

AGRONOMIC AND FUNCTIONAL TRAITS OF SOME SPECIES OF *HEDYSARUM* GENUS CULTIVATED IN A NEW HABITAT OF ALGERIA (OASES OF M'ZAB – GHARDAÏA) UNDER IRRIGATION

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SUMMARY

The present research was carried out to determine forage production and characterization of agronomic traits of three leguminous species of *Hedysarum* genus. They are cultivated for the first time in agro-ecological conditions of Saharan region in the oases of Ghardaïa (M'Zab region, Algeria). *Hedysarum coronarium* L., *H. carnosum* and *H. flexuosum* L. are chosen because they considered well adapted to marginal areas. Seeds of species were collected from different regions of Algeria. The trial was conducted in sandy soil with alkaline pH = 8.27 in autumn of 2020. Results indicate that *Sulla coronaria* showed the best results ($p < 5\%$) compared to the other species: *S. carnosum* and *S. flexuosa* for all traits, except leaf/stem ratio and SLA (*S. carnosum* takes the first place with 1.44 vs. 1.40 and 1.25, and 13.09 m²/kg vs. 8.9 and 13.07 m²/kg, respectively). The results of the correlation matrix indicated that the plant height is positively correlated with the leaf number ($p < 0.000$). The variance analysis revealed a significant variation between the species regarding the seven studied traits, except the leaf to stem ratio and dry biomass. The findings of this study encourage farmers to include this valuable culture in their production system.

Key words: Algeria – behavior – Forage production – *Hedysarum* genus – M'Zab oases – sandy soil

Sulla species are winter growing legumes and have a high nutritive potential which are not well studied and assessed (Zirmi-Zembri et Kadi 2021) especially in cultivated conditions. *Hedysarum* genus (*Fabaceae*) is an important group of temperate forages on pastures in the western Mediterranean basin of Morocco, Algeria, and Tunisia (Tola *et al.*, 2009; Liu *et al.*, 2017). *Hedysarum*, with about 300 species distributed worldwide, is a large genus within the tribe *Hedysareae* (Kishinevsky *et al.*, 2003; Issolah 2012). Plants of this genus are annual or perennial herbs, and they occur in varied habitats such as seashores or deserts (Issolah 2012; Ranjbar 2010). So, it is characterized by very large spectrum of diversity. Species of *Hedysarum* have a lot of advantages in agriculture; the main reasons for its cultivating are animal feed, soil improvement and conservation, honey production and minimizing of fertilizers (Errassi *et al.*, 2018; Slim *et al.*, 2018).

In Algeria, the forage production is limited by the climatic conditions: irregular and insufficient precipitations make this agronomy sector uncertain and difficult (Moussaouali et Hamdi-Aïssa 2017). Therefore, after colonial period, the surface of grasslands and rangelands has not stopped regression, that's why; unfortunately, the livestock remain undernourished (Abdelguerfi 2003). The cultivated forage represents only 1.94% of the forage used in feeding of cattle (Senoussi et Behir 2010), because this production is closely limited to areas under cultivation and the diversity of species. The use of forage species is usually based on grazing in the natural areas of Algeria, but in Ghardaïa (our study area) it bases only on the cultivated lucerne (*Medicago sativa* L.), which enter in dormancy in winter season; that is why necessary to introduce some other species to cover needs and the feed gap during low forage

availability and production periods. For a long time, Struffi et al. (1998) announced that Sulla plant has been successfully introduced in marginal areas including highly calcareous and semi-arid soils. In front of this situation, the use of some species adapted to actual climatic conditions is the first step in the long challenges to find solutions to remedy the problem of feed deficit (Zirmi-Zembri et Kadi 2020). In addition, climate change and increasing world population impose an urgent need to develop a various range of nodulated legumes adapting to dry areas. The farming in sandy soils is very difficult because these are characterized by low water retention, poverty in mineral elements and consequently reduction of crop yields (El-Hendawy et al. 2010; Moussaouali et Hamdi-Aïssa 2017). For this reason, in order to reach the growing demand for animal-source food, we tried to introduce, for the first time, three species of perennial legume of Sulla into this region in the raison to use them for forage production under cutting regime, like is known in the Mediterranean countries. Our study was conducted in 2020-2021 agriculture season to establish some morphological and agricultural characteristics shown by these species of *Hedysarum* genus in this new area, such as plant length, dry matter content, fresh matter content, leaf to stem ratio.

In previous work, we demonstrated that *Hedysarum coronarium* presents some important features in new habitat (arid region). This work follows that of Moussaouali et Hamdi-Aïssa (2017). Thus, the objective of this study is to assess some morphological and agronomic characters and, by the way, behavior of these collections of Sulla. The aim of the present experiment was to evaluate and characterize preliminarily the behavior and the production of *H. coronarium*, *H. flexuosum* and *H. carnosum*, legumes

of high potential for animal nutrition, cultivated in this new and marginal area of Algeria (M'Zab oases), in the raison to be used as green or dry forage for local livestock. Until now, and to our knowledge, no agronomic study concerning these species has been conducted in these same conditions only this realized by Moussaouali and Hamdi-Aïssa (2017).

MATERIALS AND METHODS

Description of experimental site

The experimental trial was conducted in the region of M'Zab, specifically in the oases of Laadira, west of Ghardaia province (latitude, 32°34' N; longitude, 3°37' E; and elevation above sea level, 450 m), situated at 600 km south of the capital of Algeria (Fig. 1), during 2020/2021 growing season. This region is known by an arid climate with scarce amounts of rainfall (< 60mm annually).

The dry period extends throughout the year, the average annual temperatures are too high, the maxima can reach to 50 °C and the minima are 2 to 3 °C recorded between December and January; which leads to strong daily and annual thermal amplitudes. The total precipitation was 51.3 mm during growth period.

Before starting our trial, the main physical and chemical characteristics of 0-30 cm of soil layer were evaluated such as *pH*, *EC*, *total limestone*, *organic carbon percentage and soil texture*. The soil texture in this study area is predominantly sandy throughout its profile (80.1 % sand, 16.2 % silt and 3.7 % clay). Others results are listed in Table 1.

The soil is almost devoid of all kinds of organic matter; nitrogen content and C/N rate are extremely

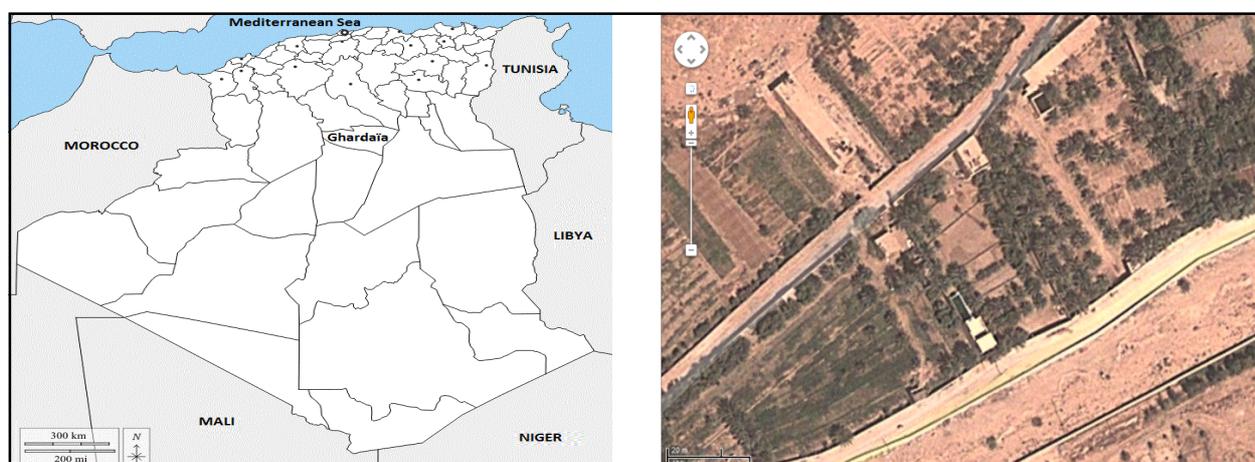


Fig. 1. Geographical map of Ghardia province and location of the study area (Google map, 2022).

TABLE 1
Physical and chemical characteristics of the experimental field soil (0-30 cm)

Depth (cm)	Bulk density (g.cm-3)	Organic matter (%)	pH	N (%)	P (ppm)	Texture
0-30	1.65	0.52	8.27	0.17	38.40	Sandy

low. According to study of Zirmi-Zembri and Kadi (2020), Sulla can grow in soils with acidity from 7.62 to 8.40.

Plant material

Three species of Sulla were used in this study, *Hedysarum coronarium*, *Hedysarum flexuosum* and *Hedysarum carnosum*, they are considered as spontaneous palatable shrubs which grow to between 110-200 cm in height and they tolerate occasional frost and drought.

Seeds of the species were collected in their natural habitat from different regions of the country (Algeria). For *H. coronarium*, the seeds were collected from the northeastern of Algeria (Sétif), those of *H. carnosum* from the southeast (Biskra) and those of *H. flexuosum* were brought from the northern of Algeria (Tizi-Ouzzou).

Experimental design, treatments and agronomic practices

A randomized complete block design with three replicates was used in this study. Each plot was 4 m wide by 6 m long with 4 plant rows of 12 plants (50 cm between plants). Three species of *Hedysarum* were randomly assigned to the main plot and subplots, respectively.

The sowing has been carried out in November 25th, 2020 directly on soil in the parcel. The irrigation has been started in the same day of seedling, three times a week (day on - two days off) in winter and day-on day-off in summer (high temperatures, dry season).

Evaluation of the quality and forage production of agronomic parameters

After 7 months of following-up, so exactly at the flowering stage (the experiment took place between November and May), five plants from each plots and from each repetition were harvested and directly weighted. The fresh weights are shown by g/plant.

The studied parameters are the following : plant height (cm), leaves number per plant, stems number per plant, weight of fresh leaves, weight of fresh stems, Leaf/stem ratio for fresh weight, forage dry yields (t DM.ha⁻¹), Specific Leaf Area (SLA: m².kg⁻¹); Leaf Dry Matter Content (LDMC : mg.g⁻¹).

These three cultivated species were followed and monitored during all phenological stages, and forage samples were taken to evaluate some morphological characters and forage dry matter yield at the beginning of the flowering stage. From each plot, five plants chosen randomly are uprooted manually during spring period, in May 2021 in the morning, to be used for analysis. Samples were transported to the laboratory of plant physiology of Ghardaïa University.

Firstly, the fresh samples were weighed (to determine the fresh weight) and the number leaves and stems are accounted, and immediately, fresh leaves and stems were separated and weighed again. These samples (leaves and stems together) were dried in an oven at 70°C for 48 hours and re-weighted again in a precision balance to determine the dry weights. One hundred thirty five plants were uprooted for the three species for this analysis. The leaf/stem ratio was calculated by dividing fresh leaves weight over fresh stems weight.

Specific leaf area (SLA) is the best indicator of the relative growth rate (RGR) and the availability of nutrients in the soil (Gondard et al., 2003) : 5 different healthy leaves were randomly harvested, photographed by the smart phone to determine their surfaces separately with an application. Then, each one (including petiole) was put in paper bag to assess its dry weight after oven drying at 70°C until the constant weight. SLA (m².kg⁻¹ DM) was assessed, using the formula of Burylo et al. (2010) and (Pérez-Harguindéguy et al., 2013), by dividing leaf area by leaf oven-dry mass.

Leaf dry matter content (LDMC, mg.g⁻¹) was also calculated by dividing leaf oven-dry mass by leaf fresh mass. This trait reflects the leaf lifetime and the ability of plant to store organic matter in its leaves (Chalmandrier, 2008). The same 5 leaves used previously to determine SLA were used to determine LDMC.

Statistical analysis

Data analysis was performed using the free software R (4.0.0) for windows. Data were

represented as the mean \pm standard deviation (SD) of the triplicates samples. ANOVA was used to analyze the recorded data and the least significant difference (LSD) to separate means ($P \leq 0.05$). To check correlation between parameters, we used the same program to calculate the coefficients of correlation.

RESULTS AND DISCUSSION

The comparative study of forage production and the phenotypic characterization of the three species of *Hedysarum* genus cultivated in the oases of M'Zab (Ghardaïa, Algeria) under irrigation for above traits revealed a large degree of variation. There is very important variability for all the parameters.

Plant height

Plant height is an important component of plant life history and affects a plant's ability to capture light (Lavorel and Grigulis, 2012).

At the uprooting operation, precisely at flowering stage, the plant height was clearly different between this three species of Sulla, the low value is registered by *H. carnosum* with 38.29 cm followed by *H. flexuosum* with 67.16 cm, and the high value is given by *H. coronarium* 69.23 cm (Fig. 2). The difference between the first and the last average is 80.98%. Ben Jeddi (2005) and Issolah and Yahiaoui (2008) were registered some very low values of height plant at some spontaneous populations of *H. coronarium* in Tunisia and Algeria (32 cm and 12.5 respectively).

The analysis of variance showed a difference highly significant between species ($p < 0.001$) at $\alpha = 5\%$. The height of cultivated plants at the end of trial is not significantly different for *Hedysarum coronarium*-*Hedysarum flexuosum*, while it's highly significant between *Hedysarum flexuosum*-*Hedysarum carnosum* and *Hedysarum coronarium*-*Hedysarum carnosum* (Table 2).

TABLE 2
Tukey multiple comparisons of means

	diff	Lower	Upper	p adj
<i>Hedysarum flexuosum</i> - <i>Hedysarum carnosum</i>	28.87	8.46	49.29	0.004
<i>Hedysarum coronarium</i> - <i>Hedysarum carnosum</i>	30.94	10.52	51.36	0.002
<i>Hedysarum coronarium</i> - <i>Hedysarum flexuosum</i>	2.07	-17.99	22.13	0.97

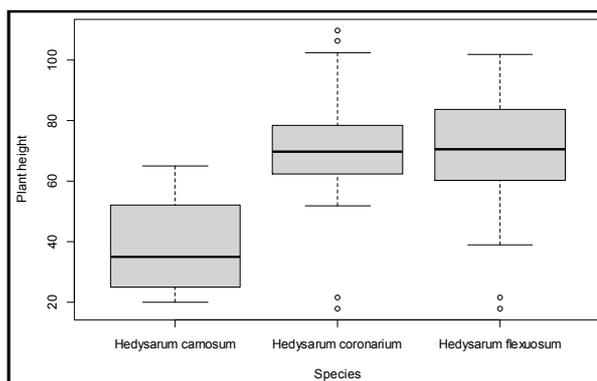


Fig. 2. Plant height of three species studied of *Hedysarum* at the flowering stage.

These values reflect the reaction of the plants in relation to the new area of growth.

Several studies show clearly how availability of water and nutrients in the soil increases significantly the height of plants, but the weak value of *H. carnosum* refers to its non-adaptability to the climatic conditions.

Leaves and stems number of plants according to the different species of *Hedysarum*

The analysis of variance showed the existence of a very highly significant difference between species ($p = 0.0002$) for the parameter leaves number per plant.

The analysis of variance showed the existence of a highly significant difference between species ($p = 0.007$) for the parameter stems number per plant.

Kruskal-Wallis test calculations gave us the following results:

Chi-squared = 15.045, $df = 2$, p -value = 0.000541 for leaves.

Chi-squared = 9.6434, $df = 2$, p -value = 0.008053 for stems.

The p -value found reflects the existence of a highly significant difference between the medians of the three species (Fig. 3).

According to the obtained results, these two traits are positively correlated ($r = 0.67$; $p < 0.000$). So species which have the high number of stems will have systematically a high number of leaves.

The lowest leaf and stem numbers are found in *Hedysarum carnosum* with an average number of 155.07 and 6.57 respectively. The highest are those of *Hedysarum coronarium* which is registered an average leaf number as 454.6 and stem number as

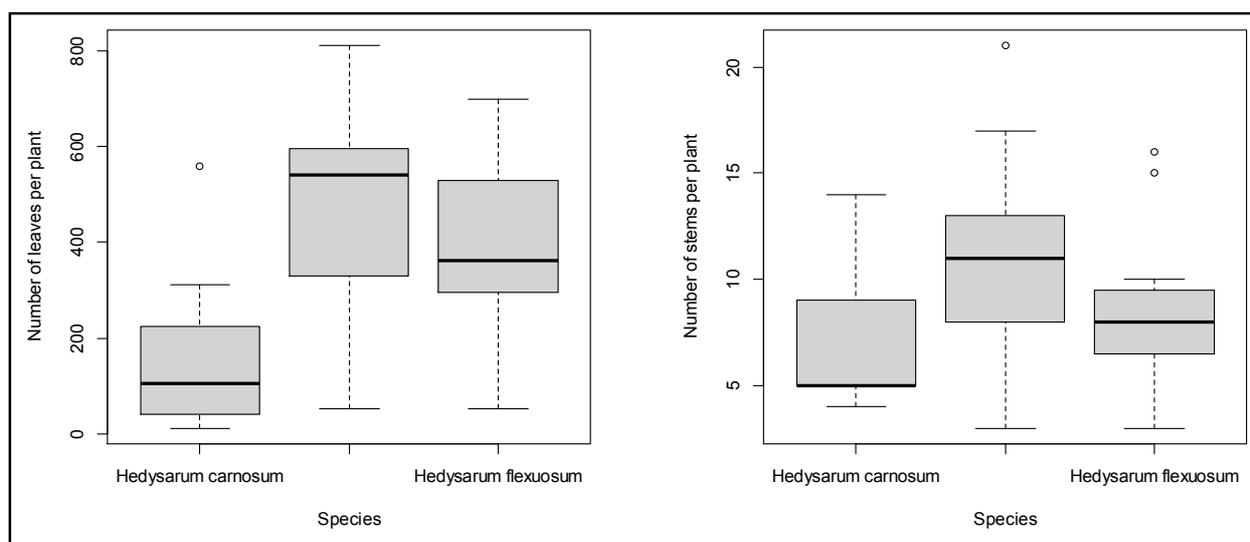


Fig. 3. Number of leaves and stems per plant for the studied species of Hedysarum.

11.07. Finally, the intermediate averages are manifested by *Hedysarum flexuosum*, with corresponding consecutive leaves and stems numbers of 389.8 and 8.53 (Fig. 3).

Leaves and stems fresh weight

The values of leaves and stems fresh weight are very important for the studied species, and very highly and positive correlation is recorded ($r=0.718$; $p<0.000$) between these traits.

The results of two way ANOVA indicate no significant difference between species for these two traits ($p=0.98$ and $0.49>0.05$ respectively). This means that the three species have nearly a similar weight of fresh leaves and stems. Furthermore, leaves fresh weight presents 57.01% of the plant fresh weight for *Hedysarum carnosum*, while it presents 53% and 49% for *H. flexuosum* and *H. coronarium* respectively.

The higher mean values for leaves fresh weight was obtained by *Hedysarum carnosum* (130.01 g) followed by *Hedysarum flexuosum* (126.61 g) and finally comes *Hedysarum coronarium* with 124.92 g (figure 4). Whereas, the highest mean value of stems fresh weight is presented by *Hedysarum coronarium* with 121.64 g, *Hedysarum flexuosum* and *Hedysarum carnosum* showed 102.66 g and 96.11 g respectively (Fig, 4). According to the previous results, *Hedysarum flexuosum* takes the middle place between other species for the studied traits. Zirmi-Zembri and Kadi (2020) recorded very low values of leaf and stem fresh weight on *H. flexuosum* populations of Kabylie region : from 30.42 g to 31.39 g for leaf fresh weight and from 56.76 g to 65.19 g for stem fresh weight.

According to the study of Rabhi et al. (2017), these parameters decrease clearly in soils poor in mineral elements especially Mg^{++} , the authors noticed that the fresh weight of plants of sulla (*H. carnosum*)

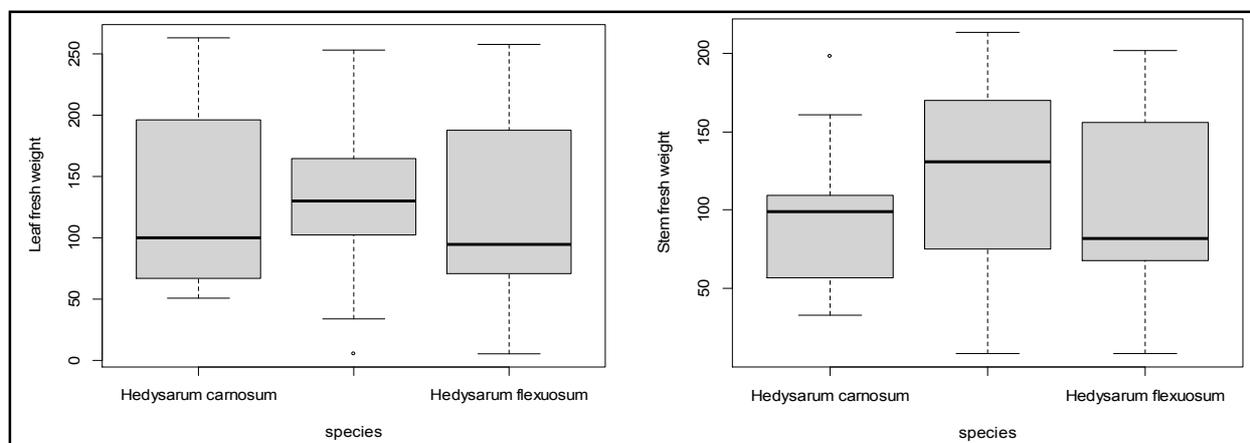


Fig. 4. Boxplots of leaf fresh weight and stem fresh weight for the studied species of Hedysarum.

was reduced by 43.6% in the deficient treatment when compared to the control.

Leaf/stem ratio for fresh weight

This trait is considered as a reference mark of the use of any plant as forage or livestock feed. It shows how plants were invested their energy to produce an enormous quantity of leaves compared to the stems or the opposite.

Our three cultivated species were showed close value of leaves to stems ratio; there is no significant difference between species ($p > 0.05$): 1.25, 1.40 and 1.44 for *H. coronarium*, *H. flexuosum* and *H. carnosum* respectively. Moreover, these values are considered as very important regard to the fodder production; that's why the literature announce that if any plant shows a value of leaf/stem ratio superior to 1 means that plant produces more leaves than stems, so it's more palatable for animals.

Moussaouali et Hamdi-Aïssa (2017) were found the same ratio in their experiment on *H. coronarium* in the region of Ghardaïa (1.28 on average); in the same way, Abdelguerfi (2002) was registered ratios which vary from 0.44 to 1.32 for some Algerian populations of Sulla. In the study of Zirmi-Zembri and Kadi (2020) on *H. flexuosum*, they found values close to ours in two different phenological stages: 0.54 at budding stage and 0.44 at flowering stage. Borreani (2003) reported that the values of leaf to stem ration on *H. coronarium* range between stages and years from 5.6 at late vegetative stage to 0.2 at seed set (Brown florets, green loment).

Forage dry yields (t DM/ha)

Sulla species have a high potential of production and forage fresh and dry yields. In this trial, three species of Sulla were produced a very important amounts of forage dry yields: 2.93, 3.18 and 3.29 t DM/ha for *H. carnosum*, *H. flexuosum* and *H. coronarium* respectively.

The analysis of variance did not reveal any significant difference between species for this parameter ($p = 0.88$).

The Algerian populations of Sulla coming from the high altitude regions are characterized by a low vegetative growth (Issolah et al. 2014).

According to the previous study of Moussaouali et Hamdi-Aïssa (2017), *Hedysarum coronarium* produces well in this region, they found

3.392 t DM/ha against 2.45 t DM/ha in its native region. Ben Jeddi (2005) has found the maximum yield reached by *H. coronarium* at the flowering stage was 6.2 t DM/ha, that's to say 40 t of fresh matter per hectare. In cultivated trials of Sulla in Tunisia, the forage dry yields varied from 0.97 to 5.49 t DM/ha (Slim et al. 2012).

This parameter is influenced by some environmental and agricultural conditions such as temperature and rainfall, variety used and year of production (the 1st or the 2nd). Moreover, forage dry yield of Sulla is closely related to the phenological stage which the forage is exploited (Kadi et al. 2011).

Specific Leaf Area (SLA)

The SLA represents the surface of luminous interception per unit of dry mass foliar. It's considered, in many cases, a good indicator of photosynthesis rate and potential relative growth rate.

In our study, the p-value found reflects the existence of a highly significant difference between the medians of SLA of these species (Table 3). The low value of SLA of *H. flexuosum* may be refers to its very important content of water in their leaves, because this parameter is calculated after oven-drying of leaves.

But, in general, these values of SLA reflect the adaptability of these three species in the new habitat, especially *H. coronarium* and *H. carnosum*, when we compare them to others taken from leaves of plants from native habitat. Despite the presence of salinity in the soil, the leaf area in not affected by this major problem (Tibaoui et al. 2018) widely existing in dried and hottest regions.

Moussaouali and Hamdi-Aïssa (2017) found value of 14,73 m²/kg MS in their study on *H. coronarium* carried out in the same region of Ghardaïa. This value is very closely similar to our values. They considered that *H. coronarium* has adopted an adaptive functional strategy in the new zone. According to Hodgson et al. (2011), this parameter informs us about the ecological strategy of plants, and it's the perfect soft plant trait used to assess soil fertility.

TABLE 3
Values of SLA recorded between species of Hedysarum (m².kg-1)

	Low value	High value	Average
<i>Hedysarum coronarium</i>	6.710	21.962	13.075
<i>Hedysarum flexuosum</i>	6.136	20.747	8.901
<i>Hedysarum carnosum</i>	7.167	23.144	13.088

The lowest SLA value (8.90 m²/kg) was found in *H. flexuosum*. These values reflect certainly the huge amount of water stored in the leaf (e.g.: leaf fresh weight = 4.71g and leaf dry weight = 0.55g; so water content represents 88.32%). Maximum SLA was observed in the annual grass *H. coronarium* and *H. carnosum* with an average of 13.07 and 13.09 m²/kg respectively. The content of water in the leaves of these species is in the rates of 83.04%.

Kruskal-Wallis test calculations gave us the following results:

Chi-squared = 44.601, df = 2, *p*-value = 2.065e-10.

The *p*-value found reflects the existence of a highly significant difference between the medians of the three groups (Fig. 5).

In order to determine between which species the difference is really occurred, we had to perform the multiple comparison tests with the Bonferroni adjustment method (Table 4).

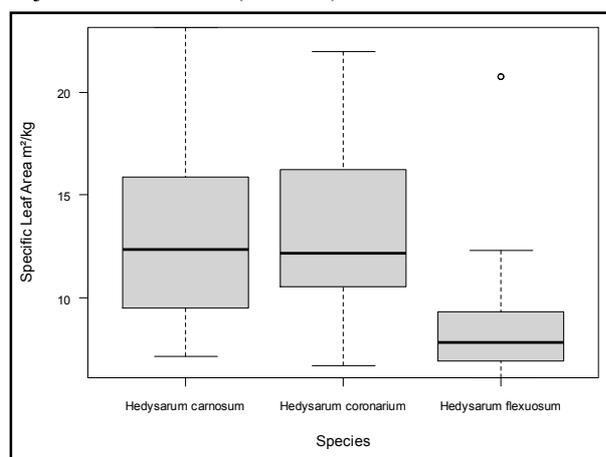


Fig. 5. Boxplots showed the difference between species in the SLA values.

TABLE 4

Level of difference in SLA values between the three cultivated species

	<i>Hedysarum carnosum</i>	<i>Hedysarum coronarium</i>
<i>Hedysarum coronarium</i>	1	-
<i>Hedysarum flexuosum</i>	4.1e-08	1.3e-08

Leaf Dry Matter Content (LDMC)

The analysis of variance of the leaf dry matter content revealed a difference highly significant between species and blocs (*p* = 2.2e-16 ; *p* = 8.844e-05).

H. coronarium shows the highest value of LDMC between these three studied species. On the other hand, *H. flexuosum* and *H. carnosum* registered lower values (Fig. 6).

LDMC varied from 70.48 to 126.37 mg/g for *H. carnosum* with an average of 101.966 mg/g between 36.85 and 139.10 mg/g for *H. flexuosum* with an average of 116.24 mg/g and for *H. coronarium* they changed between 128.52 and 248.57 mg/g with an average of 177.2 mg/g. The values of *H. coronarium* are very close to the works of Moussaouali and Hamdi-Aïssa (2017), they found an average of 172.86 mg/g in the same region.

LDMC is a feature reflecting the organic matter storage capacity within the leaf and generally at the life duration of the leaves (Chalmandrier 2008). High values of LDMC characterize plants that their foliar organs accumulate effectively nutriment and constitute very important pools of storage with weak turnover of tissues.

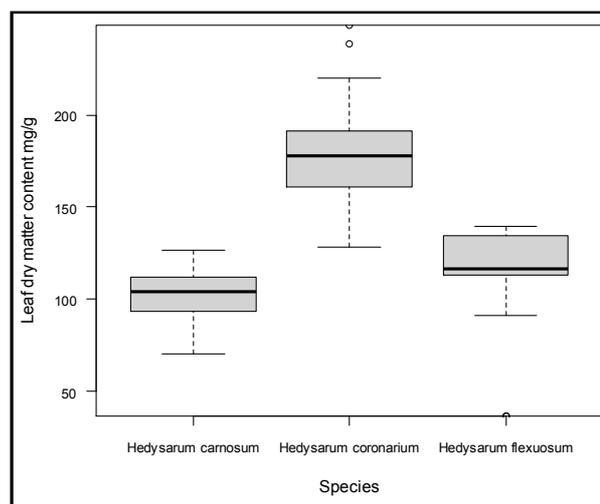


Fig. 6. Boxplots showed the difference between species in the LDMC values.

Correlation between SLA and LDMC

The study of correlation between SLA and LDMC led us to conclude that there is a relationship between these two parameters, which are very important traits in plant ecology and they are the indicator traits of resource-use strategies because they are associated with many critical aspects of survival and plant growth (Garnier et al. 2001). Some studies in controlled environments have showed the important roles played by these traits in the explanation of the variation in potential relative growth rate RGR and ecological compartment of plants (Li et al. 2005). So, it's important to assess these traits for various plant species in different environments.

TABLE 5
Coefficients of correlation between parameters of the species

	Plant height	Stem number	Leaf number	Weight of fresh leaves	Weight of fresh stems	Leaf to stem ratio	Dry Biomass t/ha
Plant height	1	0.56	0.82	0.43	0.67	-0.32	0.67
Stem number	0.56	1	0.67	0.39	0.41	-0.03	0.41
Leaf number	0.82	0.67	1	0.55	0.65	-0.16	0.65
Weight of fresh leaves	0.43	0.39	0.55	1	0.72	0.18	0.72
Weight of fresh stems	0.67	0.41	0.65	0.72	1	-0.41	1
Leaf to stem ratio	-0.32	-0.03	-0.16	0.18	-0.41	1	-0.41
Dry Biomass t/ha	0.67	0.41	0.65	0.72	1	-0.41	1

In the literature, at (high SLA, low LDMC species) means rapid biomass production and at (low SLA, high LDMC species) means efficient conservation of nutrients.

Correlation between other parameters (between species confused)

In this study, significant effects of environment were observed in the majority of growth traits between species. The growth or allometric relationship between morphological traits measured and their significance level were monitored (Fig. 7).

For the 49 possible correlations between measured parameters, 39 were significant (Table 5) with correlation values ranging from low 0.181 ($p < 0.000$) to high 0.823 ($p < 0.000$).

The most significant negative correlations were found between weight of fresh stems and leaf to stem ratio ($p < 0.01 = 0.006$) while the most significant positive correlations were observed between plant height and leaf number ($p < 0.000$).

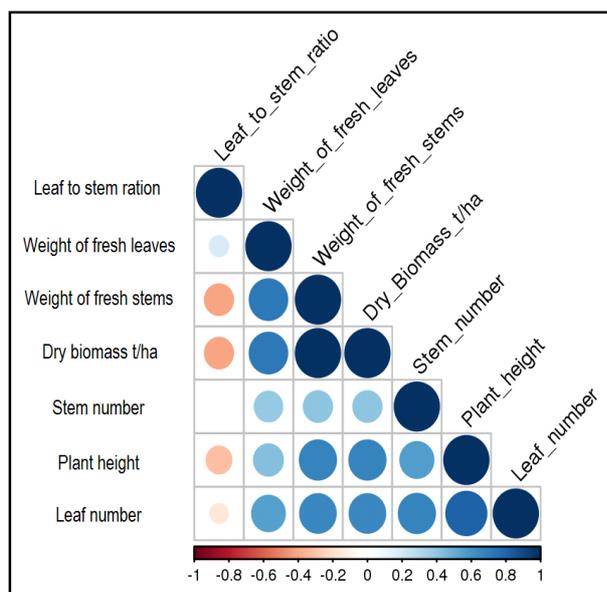


Fig. 7. Correlogram between all parameters of the three studied species of Hedysarum.

CONCLUSION

Results of the present study showed the best adaptability of these species in the agro-ecological conditions of the new habitat. *H. coronarium* (collected from Sétif province) could be considered the best species from the point of view of its plant height, leaves and stems number, plant weight, forage yield and LDMC, while *H. flexuosum* (collected from Tizi-Ouzou province) takes the second place concerning the whole of traits except SLA. These species provide values of leaf to stem ratio (approximately the half), so they can produce a huge quantity of forage yield.

The comparative study of forage production and the phenotypic characterization of the three species of *Hedysarum* genus cultivated in the new habitat under irrigation for above traits revealed a large degree of variation. There was a very important variability for all the parameters. Majority of results obtained in the present study have made possible to consider *H. coronarium* produces more forage yield crop than other species, and it's important for livestock feed and forage production calendar.

The findings of this study can be noticed for proper range management, conservation and development for this valuable species in such conditions. Sulla species can play a goal role in the sustainable development in this region habitually considered marginal. More studies and researches should be conducted to assess the chemical composition of these species in the same conditions to approve their nutritive value, and even trying to add other species of *Hedysarum* genus to the study.

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