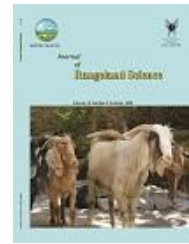




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Research and Full Length Article:

Morphological Diversity Assessment of Five Populations of Sulla (*Hedysarum flexuosum* L.) Harvested at Five Phenological Stages in Kabylie region (Algeria)

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Abstract. *Hedysarum flexuosum* L. is an important forage legume with high nutritive value. The determination of forage production in natural habitats is the most important factor for grazing management. Studies on its habitat characteristics and distribution are scarce. This research was conducted in order to evaluate the extent of pheno-morphological diversity in natural populations of *Hedysarum flexuosum* from different environments throughout Tizi-Ouzou province (Algeria), and to analyse the relationships among the diversity patterns and environmental parameters. Some morphological characters that reflect the behaviour and diversity of biogeographical origin of five natural populations of *Hedysarum flexuosum* collected *in situ* under edaphic and climatic conditions of the region of Kabylie, for five phenological stages (vegetative growth, budding, flowering, seed setting and seed ripening), were determined. The sites have been chosen following the gradient East (Souama), West (Sidi Naamane), North (Timizart), and South (Ait Toudert) and site in the Center (Bousmahel). Plant samples harvested from 12 plants for stems and leaves parameters as a replication in each phenological stage. The parameters were: leaves number per plant, leaves number per stem, stems number per plant, weight of fresh leaves, weight dried leaves, weight of fresh stems and weight dried stems, Leaf/stem ratio for fresh and dry weight, and forage dry yields. Soil samples were analysed to determine pH, EC, total limestone, organic carbon percentage and soil texture. Results showed that this species grows in soils with lime amount between 0.63 to 20%, clay or clay loam texture, acidity from 7.62 to 8.40 and organic matters from 0.21 to 2.54%. Furthermore, results showed significant effect ($p < 5\%$) of *H. flexuosum* populations (sampling sites) and phenological stages on all traits, except leaf/stem ratio (fresh weight and dry weight) for sites. The findings of this study can be noticed for proper range management, conservation and development for this valuable species in such conditions.

Key words: *Sulla flexuosa*, Populations, Morphological characters, Phenological stage, Algeria

Introduction

The Mediterranean region is very rich in biodiversity and continues to be the source of new traits, particularly resistance to drought and salinity, sought for improved production of a large number of important crops at the global level, such as legumes. Thus legumes, through their agronomic, food and ecological interests, are currently the focus of international organizations. Bioclimatic variation give rise to very diverse types of vegetation as forests, maquis, matorrals, meadows, pastures, rangelands, grasslands and steppes. The combination of several factors, especially anthropogenic actions associated with climate change causing the decline of biodiversity in the world. In North Africa, the climate has become drier and pastoralists are facing the problem livestock feed. In Algeria, forage and pastoral production is less than livestock needs (Abdelguerfi and Laouar, 1999). The area fodder in the province of Tizi-Ouzou (cultivated and natural fodder) is estimated about 15387 ha and remain insufficient, taking into account the needs of the livestock that count 70274 cattle, 113669 sheep and 38688 goats according to DSA (2019). Algeria has a very rich heritage of spontaneous forage species that, used without any improvement, could mitigate the combined effects of lack of quantity and quality of forage (Abdelguerfi and Laouar, 1999). Inputs of certain species are at the same level, or even better than some fodder resources cultivated (Zirmi-Zembri and Kadi, 2016; Kadi and Zirmi-Zembri, 2016). Therefore, the preservation and valorisation of local phylogenetic resource, particularly *Hedysarum*, could play a significant role in the increase of forage production in Algeria. The use of adapting species is the first step in the long challenges to find solutions to resorb the feed deficit.

Hedysarum flexuosum L., syn. *Sulla flexuosa* [L.] Medik. is part of the genus *Hedysarum*, it is an annual leguminous plant

originating from the western Mediterranean region and North Africa (Le Houérou, 2001). There is evidence that forage legumes, as components of mixed grass-legume swards, can provide multiple benefits to agriculture by acting at different stages in the soil-plant-animal- atmosphere system. The species plays a key role in organic production and low-input oriented agriculture, and commonly used to enhance the productivity and sustainability of farming systems. *H. flexuosum* is a multi-use species that could be considered from different viewpoints. There is a growing interest in *Sulla* in traditional and in non-traditional areas (particularly in New Zealand and Australia), due to its excellent adaptability to marginal and drought prone environments (Borreani *et al.*, 2003; Annicchiarico *et al.*, 2008). It is a forage used for grazing (De Koning *et al.*, 2010), hay (Foster, 2010) and silage production (Slim and Ben Djeddi, 2012); and good quality forage, with high protein content (Kadi *et al.*, 2015; Zirmi-Zembri and Kadi, 2020). Because of its spontaneous and uncultivated forage character, *Hedysarum flexuosum*, commonly known as *Sulla*, is unfortunately, ranked on the red list of species at high risk of extinction (Groom, 2012). The aim of the present study was to evaluate the extent of phenological diversity in natural populations of *H. flexuosum* L. from different environments throughout Tizi-Ouzou Province, and to analyse the relationships among the diversity patterns and environmental parameters of the collection sites.

Materials and Methods

Study area

The study was carried out in Tizi-Ouzou province, which is located between 36°43' and 36°91' latitude north and between 3°79' and 4°72' longitude east and covers an area of 3993 km² (Fig. 1). This province is

located in the hotspot Kabyles-Numidie-Kroumirie according to Véla and Benhouhou (2007), in the North of Algeria, 100 km east of the capital Algiers. The climate of study area, classified as Mediterranean, is characterized by summer water deficit, growth period in spring and autumn, where the average annual precipitation is around 800 to 1200 mm (ONM, 2020)

Methodology

After investigations followed by an ethnobotanical survey on the use of *H. flexuosum* in animal feed carried out in 2018 in the same region (Zirmi-Zembri and Kadi, 2019), we were able to identify the natural zones of presence of this forage legume. It is on this basis that we selected the representative stations of this study. Five stations were selected following the gradient East (Souama), West (Sidi Naamane), North (Timizart), South (Ait Toudert) and including a site in the Center (Bousmahel) of Tizi-Ouzou province (Table 1 and Fig. 1).

Determination of soil characteristics

Soil sampling was performed using a diagonal randomized method (10 samples at each site) at a depth of 0-10cm and 10-20cm (depending on depth of root development and low depth of mountainous area) in September 2018. For each station, we have formed two composite soil samples that were analyzed in the soil analysis laboratory of Mouloud Mammeri University (Tizi-Ouzou, Algeria) to determine the following characteristics:

- a) Acidity (pH), measured by a pH meter.
- b) Electrical Conductivity (EC) determined using EC meter; it is a tool to estimate soil salinity.
- c) Total limestone and Organic carbon percentage, determined using burning and weighing method (Nelson, 1982).

- d) Soil texture measurements, using a standard lab hydrometer method (SSC-Orstom).

Evaluation of the quality and forage production of *H. flexuosum*

Five populations of *H. flexuosum* were evaluated for some morphological characters and forage dry matter yield. Forage samples were taken at five phenological stages (Fig. 2) according to Zirmi-Zembri and Kadi (2020, Fig.3), including vegetative growth (stage2), Budding (stage 4), flowering (stage 6), seed setting (stage 8) and seed ripening (stage 9) over the five populations. Vegetation sampling was performed using random systematic method, between March and June 2019 in the morning. Samples were transported to the animal nutrition and animal products laboratory of Mouloud Mammeri University (Tizi-Ouzou). Then, we accounted for each 12 plants the number of leaves and stems (LN/P, LN/S, SN/P) and, immediately, separated fresh leaves and stems were weighed (WFL, WFS). To determine the leaves and stems dry weight (WDL, WDS), fresh samples were oven dried at 60°C for 48 hours. Three hundred plants were harvested for the five stages and for the five populations.

To determine the leaf /stem ratio (fresh and dry weight), we divided leaves weight (fresh and dry) over stems weight (fresh and dry) respectively.

To determine fresh yield, a quadrat 50×50 cm randomly laid out in each plot (Fig.2) and plants manually clipped at ground level using a small scythe. The plants from each plot were weighed separately to determine fresh yields of populations for each phenological stages (kg/0.25 m²). These samples were oven dried at 60°C for 48 hours to get dry matter (DM) percentage and forage dry matter yield. The data were then converted to ton/ha.

Statistical Analyses

All statistical analyses were performed using R software.3.6.1. For all measured parameters, differences between the stations and phenological stages (soil and

morphological parameters) were calculated using Two-way and/or one-way analysis of variance (ANOVA) and means comparisons were made using Tukey pairwise test ($P \leq 0.05$).

Table1. Geographic and topographic data of prospected populations for morphological analysis of *H. Flexuosum*

| Stations | Latitudes | Longitudes | Elevation (m) | Topography | Exposition |
|--------------|----------------|---------------|---------------|------------|------------|
| Ait toudert | 36°32'27.05" N | 4°08'48.51" E | 372 | Slope | South-East |
| Bousmahel | 36°40'54.03" N | 4°08'54.42" E | 186 | Slope | South-West |
| Sidi naamane | 36°45'51.11" N | 3°59'59.54" E | 110 | Slope | South-East |
| Souamaa | 36°38'53.38" N | 4°21'11.55" E | 340 | Slope | South-East |
| Timizart | 36°45'32.71" N | 4°14'30.27" E | 219 | Slope | South-West |

N: north; E: east

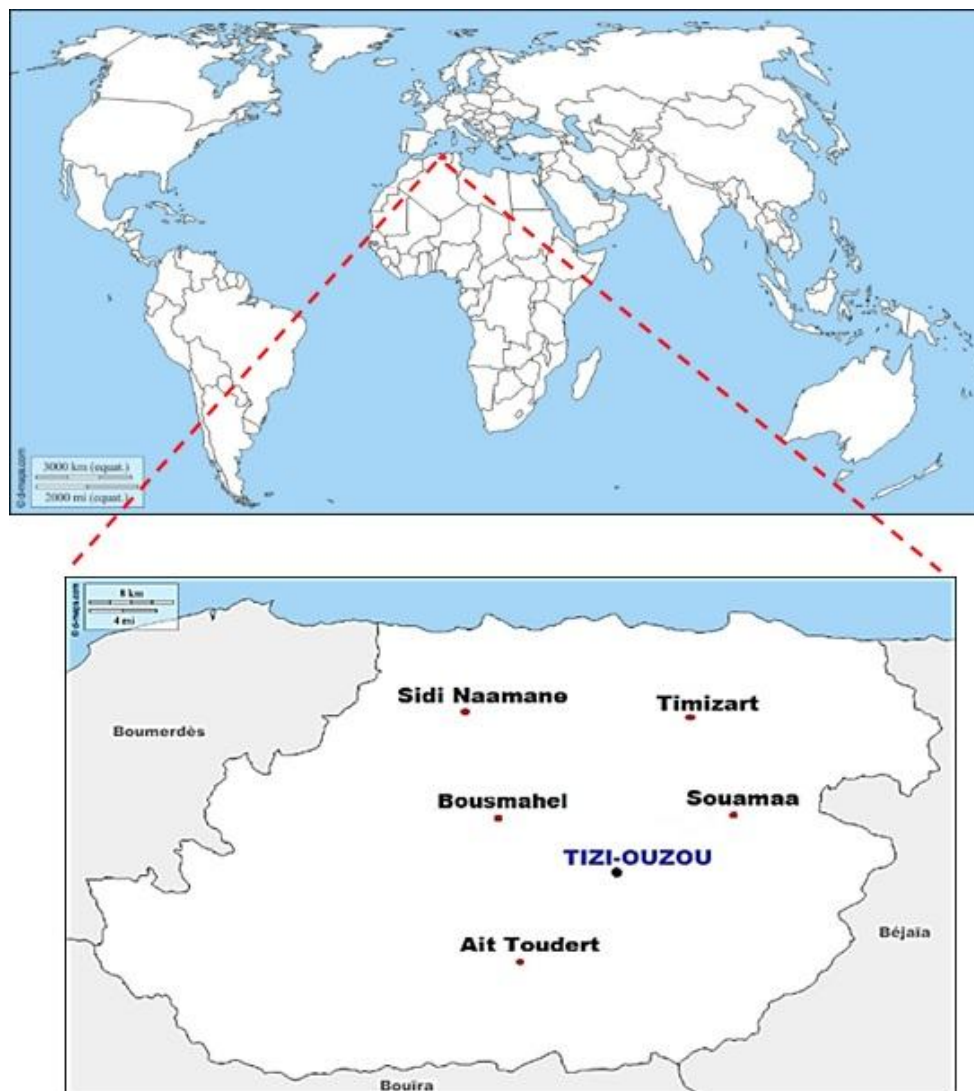


Fig.1. Geographical location of the five stations of *Hedysarum flexuosum* in Tizi-Ouzou province, Algeria



Fig. 2. A, B, C, D and E: Different harvesting stages of *Hedysarum flexuosum*. A-vegetative growth (stage 2), B- Budding (stage 4), C-flowering (stage 6), D-seed setting (stage 8) and E- seed ripening (stage 9), F: quadrat 0.5×0.5 m, G: weighing forage fresh yield

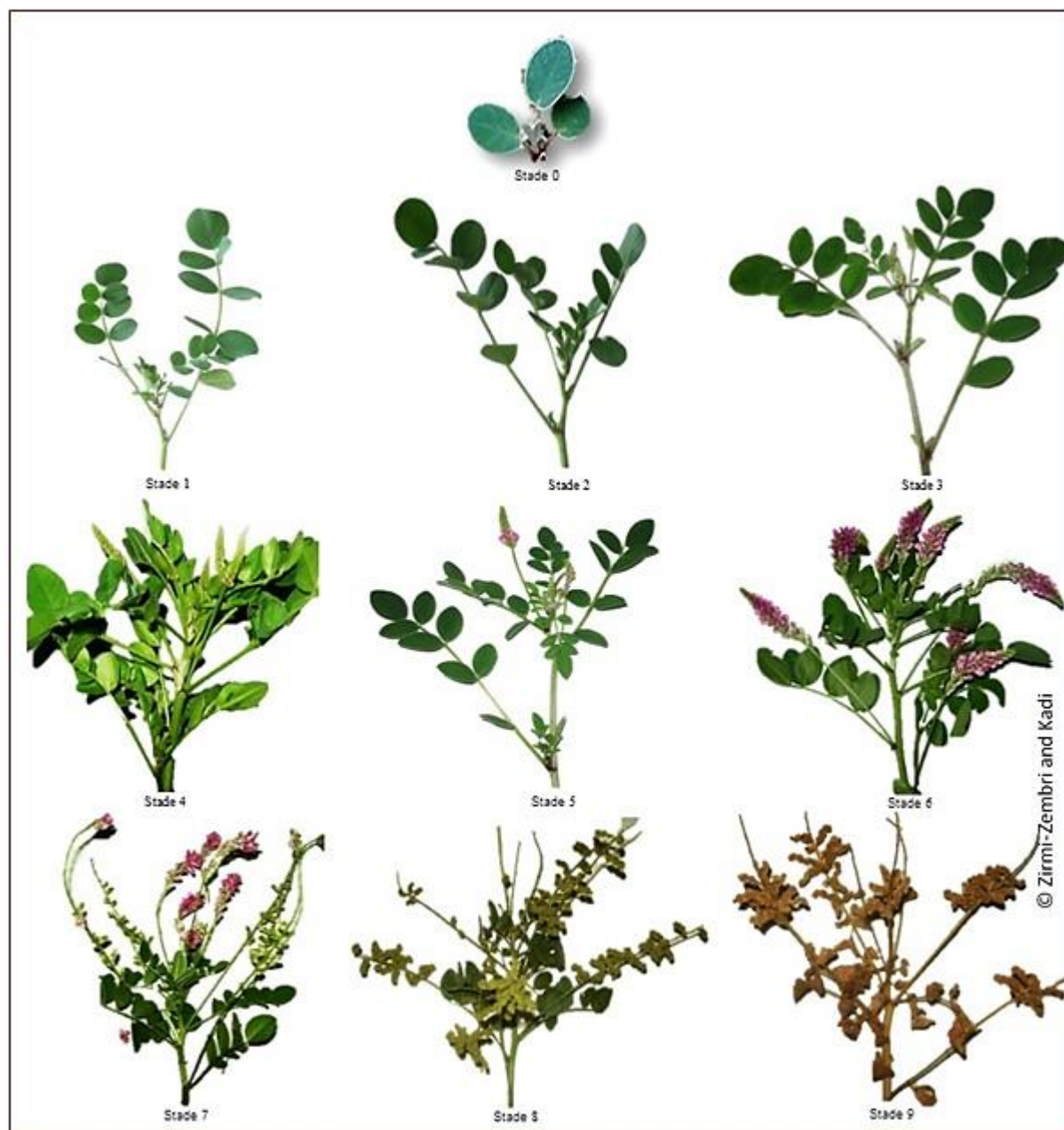


Fig.3. Aspect of *Hedysarum flexuosum* according to phenological stage (Zirmi-Zembri and Kadi, 2020)

Results

Results showed that the five stations of *Hedysarum flexuosum*, located in the province of Tizi-Ouzou, were at altitude of 110 to 372 m above sea level. Abdelguerfi (2002) reported that *H. flexuosum* has never been encountered at more than 600 m altitude. The sites were slope topography and oriented southeast for the populations of Sidi Naamane, Souama and Ait Toudert,

while Bousmahel and Timizart populations have a southeast exposure. Tizi-Ouzou province constitutes one of the most wooded in the country (38% afforestation rate), due to favorable bioclimatic conditions (sub-humid and humid).

Soil parameters

According to the results obtained from the analysis of soil properties (Table 2), there

were significant differences between the five sites for all traits such as acidity, organic matter percentages (10-20cm) and total limestone ($p < 0.001$). Electrical conductivity and organic matter percentage in depth 0-10cm ($p < 0.01$). There was no significant difference between the means of EC (10-20cm) from the five soils of the stations studied. The mean value of EC oscillates between 141.30 to 178.95 $\mu\text{s/cm}$, in 0-10cm depth and 119.40 to 137.70 $\mu\text{s/cm}$ in 10-20cm depth.

The five soils are alkaline with acidity ranged from 7.62 to 8.36 in 0-10cm depth and 7.86 to 8.40 in 10-20cm depth. It can be seen from data in Table 2 that soils pH of Ait Toudert (8.32), Bousmahel (8.31) and Timizart (8.36) reported significantly higher than Souama (7.62) and Sidi Naamane (7.80) soils in 0-10cm depth. Concerning soils from the depth of 10-20cm, the trend is the same as for the depth 0-10cm with higher alkalinity (Souama; 7.86; Sidi Naamane: 8.17; Ait Toudert: 8.32; Bousmahel: 8.35 and Timizart: 8.40). The highest value was obtained on Timizart site and the lowest was from Souama.

On the other hand, for total limestone, there was a great variability from soil to another, Sidi Naamane and Souama obtained the minimum value i.e. 0.63%, 3.13% in 0-10cm depth and 1.88%, 0.63% in 10-20cm depth respectively. Bousmahel and Timizart stations obtained the largest percentages with 17.5% in 0-10cm depth and 18.75%, 20% respectively. Nevertheless, Ait Toudert soil presented average values intermediate of

total limestone, 10.63% and 9.38%, respectively from depths 0-10cm and 10-20cm.

We also noticed that results of edaphic data showed that the soils of the five areas presented significant differences for organic carbon percentage. For the 0-10 cm depth, the percentage of organic matter divided the five Sulla soils into two groups; Souama, Bousmahel, Ait Toudert and Sidi Naamane dosing the highest values 2.54, 2.33, 2.22 and 2.22% respectively, in contrast, - Timizart presented the lowest rate (1.80 %). More in depth, from 10-20 cm, there is a clear trend of decreasing of the organic matter rates for all the soils of the five stations. The soils of Ait Toudert and Timizart dosed the lowest rates: 0.21% and 0.53% respectively. On the other hand, the soils of Bousmahel and Souama contained the highest percentages (2.12%). The soil of Sidi Naamane presented an intermediate organic matter rate (1.59%) between the two preceding groups of soils.

Soil texture were detailed in Table 3, the clay content was twice as high in Ait Toudert soil than in the other four soils, with 60.52% against (31.26 to 37.36%) respectively in 0-10cm depth. While at 10-20cm depth, Souama (45.16%) and Timizart (59.73%) soils dosed more clay, nevertheless, Ait Toudert, Bousmahel and Sidi Naamane soils stays in the same percentages with the first depth. As a result, all the soil in *Hedysarum flexuosum* habitats was clayey or clayey-loamy texture.

Table 2. Values of soil physico-chemical parameters in five habitats of *H. flexuosum*

| Sits | pH | | EC ($\mu\text{s/cm}$) | | Total limestone (%) | | Organic Matter (%) | |
|--------------|--------|---------|-------------------------|----------|---------------------|---------|--------------------|---------|
| | 0-10cm | 10-20cm | 0-10cm | 10-20cm | 0-10cm | 10-20cm | 0-10cm | 10-20cm |
| Ait Toudert | 8.32 a | 8.32 a | 149.85 b | 137.70 a | 10.63 b | 9.38 b | 2.22 a | 0.21 c |
| Bousmahel | 8.31 a | 8.35 a | 160.45 ab | 128.50 a | 17.50 a | 18.75 a | 2.33 a | 2.12 a |
| Sidi Naamane | 7.80 b | 8.17 b | 178.95 a | 123.55 a | 0.63 d | 1.88 c | 2.22 a | 1.59 b |
| Souama | 7.62 c | 7.86 c | 174.65 a | 119.40 a | 3.13 c | 0.63 d | 2.54 a | 2.12 a |
| Timizart | 8.36 a | 8.40 a | 141.30 b | 130.55 a | 17.50 a | 20.00 a | 1.80 b | 0.53 c |
| SEM | 0.08 | 0.06 | 3.80 | 1.96 | 1.90 | 2.14 | 0.06 | 0.21 |
| P value | <0.001 | <0.001 | <0.01 | 0.145 | <0.001 | <0.001 | <0.01 | <0.001 |

a, b, c, d Means in a column with different superscripts are significantly different ($p < 0.05$).

Table 3. Soil texture in the five stations of *H. flexuosum*

| Stations | 0-10cm | | | 10-20cm | | | Soil texture |
|--------------|----------|----------|----------|----------|----------|----------|--------------|
| | Clay (%) | Silt (%) | Sand (%) | Clay (%) | Silt (%) | Sand (%) | |
| Ait Toudert | 60.52 | 26.24 | 13.24 | 58.99 | 28.44 | 12.57 | clayey |
| Bousmahel | 31.26 | 37.89 | 30.85 | 33.86 | 33.58 | 32.56 | Clayey loam |
| Sidi Naamane | 37.10 | 25.61 | 37.29 | 33.99 | 27.52 | 38.49 | Clayey loam |
| Souama | 31.57 | 52.99 | 15.44 | 45.16 | 40.47 | 14.37 | Clayey loam |
| Timizart | 37.36 | 42.05 | 20.59 | 59.73 | 23.77 | 16.50 | clayey |

Agronomic traits and forage production of *Hedysarum flexuosum*

The results of two way ANOVA as shown in Table 4 presented significant differences among phenological stages, locations and location by phenological stages for all traits ($P < 0.01$), except leaf/stem ratio (fresh weight and dry weight) for locations .

Table 5 displays the results between the phenological stages. The higher mean values for leaf number per plant was obtained in flowering and seed setting stages (59.80 and 66.42) respectively, followed by budding stage (40.48) and lowest leaf number was found at seed ripening and late vegetative stages (26.63 and 19.13). Another important parameter leaf number per stem divided harvested stages to three groups, first which included seed setting (9.30), vegetative (8.61) and flowering (8.15) stages, secondly budding stage (6.46) and finally seed ripening stage (3.09).

Regarding stem number per plant, we noticed two groups; the first is composed only late vegetative stage with the lowest value (2.07), however second group is represented by budding, flowering, seed setting and seed ripening stages (6.84, 7.93, 8.04 and 7.03 respectively).

In addition, budding stage recorded the highest leaves fresh weight (57.38g) followed by flowering (50.58g), late vegetative (41.88g), than seed setting (19.71g); in contrast, the seed ripening registered the lowest value (4.26g). However, the phenological stage effect according to stem fresh weight, can be divided into several groups, first composed the highest value, measured at flowering

stage (112.28g), then budding (106.23g), followed by seed setting stage (91.14g) and the last group, included seed ripening (30.68g) and late vegetative stages (24.38g). Leaves dry weight was important at budding (8.10g) and flowering (8.34g) stages and minimum at seed setting (4.88g) and seed ripening (3.52g). Late vegetative stage with 5.85g obtained the intermediate value. Whereas DWS was highest at seed setting (18.25g) and seed ripening stages (16.33g), then flowering (12.90g) and budding (11.58g) stages ensued. Finally, with 2.33g of stem dry weight was weighed from late vegetative stage.

The values of leaf/stem ratio was clearly, most important for late vegetative stage (2.03 fresh weight and 3.19 dry weight), then we found budding (0.54 fresh weight and 0.78 dry weight) and flowering stages (0.44 fresh weight and 0.65 dry weight). Lowest stages composed of seed setting (0.19 fresh weight and 0.22 dry weight) and seed ripening (0.10 fresh weight and 0.15 dry weight).

The results, as shown in Table 6, indicate a significant difference between the studied populations (locations), for all of traits ($p < 0.01$) except leaf/stem ratio (fresh weight and dry weight).

Overall, these results reveal differences between different parameters of the five populations of *Hedysarum flexuosum*. Three groups stand out, the first group with the lowest values grouping the populations of Sidi Naamane and Souama leaf number per plant: 31.54 and 35.79; stem number per plant: 4.66 and 5.27; leaves fresh weight: 31.39g and 30.42g; stem fresh weight:

65.19g and 56.76g; leaves dry weight: 4.05g and 4.57g; stem dry weight: 10.29g and 10.43g, respectively). However, Ait Toudert and Bousmahel populations presented the highest values (leaf number per plant 43.64 and 47.28; stem number per plant: 7.28 and 7.62; leave fresh weight: 55.75g and 40.04g, stem fresh weight: 96.92g and 86.41g; leave dry weight: 8.59g and 7.10g; stem dry weight: 14.94g and 16.56g respectively). On the other hand, it has been noted for Timizart population intermediate values (leave number per plant: 42.26; stem number per plant: 6.53; leave fresh weight: 43.67g; stem fresh weight: 79.99g; leave dry weight: 6.67g and stem dry weight: 12.40g).

The value of leaf number per stem was higher in Timizart station (9.03), followed by Souama with intermediate value (8.06) and finally the lowest value were registered from Bousmahel, Sidi Naamane and Ait Toudert populations (6.77, 7.11 and 7.14 respectively).

What stands out in figure 4,5,6,7,8 and 9 is the global view of the evolution of

agronomic traits (leaves number per plant, stems number per plant, weight of fresh leaves, weight dried leaves, weight of fresh stems and weight dried stems) of *Hedysarum flexuosum* in the five stations (Ait toudert, Bousmahel, Sidi Naamane, Souamaa and Timizart) for the five phenological stages (vegetative, Budding, flowering, seed setting and seed ripening).

Forage yield in Souama, Timizart, Ait Toudert was average, ranging from 21 to 25 tons DM.ha⁻¹ (Fig. 10). The most productive population was Bousmahel population, with the mean for the five stages that was 37 tons DM. ha⁻¹, while the least productive population, with up to 20 tons DM.ha⁻¹ was Sidi Naamane population. Results showed that this species product a forage dry yield between 18 to 37 tons DM per hectare (Fig.10). The DM yields of the fives populations of this legume at a particular harvest stage are influenced by the environmental conditions and utilisation pattern.

Table 4. Summary of two-way ANOVA and level of significant MS for leaves and stem production of *H. flexuosum* harvested at five phenological stages in 5 research stations in Kabylie region

| SOV | DF | Leaves No./plant | Leaves No./Stem | Stem No./plant | Leaves FW | Stem FW | LSR FW | Leaves DW | Stem DW | LSR DW |
|--------------------|----|------------------|-----------------|----------------|-----------|----------|---------|-----------|-----------|----------|
| Phenological stage | 4 | 174994** | 1467.7** | 1685.7** | 122176** | 495430** | 128.9** | 1692.6** | 12099.1** | 318.06** |
| Location | 4 | 33569** | 18.95** | 540.8** | 16299** | 76537** | 6.05 | 724.8** | 3344.5** | 15.12 |
| Stage x Region | 16 | 15255** | 397.2** | 409.4** | 10223** | 85377** | 20.9** | 426.5** | 2587.4** | 36.18** |

*Significant at P < 0.05, ** Significant at P < 0.01, LSR= leaf/stem weight ratio, FW= fresh weight, DW= dry weight

Table 5. Mean comparison among five phenological stages for leaves and stem production of *H. flexuosum* averaged over 5 research stations in Kabylie region

| Phenological Stages | Leaves No/ plant | Leaves No./Stem | Stem No /plant | Leaves FW (g) | Stem FW (g) | LSR FW | Leaves DW(g) | Stem DW(g) | LSR DW |
|---------------------|------------------|-----------------|----------------|---------------|-------------|--------|--------------|------------|--------|
| Late vegetative | 19.13 c | 8.61a | 2.07b | 41.88b | 24.38c | 2.03a | 5.85b | 2.33c | 3.19a |
| Budding | 40.48 b | 6.46b | 6.84a | 57.38a | 106.23ab | 0.54b | 8.10a | 11.58b | 0.78b |
| Flowering | 59.80 a | 8.15a | 7.93a | 50.58ab | 112.28a | 0.44bc | 8.34a | 12.90b | 0.65b |
| Seed setting | 66.42 a | 9.30a | 8.04a | 19.71c | 91.14b | 0.19cd | 4.88bc | 18.25a | 0.22c |
| Seed ripening | 26.63 c | 3.09c | 7.03a | 4.26d | 30.68c | 0.10d | 3.52c | 16.33a | 0.15c |

Means, within columns, followed by the different letter are significantly different (P<0.05).

LSR= leaf/stem weight ratio, FW= fresh weight, DW= dry weight

Table 6. Mean comparison among five stations for leaves and stem production of *H. flexuosum* averaged over five phenological stages in Kabylie region

| Locations | Leaves No/ plant | Leaves No./Stem | Stem No/plant | Leaves FW (g) | Stem FW (g) | LSR FW | Leaves DW(g) | Stem DW(g) | LSR DW |
|--------------|------------------|-----------------|---------------|---------------|-------------|--------|--------------|------------|--------|
| Ait Toudert | 43.64ab | 7.14b | 7.28a | 55.75a | 96.92a | 0.73a | 8.59a | 14.94a | 0.58a |
| Bousmahel | 47.28a | 6.77b | 7.62a | 40.04b | 86.41ab | 0.51a | 7.10a | 16.56a | 0.43a |
| Sidi Naamane | 31.54b | 7.11b | 4.66c | 31.39b | 65.19bc | 0.58a | 4.05b | 10.29b | 0.63a |
| Souamaa | 35.79ab | 8.06ab | 5.27bc | 30.42b | 56.76c | 0.58a | 4.57b | 10.43b | 0.64a |
| Timizart | 42.26ab | 9.03a | 6.53ab | 43.67ab | 79.99ac | 0.73a | 6.67a | 12.40ab | 0.53a |

Means, within columns, followed by the different letter are significantly different (P<0.05).

LSR= leaf/stem weight ratio, FW= fresh weight, DW= dry weight

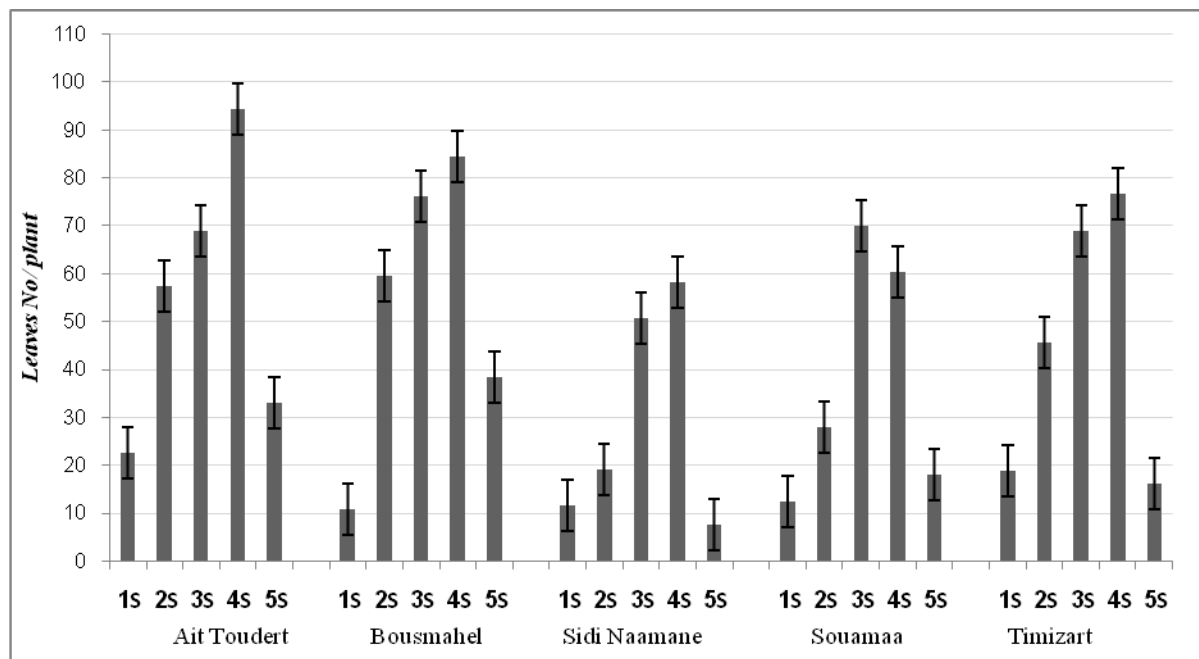


Fig. 4. Mean of leaves number per plant in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

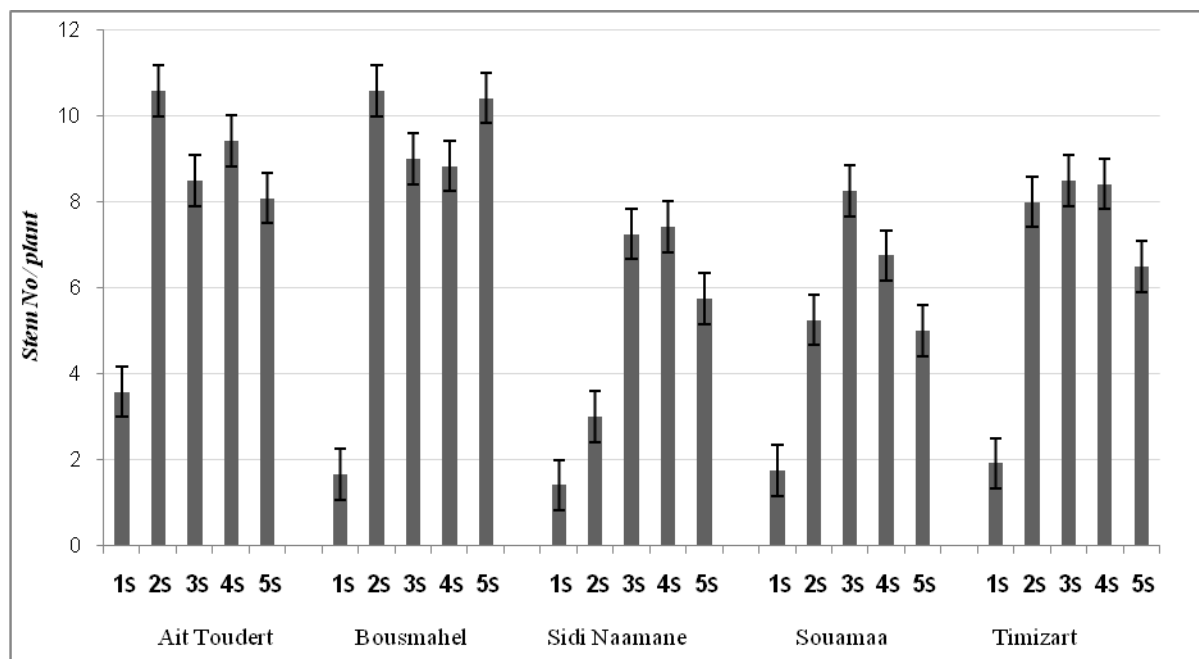


Fig. 5. Mean of stems number per plant in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

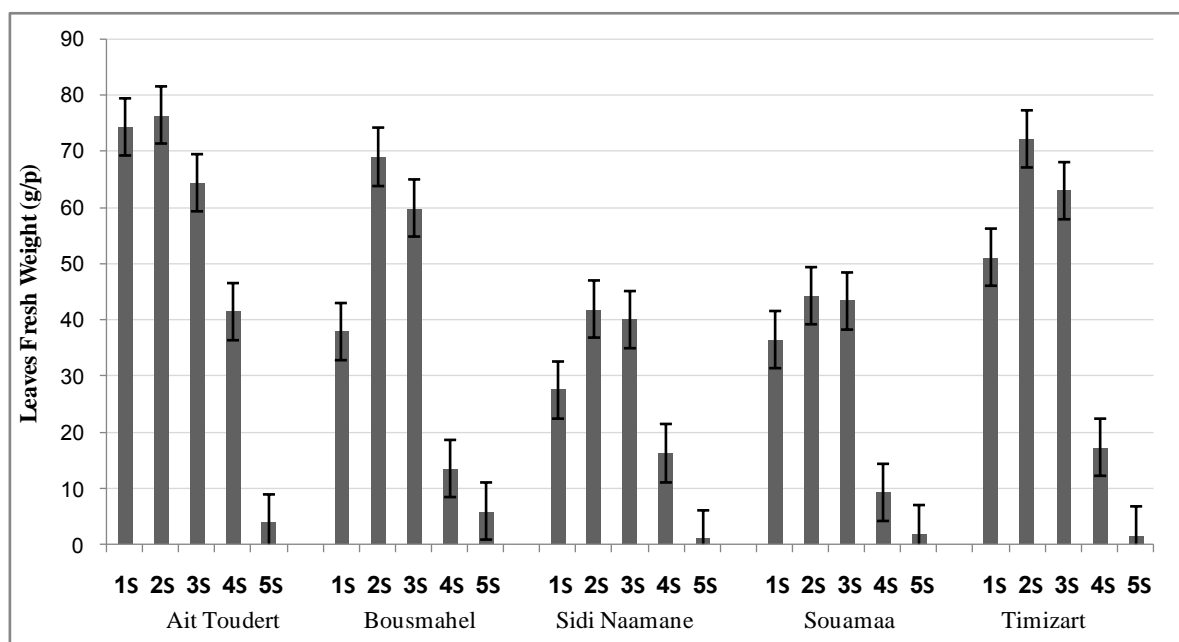


Fig. 6. Mean of leaves fresh weight in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

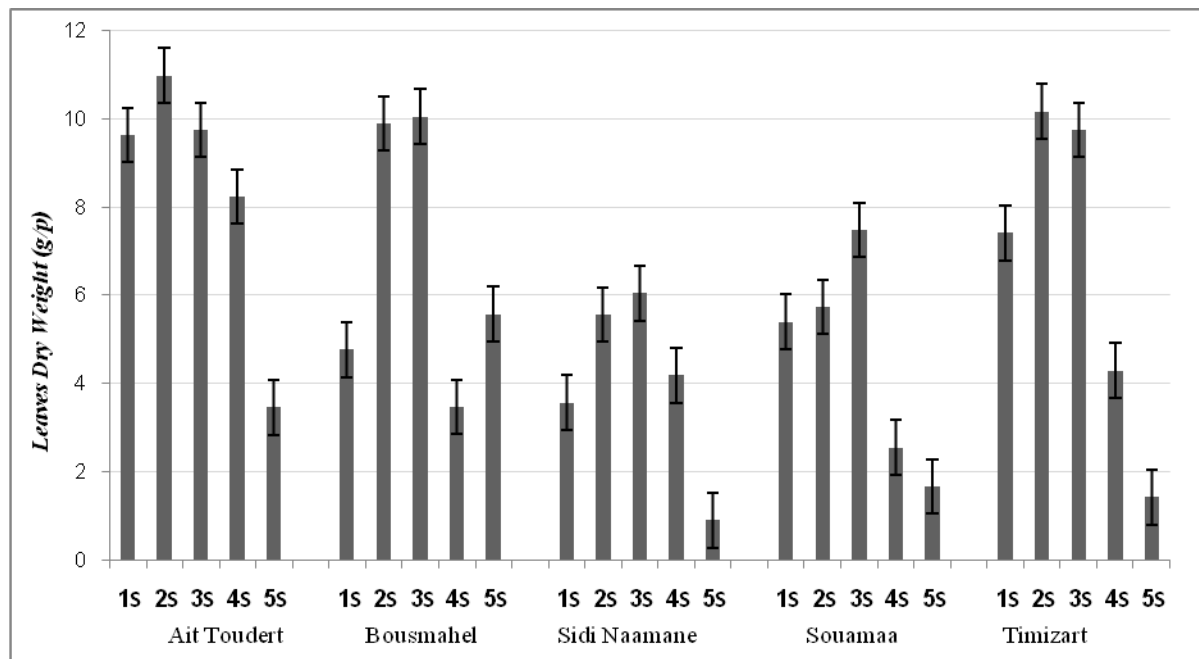


Fig. 7. Mean of leaves dry weight in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

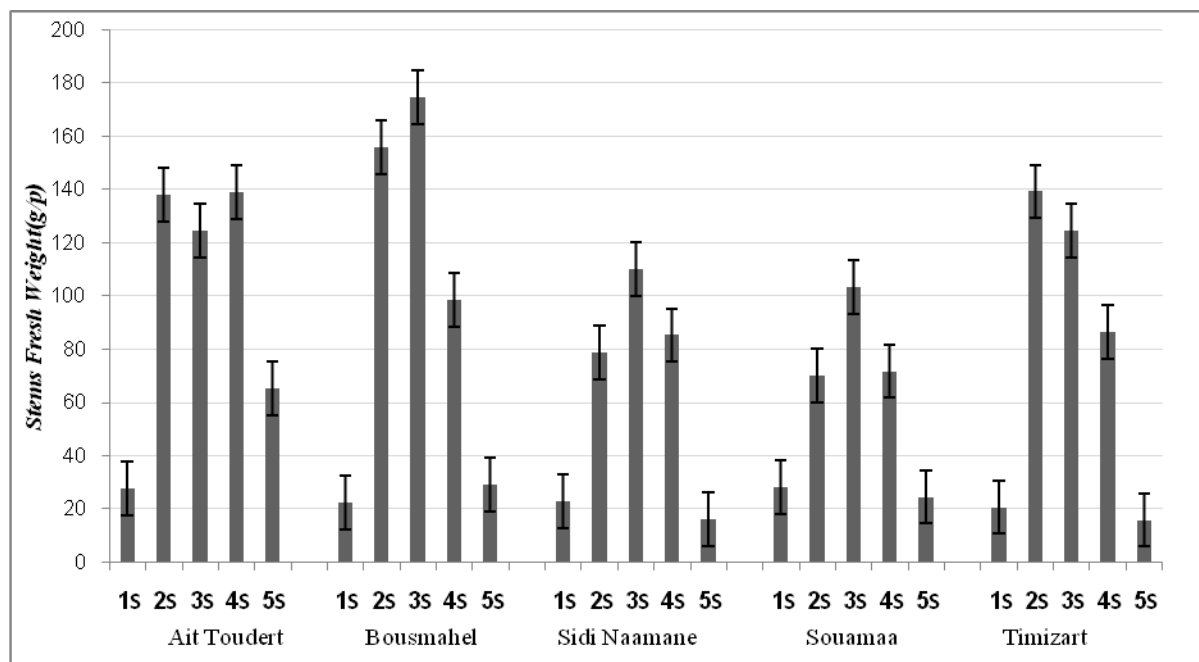


Fig. 8. Mean of stems fresh weight in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

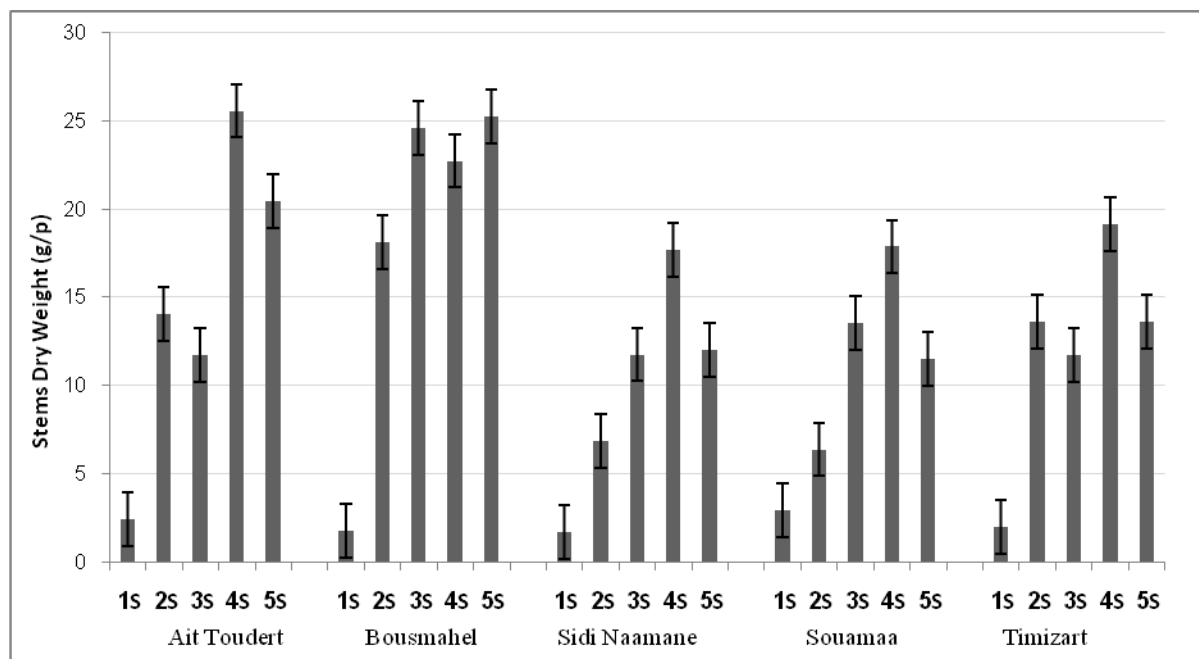


Fig. 9. Mean of stems dry weight in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

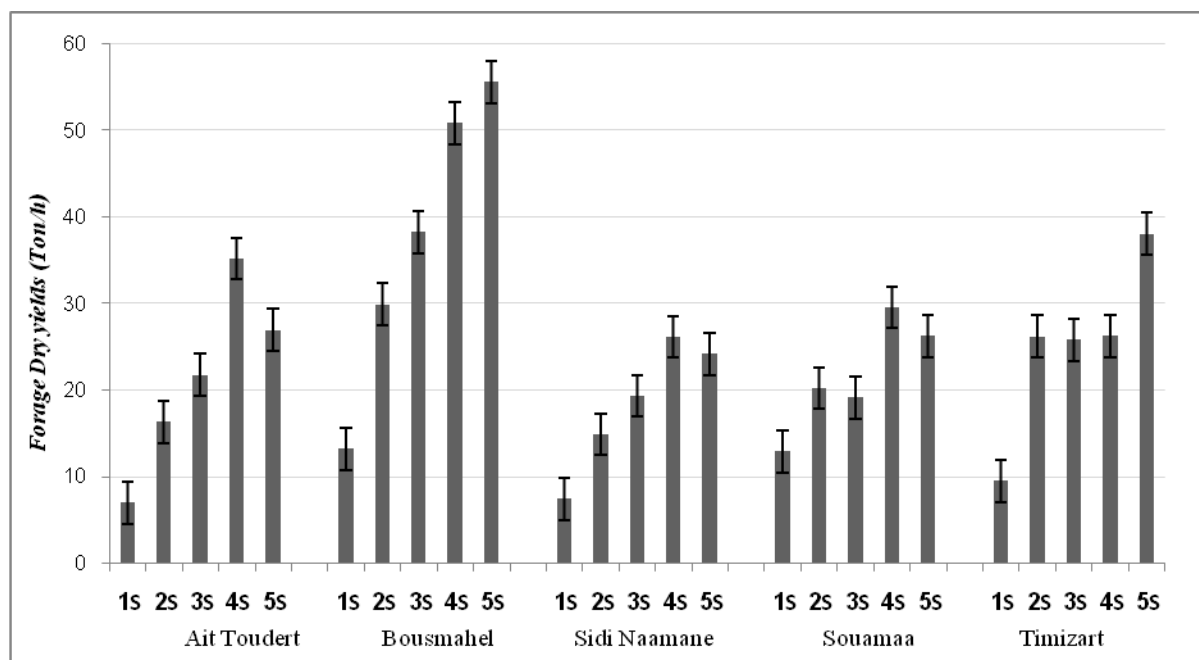


Fig. 10. Mean of forage dry matter yield in five phenological stages of *H. flexuosum* in five stations in Kabylie region. S₁ to S₅= vegetative, Budding, Flowering, Seed setting and Seed ripening, respectively

Discussion

Pastoralists used *H. flexuosum* species in green, pasture or forage at trough and/ or dry in form of hay in different phenological stages, from late vegetative stage to seed

ripening stage. A better knowledge of forage legume physiology in different environments is necessary to exploit their potential, *H. flexuosum* is a winter growing legume, here in Kabylie region, and few studies have

assessed *H. flexuosum* characteristics. Based on the findings of this research, result of analyses of variance showed a high variation ($P < 0.01$) of the physical and chemical characteristics between the different soils of the five populations of *H. flexuosum* except EC. On the other hand, results of edaphic data showed that the soil of the five areas had differences with each other for organic carbon (percentage), lime amount and soil texture. According to laboratory analyses results of soil properties (Table 2), *H. flexuosum* grows often in Kabylie region with clayey or clayey loamy soil texture. The results showed that this species grows in soils with lime amount between 0.63% to 20%, acidity from 7.62 to 8.40 and organic matters from 0.21% to 2.54%. Our results agree with the finding of Abdelguerfi-Berreki *et al.* (1991) who showed that this species grows on soils with average pH 7.93, and a limestone content total less than 20%. *H. flexuosum* is common on soils rich in clay with an average of 34.9%, which indicates that the texture is fine to very fine. Its location in certain regions would be due to the specific requirements of the species and/or the Plant/ Rhizobium pair. A two-way ANOVA revealed significant at $P < 0.01$ differences between five harvested stages. We have noticed that the number of leaves changes irregularly, increased at the beginning of the cycle until the beginning of budding, then this parameter increases in the seed ripening phase, the highest values noticed between budding and seed setting stages, finally, decrease towards haymaking. This means that the best operating period is between late budding and seed setting, however, Borreani *et al.* (2003), who studied the relationship between the composition of dry matter and the stage of development, have shown that the preferable stage is between stages 4 (budding) and 5 (early flowering). Leaf/stem ratio fresh weight higher than 1 means that the plants have invested more for the foliage production,

which is sought after for fodder intended for green feed and provides fodder quality. The data in Table 5, showed that the number of stems evolve with the development of the plant. Then we noticed a drop which is due to the withering of some secondary axes and ramifications probably caused by the decrease in soil water reserves which no longer reach full coverage, therefore we have witnessed the withering of the stems and ramifications less vigorous. The ratio fresh weight found oscillate between 0.10 to 2.03 from five stages and 0.51 to 0.73 from stations. These results agree with the results of Borreani *et al.* (2003) who states that Sulla has a high growth rate in the reproductive phase. The leaf/stem ratio decreases with development and this is due to the accumulation of raw cellulose more in the stems than in the leaves. In addition, results reflect those of Moussaouali and Hamdi Aissa (2017) who also found that leaf/ stem ratio fresh weight for 20 populations of *Sulla coronaria* at flowering stage varying from 0.81 (El Tarf) to 1.28 (Ghardaia). Leaf/stem ratio dry weight of *H. flexuosum* from the five stations was ranged (0.15 to 3.19) and from the five populations (0.53 to 0.64), this study produced results which corroborate the findings of Abdelguerfi (2002) who reported leaf/stem ratio oscillating between 0.44 and 1.32 from 17 populations of *Sulla coronaria*.

There were highly significant inter-population differences among natural Sulla populations in Kabylie region for most of the recorded pheno-morphological traits, variance analyses performed on the variables showed a very significant population effect reflecting the phenotypic variability between the five populations (Table 6). Ben Fadhel *et al.* (1997) associate this variability with the preferentially allogamous regime of the species; the numerous hybridizations would be at the origin of the genotypic diversity observed. Bousmahel and Ait Toudert populations were presented, by far, the

highest characteristics, including yield. The phenotypic expression of a population depends on its genotype combined with pedo-climatic characteristics. Berrekia *et al* (1989) found that some *H. flexuosum* characters seem dependent on a stable genetic determinism, which is expressed whatever either the environment, it follows that certain morphological types can also be identified, as part of a selection, in relation to the conditions of the original environment. In addition, biomass superiority of Bousmahel population probably would be due to the soil characteristics; rich in organic matter >2 %, PH > 8.30 and the total limestone: 17.5 to 18.75%. The forage dry yield obtained is high (mean oscillate between 10 and 34 t DM.Ha⁻¹), with peaks of 55 t DM. Ha⁻¹ recorded for Bousmahel population; while in Australia, under ideal conditions of moisture and temperatures, Sulla can produce up to 10 t DM.Ha⁻¹ in the first year and over 20 t DM-ha⁻¹ in the second year (De Koning *et al.*, 2010). Our study focused on some pheno-morphological characteristics of five populations of *H. flexuosum* from the Tizi-Ouzou region, has shown that these populations presented very remarkable specificities, in particular as regards vegetative development for all of traits and reveal than populations possess a high fodder production capacity, therefore he would be very interested in enhance. Indeed, our observations have shown that Sulla allows a very good forage production without additional water supply.

Conclusion

The analysis of the morphological variation of some natural population of *H. flexuosum* in Algeria has for principal objective to understanding the diversity of this spontaneous forage resource, which is widely used in livestock feeding in this rearing area. A better knowledge of developmental morphology and quality

changes of *H. flexuosum* in different environments is necessary to optimize its potential for livestock production. The results recorded in this study will contribute to valorize and develop *H. flexuosum* in the marginal, isolated and deprived areas. The pheno-morphological and agronomic diversity observed among the Sulla accessions suggest a high fodder potential, which could be used to provide valuable materials for breeding programs aimed at Sulla improvement for Mediterranean environments. Conversely, this study provides a foundation for developing characterization and conservation strategies for the Sulla diversity present in different areas of Tizi-Ouzou province.

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