.

S.2012). However, mineral nutrition required for legumineuse-rhizobium symbiotisis seem to be more complex since the concentration levels of some soil nutrients such as K (-0.65*) that could obstruct the nitrogen fixation (Fajri, 2006).

Similarly, significant differences (*p<0.05*) were registered in the contents of crude fiber (Table 4). The lowest value was registered for ecotype Beni Gorfet (14.90%DM), while, the highest value was recorded for ecotype Ksar Sghir (28.98%DM), in compliance with fiber fractions i.e. NDF, ADF and ADL (Table 4). This variation could presumably due growth environments (Nelson & Moser. 1994; Ritchie *et al*., 2006; Temel *et al*., 2015),including soil properties. Thus, a decrease in NDF content were registered in plants growing in high levels of Ca(-0.68*), Mg (-0.73*) and Mn **(**-0.61*),an essential element for some lignin-degrading enzymes such as Mn-peroxidases (Fioretto *et al*., 2005). Instead, soils with low Mglevel produced plants with high cellulose (-0.81**) content, which is likely arising from an enhancement of root cell wall invertase activity, this enzyme was shown to play primary role in cellulose biosynthesis under magnesium deficiency (Farhat *et al*., 2016). On the other hand, high level of cell wall constituent's content, including cellulose is known to have a negative effect on voluntary food intake and digestibility.

The results of the *in vitro* enzymatic digestibility of five ecotypes of Sulla are presented in Table 5. There were significant ($p<0.05$) differences in terms of dry and organic matter digestibility among the five evaluated ecotypes and values are generally higher in comparison with those reported in (*Hedysarum coronarium* L.) using pepsin-cellulase method (Selmi *et al*., 2010) and other forage species currently used for pasture using in vitro gas production to evaluated their digestibility (Selmi *et al*., 2010; Arab *et al*., 2009). The highest organic matter digestibility (OMD) was obtained in the ecotype Beni Gorfet (76% OM), while the lowest OMD (44.66%M) was observed in the ecotype Ksar Sghir, in correlation (Table 6), with the ADF (-0.94***), ADL (-0.88***) and extractable condensed tannins (ECT)

Table 5. Dry matter, organic matter and crude protein digestibility of different Sulla ecotypes.

Ecotypes	**DMD (%DM)**	**OMD (%OM)**	**CPD (%DM)**
Khandak Lihoudi	63.00±0.63[c]	58.86±0.71[c]	13.816±0.18[b]
Ksar Sghir	50.40±0.47[e]	44.66±0.53[e]	9.120±0.21[e]
Melloussa	58.20±0.30[d]	53.46±0.33[d]	11.168±0.20[c]
Boukhalef	68.40±0.05[b]	64.95±0.06[b]	14.813±0.17[a]
Beni Gorfet	78.20±0.36[a]	76.00±0.41[a]	10.911±0.10[d]
S.E.M[1]	2.508	2.828	5.526
Sig.	***	***	***

Means with different letters within a column are significantly different (*p*<0.05); DMD, dry matter digestibility; OMD, Organic matter digestibility; CPD, crude protein digestibility. [(1)]S.E.M., standard error of the means. Sig., *** Level of significance, *p*<0.05.

Table 6. Pearson's correlation coefficients between nutritional compounds and organic matter and crude protein digestibility of different Sulla ecotypes.

	OMD (%OM)	**CPD (%DM)**
CP	0.45[NS]	0.92***
CF	-0.84**	-0.10[NS]
NDF	-0.80**	0.17[NS]
ADF	-0.94***	-0.13[NS]
ADL	-0.88***	-0.44[NS]
HEM	-0.02[NS]	0.54[NS]
CEL	-0.83**	0.08[NS]
TEP	-0.43[NS]	0.07[NS]
ECT	-0.90***	-0.48*

CP, crude protein; CF, crud fiber NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, and acid detergent lignin; HEM, hemicellulose; CEL, cellulose; NFC, Non-fibre carbohydrates; TEP, total extractible phenols; ECT, extractable condensed tannins. (*), (**), (***) and (NS) are respectively level of significance, *p<0.05*; *p<0.01*; *p<0.001*; and *P>0.05*.

(-0.90***) content. The last ECT shows abroad range (0,11-4,38%DM) of variation(Table 3),in agreement with those of *Hedysarum coronarium* L. when examined at different phenological stages (Amato et al., 2005) using butanol-HCl method. This seems to be related to some soil mineral concentration such as Ca (-0.90***), Mn (-0.99**), as shown in Table 7.

Conclusion

The results of the present study show that *Hedysarum flexuosum* L. ecotypes of northwestern Morocco are well depend on the soil type of their natural habitats and grown preferably in clay, alkaline and calcareous soil. In addition, the study highlights some significant correlation between plant quality and some soil mineral content. Thus, cultivating *Hedysarum flexuosum* L., in soil rich in (Ca), (Mg), (Mn) and (Fe) and low in (K), found to be advantageous in increasing crude protein up to (21.7%DM), in contrast, decreasing fiber content, i.e. NDF, ADF and ADL to (36.01%DM), (22.51%DM) and (15.94%DM) respectively. Therefore, these findings will constitute a useful tool for improvement of agro-pastoral ecosystems through the domestication of natural forage plants in a specific soil that favor high nutritive value.

Table 7. Pearson's correlation coefficients between nutritional compounds and soil chemical composition of five ecotypes of Sulla growing in natural habitat.

	Soil properties													
	P_2O_5	K_2O	N	Ca	Mg	Na	S	Fe	I	Mn	Al	Si	OM	$CaCO_3$
CP	0.91***	-0.65*	-0.09^{ns}	0.54*	0.28*	-0.13^{ns}	-0.32^{ns}	0.76***	-0.95***	0.68*	0.88^{ns}	-0.91***	0.12^{ns}	0.23^{ns}
CF	-0.45*	0.84**	0.25^{ns}	-0.77**	-0.66*	-0.15^{ns}	-0.11^{ns}	-0.28^{ns}	0.19^{ns}	-0.89**	-0.33^{ns}	0.39^{ns}	0.40^{ns}	0.45^{ns}
NDF	-0.24^{ns}	0.61*	0.27^{ns}	-0.68*	-0.73*	-0.06^{ns}	-0.37ns	-0.20*	-0.12^{ns}	-0.68*	-0.14^{ns}	0.19^{ns}	0.52^{ns}	0.62*
ADF	-0.29*	0.43^{ns}	-0.03^{ns}	-0.54*	-0.64*	0.09^{ns}	-0.67*	-0.33*	0.04^{ns}	-0.61*	-0.49^{ns}	0.25^{ns}	0.25^{ns}	0.31^{ns}
ADL	-0.24^{ns}	0.41^{ns}	-0.46^{ns}	-0.26^{ns}	-0.22^{ns}	0.36^{ns}	-0.56*	-0.14^{ns}	0.29^{ns}	-0.62*	-0.69*	0.19^{ns}	-0.23^{ns}	-0.20^{ns}
HEM	0.01^{ns}	0.48^{ns}	0.56*	-0.44^{ns}	-0.36^{ns}	-0.25^{ns}	0.36^{ns}	0.15^{ns}	-0.29^{ns}	-0.37^{ns}	0.51^{ns}	-0.04^{ns}	0.58*	0.67*
CEL	-0.29*	0.38^{ns}	0.24^{ns}	-0.64*	-0.81**	-0.10^{ns}	-0.64*	-0.40*	-0.13^{ns}	-0.53^{ns}	-0.29^{ns}	0.25^{ns}	0.53^{ns}	0.60*
ECT	-0.81**	0.88***	0.36^{ns}	-0.90***	-0.78**	-0.45*	-0.03^{ns}	-0.70*	0.55*	-0.99***	-0.68*	0.77**	0.40^{ns}	0.36^{ns}
TEP	-0.58*	0.38^{ns}	0.79**	-0.82**	-0.96***	-0.75**	-0.09^{ns}	-0.76***	0.03^{ns}	-0.52^{ns}	-0.12^{ns}	0.58*	0.82**	0.79**

CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, and acid detergent lignin; CF, crud fiber; EE, ether extract; HEM, hemicellulose; CEL, cellulose; TEP, total extractible phenols; ECT, extractable condensed tannins. N, nitrogen; Ca, Calcium; Mg, Magnesium; Na, Sodium; S, Sulfur; Fe, iron; I, iodine; Mn, Manganese; Al, aluminum; Si, Silicon; OM, organic matter. * Level of significance, *p<0.05*. ** Level of significance, *p<0.01*. *** Level of significance, *p<0.001*. ns , *P>0.05*.

References

Abdelguerfi-Berrekia R, Abdelguerfi A, Bounaga N, Guittonneau GG (1991) Répartition des espèces spontanées du genre Hedysarum L. en Algérie, en relation avec certains facteurs du milieu. Fourrages **126:** 187-207.

Abdelguerfi-Berrekia R, Abdelguerfi A, Bounaga N, Guittonneau GG (1988) Contribution à l'étude des espèces spontanées du genre *Hedysarum* L. en Algérie. Etude auto écologique. Ann. Inst. Nat. Agro. El-Harrach. **12:** 191-219.

Abolhassan Fajri (2006) Effects of Different Rates of Potassium on Nitrogen Fixation and Agronomic Traits of Three *Medicago sative* Varieties. *Pakistan Journal of Biological Sciences* **9:** 2881-2886.

Amato G, Di Miceli G, Giambalvo D, Scarpello C, Stringi L (2005) Condensed tannins content in sulla (*Hedysarum coronarium* L.) as affected by environment, genotype and growth stage. In: Bullitta S (Ed.), Bioactive compounds in pasture species for phytotherapy and animal welfare. Digital Space Publishing, Sassari, 41-51.

AOAC (1990) Official Methods of Analysis. 14th ed. Assoc. Offic. Anal. Chem. Arlington, VA.

Arab H, Yacoub F, Mehennaoui S (2009) Seasonal changes in chemical composition and in vitro gas production of six plants from Eastern Algerian arid regions. Livestock Research for Rural Development **21(4):** 11pp.

Arpana N, Kumar SD, Prasad TN (2002) Effect of seed inoculation. fertility and irrigation on uptake of major nutrients and soil fertility status after harvest of late sown lentil. J Applied Biol **12:** 23-26.

Aufrère J, Graviou D, Demarquilly C, Vérité R., Michalet-Doreau B., Chapoutot P (1989) Aliments concentrés pour ruminants: prévision de la valeur azotée PDI à partir d'une méthode enzymatique standardisée. INRA Prod Anim **2:** 249–254.

Borreani G, Roggero PP, Sulas L, Valente ME (2003) Quantifying morphological stage to predict the nutritive value in Sulla (*Hedysarum coronarium* L.). Agron J **95:** 1608-1617.

Casella S, Gault RR, Reynolds KC, Dyson JR, Brockwell J (1984) Nodulation studies on legumes exotic to Australia (*Hedysarum coronarium* L.) FEMS Microbiology Letters **22:** 37- 45.

Dhane Fitouri S, Trabelsi D, Saïdi S, Zribi K, Ben Jeddi F, M'hamdi R (2012) Diversity of rhizobia nodulating sulla (*Hedysarum coronarium* L.) and selection of inoculant strains for semi arid Tunisia. Annals of Microbiology **62:** 77-84.

Douglas GB, Stienezen M, Warghorn GC, Foote AG, Purchas RW (1999) Effect of condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) and Sulla (*Hedysarum coronarium*) on body weight. carcass fat depth. and wool growth of lambs in New Zealand. N.Z. J. Agric. Res. **42:** 55–64.

Farhat N, Sassi H, Zorrig W, Abdelly C, Barhoumi Z, Smaoui A, Rabhi M (2015) Is excessive Ca the main factor responsible for Mg deficiency in Sulla carnosa on calcareous soils? J Soils Sediments **15:** 1483–1490.

Farhat N, Smaoui A, Maurousset L, Porcheron B, Lemoine R, Abdelly C, Rabhi M (2016) Sulla carnosa modulates root invertase activity in response to the inhibition of long-distance sucrose transport under magnesium deficiency. Plant Biol **18(6):** 1031-1037.

Fioretto A, Di Nardo C, Papa S, Fuggi A (2005) Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biol Biochem **37:** 1083–1091.

Flores F, Gutierrez JC, Lopez J, Moreno MT, Cubero JI (1997) Multivariate analysis approach to evaluate a germplasm collection of Hedysarum coronarium L. Genet Resour Crop Ev **44:** 545–555.

Gagnon B, Bélanger G, Nolin MC, Simard RR (2003) Relationships between soil cations and plant characteristics based on spatial variability in a forage field. Canadian journal of plant science **83:** 343–350.

Gasmi-Boubaker A, Selmi H, Mosquera Losada R, Ben Youssef S, Zoghlami A, Mehdi W, Rekik B, Rouissi H, Rigueiro-Rodriguez A (2012),. Nutritive value of whole plant (stem and leaves) of *Hedysarum coronarium L. Medicago truncatula L. Vicia sativa L* and *Pisum sativum L* grown under Mediterranean conditions. Livestock Research for Rural Development **24:** 172.

Gutierrez-Mas JC (1983) La Zulla. La reina de las forrajeiras de secano. Agricultura **11:** 576-677.

Hopkins BG, Ellsworth JW (2005) Phosphorus Placement for Sugar beet in Calcareous Soil. In: Murphy L (Ed.), Fluid Forum Proceedings. Fluid Fertilizer Foundation, Manhattan, Kansas, **22:** In Press

Kishinevsky BD, Nandasena KG, Yates RJ, Nemas C, Howieson JG (2003) Phenotypic and genetic diversity among rhizobia isolated from three *Hedysarum* species: H. *spinosissimum*. H. *coronarium* and H. *flexuosum*. Plant Soil **251:** 143–153.

Laamouri A, Elaloui M, Ennajah A, Bouabdelly N (2015) Study of mineral and nutritional components of some leguminous herbaceous and shrubs species in Tunisia. IJAAR **6(4):** 1-7.

Labidi S, Ben Jeddi F, Tisserant B, Debiane D, Rezgui S, Grandmougin-Ferjani A, Lounès-Hadj Sahraoui A (2012) Role of arbuscular mycorrhizal symbiosis in root mineral uptake under $CaCO_3$ stress. Mycorrhiza **22:** 337–345.

Labidi S, Jeddi FB, Tisserant B,Yousfi M, Sanaa M, Dalpé Y, Sahraoui ALH (2015) Field application of mycorrhizal bio-inoculants affects the mineral uptake of a forage legume (*Hedysarum coronarium* L.) on a highly calcareous soil. Mycorrhiza **25:** 297–309.

Lila M, Barrière Y, Traineau R, Allerit S (1986) Mise au point et étude d’un test enzymatique de la digestibilité de fourrages pauvres ou riches en amidon. *Agronomie* **6:** 285–291.

Lupi F, Casella S, Toffanin A, Squartini A (1988) Introduction of Rhizobium ‘hedysari’ in alkaline clay-loam soil by different inoculation techniques. Arid Soil Res Rehabil **2:** 19–28.

Macolino S, Lauriault LM, Rimi F, Ziliotto U (2013) Phosphorus and potassium fertilizer effects on alfalfa and soil in a non-limited soil. Agronomy Journal **105:** 1613–1618.

Makkar HPS (Ed.) (2000) Quantification of Tannins in Tree Foliage. A laboratory manual. FAO/IAEA, Vienna.

Mengel K, Kirkby EA (2001) Principles of plant nutrition. Kluwer Academic, Dordrecht, The Netherlands.

Moore G, Sanford P, Wiley T (2006). Perennial pastures for Western Australia. Bulletin No. 4690. Depai Lucent ent of Agriculture and Food. Government of Western Australia, Perth, Australia.

Nelson CJ, Moser LE (1994) Plant factors affecting forage quality. In: Fahey JGC (Ed.), Forage Quality, Evaluation, and Utilization. ASA, CSSA, SSSA, Madison, Wisc., 115–154.

NF ISO 11261 (1995) Dosage de l’azote total - Méthode de Kjeldahl modifiée. In Qualité des sols, 1996, AFNOR, Paris, 257-260.

Porter LJ, Hristich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry **25:** 223-230.

Ritchie JC, Reeves JB, Krizek DT, Foy CD, Gitz DC (2006) Fiber Composition of Eastern Gama grass Forage Grown on a Degraded, Acid Soil. *Field Crops Research* **97:** 176–181.

Selmi H, Gasmi-Boubaker A, Mehdi W, Rekik B, Ben Salah Y, Rouissi H (2010) Composition chimique et digestibilité *in vitro* des feuilles d'*Hedysarum coronarium* L, *Medicago truncatula* L, *Pisum sativum* L et *Vicia sativa* L. *Livestock Research for Rural Development 22: 116.*

Shaul O (2002). Magnesium transport and function in plants: the tip of the iceberg. Biometals **15:** 309–323.

Shi W, Wang X, Yan W (2004) Distribution patterns of available P and K in rape rhizosphere in relation to genotypic difference. Plant Soil **261:** 11-16.

Spear JW (1994) Mineral in Forages. In: Fahey J.G.C (Ed.), Forage Quality, Evaluation, and utilization. National Conference on Forage quality, Evaluation and Utilization, Lincoln, 281-317.

Sulas L, Seddaiu G, Muresu R, Roggero PP (2009) Fixation de l'azote du sulla en conditions méditerranéennes. Agronomy Journal **101:** 1470-1478.

Temel S, Surme M, Tan M (2015) Effects of growth stages on the nutritive value of speccific halophyte species in saline grasslands. J Anim Plant Sci **25:** 1419-1428.

Tola E, Henriquez-Sabà JL, Polone E, Dazzo FB, Concheri G, Casella S., Squartini A (2009). Shovel roots: a unique stress-avoiding developmental strategy of the legume plant *Hedysarum coronarium* L. Plant Soil **322:** 25–37.

Underwood EJ, Suttle NF (1999) The Mineral Nutrition of Livestock. CABI Publishing, New York.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber. neutral detergent fiber and non-starch carbohydrates in relation to animal nutrition. J Dairy Sci **74:** 3583–3597.

Yin XH, Vyn TJ (2003) Potassium placement effects on yield and seed composition of no-till soybean seeded in alternate row widths. Argon J **95:** 126-132.