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Use of stress indicators to analyze *Lygeum spartum* Loefl. ex L. responses to Edapho-climatic conditions in west Mediterranean steppic rangelands: Case in central steppe of Algeria

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Abstract

Lygeum spartum Loefl. ex L., is a perennial spontaneous fodder species present in many steppe of the western Mediterranean basin. Because it can provide relatively high livestock productivity, the plant is proposed for the rehabilitation of the weakened pastures. Knowledge of *L. spartum*'s responses to abiotic factors that can be around it, could help for the sustainable rehabilitation of pastures threatened with desertification. During two seasons, autumn and spring, in two sites, and under two bioclimate semi-arid and arid, at Laghouat (Algeria), clump size and physiological parameters of *L. spartum* are analyzed. On its fresh leaves are performed measurements of water content, total chlorophyll, soluble sugars and proline. For both sites are carried out physicochemical soil analysis and climatic synthesis. Aridity Index for the two sites are respectively 0.26 and 0.06. Both soils, are sandy textured, alkaline, non saline, have a cation exchange capacity <7.5meq/100g, and active $CaCO_3$ contents >5%. Depending on the bioclimate and the season, *L. spartum*'s clumps volume are between 0.013-0.313m^3, in its leaves: water content is ranged between 8.20-24.16%, chlorophyll and soluble sugars content were respectively in the range of 0.85-1.31mg/g FM; 17.45-89.9mg/g FM, and the accumulated proline content between 2.6910^{-8}-10^{-3}mmol/g FM. The behavioral feature of *L. spartum* is an osmotic adjustment under drought stress. The plant is more constraint under the arid bioclimate where its growth is slow down. The findings show that aridity increase would be threatening for *L. spartum*.

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Introduction

North African semiarid and arid steppe rangelands occupy more than 600000km², of which about 34% in Algeria, 31% in Libya, 19% in Morocco, 11% in Tunisia and 5% in Egypt (Le Houérou, 1990). Nomadic pastoralism is the traditional form of their land use. In these rangelands rains are unreliable from year to year, and herders conduct long distance movements across hundreds of kilometers looking for a free feed unit (Aidoud *et al.,* 2006). Many areas in these rangelands have been exposed to severe natural and human impacts, including drought and, conversion of the land use and overgrazing (Ghiloufi *et al.,* 2015). It is estimates assume that at least 50% of the original vegetation in North African steppes were clearing during the last century (Wesche *et al.,* 2016), threaten their existence with the risk to desertification. In Algeria semiarid and arid steppe rangelands occupy more than 20 million ha, and are home to more than 30% of the country's population whose main activity is sheep farming with more than 25000000 head of sheep. The inventory drawn up by the Algerian Ministry of Agriculture in 2010, lists five classes of pasture steppe land: the pasture classified as good representing 5% of the total surface of the steppe, the pasture classified medium representing 10.9%, the degraded pasture covering 7.84% of the overall surface of the steppe, but the largest area of the Algerian steppe is classified as very degraded with 57.75% of the total area of the steppe, the rest being unproductive areas (MADR, 2010). As a result, this territory which was for many centuries the space of large nomadic of ovine's flocks, has undergone strong conversions (Nedjraoui and Bédrani, 2008), its landscape and biodiversity have been altered (Aidoud *et al.,* 2006). In favor of silting or erosion of productive lands (Houyou *et al.,* 2014); facials of palatable species such as *Stipa tenacissima* L, *Artemesia herba alba* Asso., and *Lygeum spartum* Loefl. ex L. have disappeared and replaced by others which are indicators of degradation such as *Atractilys serratuloides* Sieber ex Cass., *Peganum harmala* L., *Thymelea microphylla* Coss., and *Stipa parviflora* Desf. (Nedjraoui and Bédrani, 2008). The major remark about these rangelands is that of a decrease of their vegetation cover, their pastoral production has been globally marked by a significant decline of about 60 to 80%, especially over the last five decades, the natural pastoral resource of these steppes became less than 30% of the livestock's food ration (Aidoud *et al.,* 2006).

The policies of improving pastoral production and combating land degradation of steppes in Algeria were numerous but the results are far from the undertaken endeavors (MADR, 2010). Many replanting failed down because of their copying on models from other parts of the world and because of the used plant species that were not adapted to Algerian environmental conditions. Thus, the use of local species rather than introduced ones would probably be the most sustainable and the least expensive way to recover and preserve these weakened lands.

Groupings of *Lygeum spartum* Loefl .ex. L. (*L. spartum*), a Poacea are abundantly present in many semiarid and arid Northern African steppes (Le Houérou, 1990). This native perennial specie is proposed for the rehabilitation and revegetation of the degraded pastures (Hooke and Sandercock, 2012; Nedjimi, 2016). *L. spartum* is an important perennial pastoral grass, the plant provides a natural fence against desert extension, also it is adapted to dry climates and is resistant to salt stress during its germination (Nedjimi, 2016) and its growth (Conesa *et al.,* 2007). *L. spartum* constitutes also an important pastoral resource, its associations with other annual species and small Chamaephytes constitute a valuable source of ovine livestock forage, it can provide relatively high livestock productivity (110kg dry material / ha / year), offering a livestock carrying capacity of about (2 to 5eq.Ovine/ ha) (Nedjraoui, 2004).

L. spartum has been subject of many studies, emanating on its ecology biology and its phyto-sociology (Harche *et al.,* 1990; Aidoud *et al.,* 2006), but it's physiological tolerance mechanisms to soil and climatic conditions that can being around it remain until now unknown.

Stress tolerance is exhibit in some plant species by the development of morphologically and anatomical structures (Zandalinas *et al.,* 2018) which constitute a basic strategy for their adaptation and tolerance at specific conditions (Johnson *et al.,* 2005), such as leaves during photosynthetic processes (Schurr *et al.,* 2006) and roots in anchoring the plant deep in the soil in favor to a mineral nutrition process. Stress resistance can also display by physiological or biochemical mechanisms by synthesis of metabolites and organic compounds that allow to plants to survive (Zhuo *et al.,* 2007). Like proline, which is one of the most frequently compatible solutes accumulated in plants in response to various environmental stresses, and plays a key role in plant tolerances (Garg *et al.,* 2018; Guo *et al.,* 2018). Proline synthesis is an adaptive strategy frequently observed on cells (Hasegawa *et al.,* 1994) and in whole plants to limit the effects of stress or to demonstrate tolerances to various constraints (Araujo *et al.,* 2015). Its accumulation was observed by (Naeem *et al.,* 2017) in *Zea mais* in response to water stress and also reported by (Xiong *et al.,* 2018) in *Brassica napus* under ionic stress. The chlorophyll pigment content is also a good indicator of plant performance in the face to abiotic stress (Shu *et al.,* 2013), ion stress (Khan *et al.,* 2009) and water deficit (Zegaoui *et al.,* 2017). Numerous research studies have shown that soluble sugars play a central role in controlling the tolerance metabolism of plants under various environmental constraints (Abdel-Latif and El Demerdesh, 2017). Wyn Jones and Storey (1978a) reported accumulation of soluble sugars with that of proline in barley cultivar leaves in response to ion stress. Therefore proline, chlorophyll and soluble sugars contents constitute good parameters for detecting reactions of plants subjected to various environmental constraints. In this work the concentrations of proline, chlorophyll and soluble sugars are analyzed in the fresh leaves of *L. spartum* during two seasons: spring and autumn and under two bioclimates: arid and semiarid at Laghouat Province which is located in the central Algerian steppic rangelands. The bioclimates type in North African steppes including those from Algeria, are ranged from the lower cool semi-arid to the upper cool arid (Le Houérou 1990). Factors of climate are involved in the processes forming soil in Algeria (Djili and Hamdi-Aissa, 2017), this implies that characteristics of the Algerian steppic soils would change according bioclimate type. However, we assume that *L. spartum* has preferences for Edapho-climatic conditions in the Algerian steppe. Currently Africa faces significant climatic variations (Hendrix, 2017; Sonwa *et al.,* 2017), some areas are expected to significantly become drier, annual precipitation are projected with a decrease (Goulden *et al.,* 2009), thus North African semiarid and arid rangelands could be affected by these trends. Therefore the Algerian steppe is quite concerned by these climatic variations. These climatic variations must be taken into account for the sustainable development of threatened areas. In this work, we assume that climatic variations with soil characteristics would affect the physiological responses of *L. spartum*. Our objective is to characterize, soil and climate conditions, which would be the least constraining for *L. spartum* in its natural environment in the Algerian steppic rangelands.

Materials and methods

Study sites

This study was carried out at Laghouat, a central southern province at Saharan Atlas Mountains between the isohyets 350mm north and 80mm in the south. The province covers a total area of 25,052km² and is home to a population of 636,379 people whose main activity is extensive sheep farming. The steppe pastures of Laghouat occupy 1,531,776ha supporting 1,550,112 head of sheep, but only 11% of these pastures are in good condition, 89% are degraded to varying degrees. To carry out this work, two sites were selected (Fig. 1). This choice was made based on differences in their respective bioclimates: the site of Sebgag in the north of the Province is characterized by a semi-arid bioclimate, and the site of Houita in the south of Laghouat Province is characterized by an arid bioclimate (Table 1, Fig. 2a and Fig. 2b). The region of Sebgag supports 33,033 head of sheep on a pasture area of 385,000ha; while the Houita region supports 22,011 head of sheep on a pasture area of 450,00(ha). Climatic data (1995-2016) (Data of Algerian Government office of Meteorology 2017) reveal that more than 77% of rainfall in Sebgag and

Houita occurs between autumn and spring, from October to April. (Fig. 2a and Fig. 2b) display annual dry period of six months from May to October at Sebgag under the semi-arid bioclimate and annual dry period of eleven months from January to November at Houita under the arid bioclimate. Average monthly temperatures and monthly total rainfalls at Sebgag and at Houita during the years of our field measurements are shown in (Fig. 3a and Fig. 3b). At each site, the field work took place in a steppic rangeland closured for grazing and guarded since 2005.

Table 1. Climatic synthesis of the study sites (1995-2016): Data of the national office of meteorology.

Site	Sebgag	Houita
Coordinates	34°01'36" N,1°55'41"E, Elevation 1248 m	33°37'36"N, 2°26'23"E, Elevation 906 m
Q2*	26.61	19.17
P(mm)	287	167
TM (°C) July	32.85	37.00
Tm(°C) January	-4.24	7.13
Tm_{max}(°C)	25.07	32.11
Tm_{min}(°C)	2.63	8.12
UNEP Aridity Index**	0.26	0.06
Bioclimate	Semi-arid	Arid

Q_2: Rainfall Quotient Emberger for Mediterranean climate modified by Stewart, P(mm): annual rainfall average; TM (°C): Average maximum temperature for the hottest month; Tm (°C): Average minimum temperature for the coldest month; Tm_{max} (°C): Annual maximum temperature average ; Tm_{min} (°C): Annual minimum temperature average. *: 3.43 P/(TM - Tm) (Stewart 1969); **: P/ETP, ETP calculated by the Thornthwaite method (Thornthwaite 1948).

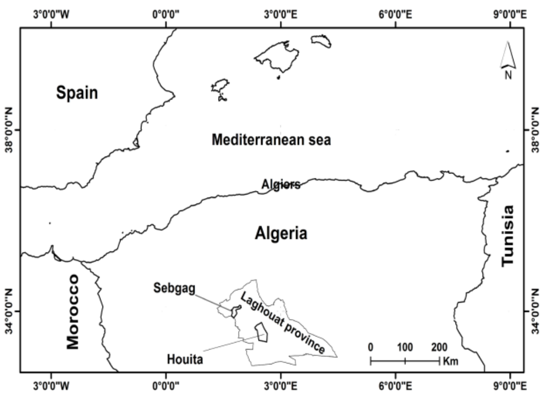

Fig. 1. Location of the study sites at Laghouat Province in Algeria.

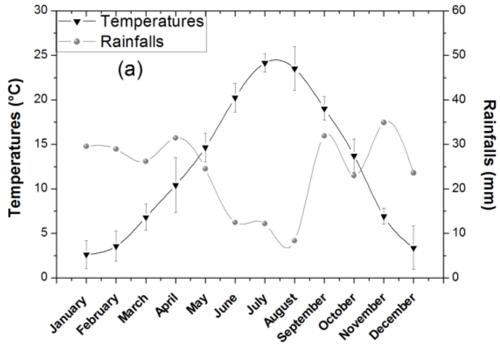

Fig. 2a. Climate graph of Sebgag, Semi-arid bioclimate (1995-2016).

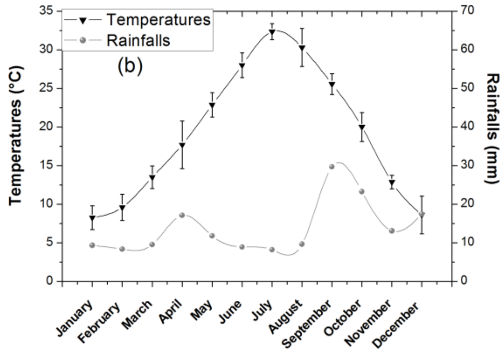

Fig. 2b. Climate graph of Houita, Arid bioclimate (1995-2016).

Field Sampling and Vegetation Analysis

Plant clumps volume measurement

The field work took place during midOctober 2016 (autumn) and mid-April 2017 (spring), between 8:00am and 10:30am. At each site, an area of (100×100m) was delimited. In these areas, during each season, we measured the following using a graduated ruler and a tape measure: the height of the apex, the largest diameter and the smallest diameter of twenty clumps of *Lygeum spartum* selected in complete randomization. We estimated the clump volume of each plant as the volume of a cylinder, using the product of the surface area occupied by the plant by its height:

Clump Volume = height of the apex $\times\pi\times$[(largest diameter + smallest diameter)/2]^2

Collection of leaves from plants

For each plant whose clump measurements were recorded, we took some fresh leaves from the clump in a randomly selected non-destructive section. The method of cutting vegetation to the ground level has been avoided because it can destroy the plant. A plant can be evaluated via the reference unit, which corresponds to a part of the plant whose foliage is reference with respect to the plant to be sampled; this cutting method is non-destructive and suitable for scientific research (National Academy of Sciences-Research Council, 1962). Some of the fresh cut leaves samples were transported to the laboratory in liquid nitrogen for analyses; the other parts were used to measure their water contents.

Determination of water contents in the fresh leaves of plant

Using a balance with a precision of (0.01g), the fresh weight of the sample leaves was recorded in the field and was then carefully kept in paper bags for the measurement of their dry weight. After having placed these sample leaves in the laboratory in an oven at 70°C and weighed after 48h, we determined their dry weight. The water content in the leaves of the two plant species was determined by the method described in Garnier and Laurent (1994):

Leaf water content (%) = (leaf fresh weight – leaf dry weight/leaf fresh weight) × 100

Determination of accumulated proline

The method we used is the one described by Monneveux and Nemmar (1986) and is based on the proline-ninhydrin oxidation reaction. To extract the solute, 100 mg of fresh material (FM) in 2 ml of methanol (40%) was heated at 85°C in a water bath for 1h. After cooling to 1ml of extract, we added 1ml of acetic acid, 25mg of ninhydrin, 1ml of mixture (120ml of H_2O, 300ml of acetic acid, 80ml of Ortho-phosphoric acid), followed by boiling for 30min at 100°C. After cooling, 5ml of toluene was added to the solution and shaken, then the upper phase was recovered, and 5mg of anhydrous sodium Na_2SO_4 was added. The optical density (OD) was determined using a spectrophotometer (528nm).

Determination of soluble sugars content

According to the method of Dubois (1956), 5ml of ethanol (80%) was added to 100mg of fresh material and placed in a water bath for 30min at 70°C. To 1ml of this solution, we added 1ml of phenol (5%) and 5ml of sulfuric acid (96%). Absorbance measurements were made at 640nm.

Determination of total chlorophyll content

Extraction by grinding in acetone (80%) was performed according to the method of Arnon (1949) and then measured by spectrophotometry at 645 and 663nm. The total chlorophyll content was determined according to the equation:

Total chlorophyll (T Chl) = 20.2 × OD (645nm) + 8.02 × OD (663nm).

Soil sampling and analysis

Using a hand auger, soil samples were collected in autumn 2016 from the top 30cm soil in the two study areas (Aubert, 1978). On the basis of our observations in the two sites: the plants roots can reach 30cm into the soil profile, this sampling soil depth was considered suitable for our measurements. In each of the two sites, 20 samples were taken randomly. For each collected soil sample, soil moisture, pH, Electrical conductivity (EC), Active $CaCO_3$, organic matter content (MO), and cation exchange capacity (CEC). Soil moisture content was calculated from the mass difference before and after drying at 105°C for 24h. Measurements of pH and EC were made in an aqueous solution (10g soil dissolved in 50ml distilled water, shaken for 30min, and then measured using a digital pH meter (HI_2OO_2) and conductivity meter (YSI Inc, OH, US). The total $CaCO_3$ content was determined by gasometry using a Bernard calcimeter. To determine active limestone (Active $CaCO_3$) the used method is that describe by Drouineau (1942): a sample of soil is stirred with a known amount of (N/5) ammonium oxalate. After filtration, the solution is titrated with permanganate before and after contact with the soil. The difference between the two titrations corresponds to the amount of calcium carbonate reacted on the ammonium oxalate. The MO content was measured by calcination method for 5 h at 650°C. For determination of (CEC) the used method is that proposed by Metson (1956): The saturation of the exchange sites by ammonium is carried out by percolating ammonium acetate (1mol/l) solution through a portion of 2.5g of soil. Particle size distribution analyses were performed by sieving and by sedimentation using a Robinson pipette.

Statistical analyses

Results were subject to one-way ANOVA. Grouping and significant differences for all statistical tests were evaluated at the level of P ≤0.05 (Tukey 95%). Pearson correlations between parameters measured on plant species were carried out at a degree of 5%. These statistical analyses, were carried out using the version 17.1.0 of the statistical software Minitab and the version 2014.5.01of XL STAT.

Results

Temperatures and Precipitations in the study sites during our field measurements autumn 2016 and Spring 2017

Data from the Algerian National Meteorological Office show (Fig. 3a) a seasonality of average temperatures in the two study sites. The average minimum temperatures are recorded in January. Average maximum temperatures are observed in July. An increase in temperatures is observed between February and July and a lowering of temperatures is noticed between August and December. (Fig. 3b), shows that monthly precipitation were irregularly distributed during the years of our field measurements. The site under the semiarid bioclimate was relatively more watered than the site under the semiarid bioclimate. The period between December 2016 and April 2017 was the rainiest under both bioclimates, the maximum monthly rainfall are observed during March under the semiarid bioclimate (41mm), and during April 2017 under the arid bioclimate (17.6mm). Between spring and autumn 2016 (March to September 2016), we observe (Fig. 3b and Fig. 4), a cumulative rainfalls of 74 and 28mm respectively under the semiarid bioclimate and under the arid bioclimate. Between autumn 2016 and spring 2017 (October 2016 to April 2017), we observe (Fig. 3b and Fig. 4), a cumulative rainfalls of 163 and 56.5mm respectively under the semiarid bioclimate and under the arid bioclimate.

Soil physicochemical parameters of the study sites

Site soil parameters are shown in (Fig. 4 and Table 2). Under both bioclimates, the soils were wetter in spring (Fig. 4), the most watered soil is noted under semiarid bioclimate, with a moisture content of 6.96%. In autumn, the lowest water content (0.92%) was observed for the soil under the arid bioclimate (Fig. 4). The observed difference for soil moisture, between soil sites was highly significant (P <0.001).

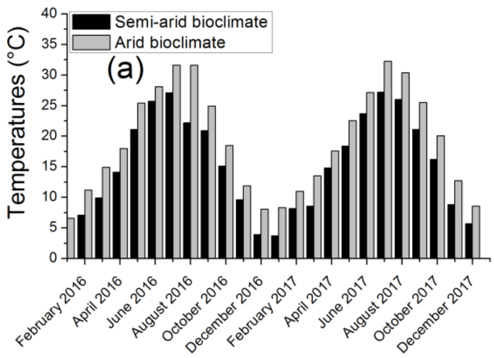

Fig. 3a. Average Monthly Temperature (°C) Sebgag (semiarid bioclimate) and at Houita (arid bioclimate) during 2016- 2017 (Years of our field measurements).

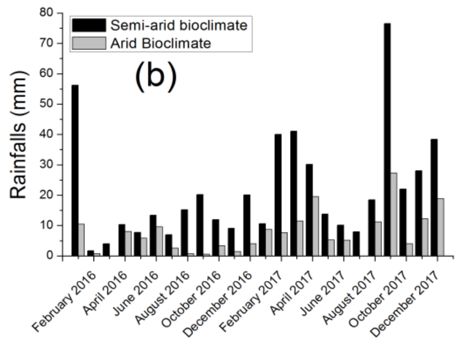

Fig. 3b. Monthly total rainfalls (mm) at Sebgag (semiarid bioclimate) and at Houita (arid bioclimate) during 2016-2017 (Years of our field measurements).

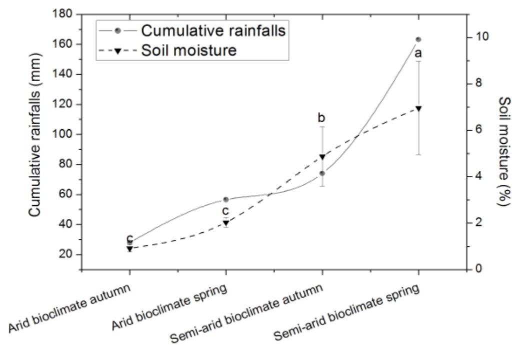

Fig. 4. Seasonal variation in soil moisture (%) at Sebgag (semiarid bioclimate) and at Houita (arid bioclimate), and seasonal variation of cumulative rainfalls (mm) at Sebgag and at Houita during years of our field measurements.

The electrical conductivity of the soil in the arid rangeland was twice high as that of the soil of the semi-arid rangeland, and the observed difference was highly significant (P <0.001) (Table 2). Soil pH in both rangelands is alkaline with a maximum of 9.31 observed for soil of the arid rangeland. Between soil pH of the arid rangeland and soil pH in the semi-arid rangeland ANOVA revealed a significant difference (P<0.001). Soils of both rangelands had low organic matter content of approximately 0.3% and a non-significant difference (P = 0.48).

Both soils are slightly calcareous. The highest total CaCO3 content of 7.98% is observed for the soil in the arid rangeland, and a relatively lower content of 6.91% is noted for the soil of the semi-arid rangeland (Table 2). ANOVA revealed a non-significant difference in total limestone content between the two soils (P = 0.33). The active limestone content is greater in soil of arid rangeland with (7%), and a significant difference between the two soils was observed. The cation exchange capacity (CEC), was weaker for the soil of the arid rangeland where the higher content of 6.86meq/100g is observed. Particle size distribution revealed that both soils were sand-textured with sand content > 63% and a significant difference only for the clay content at its lowest levels (Table 2).

Table 2. Physicochemical soil parameters (depth of 0-30 cm) of the study sites in Algeria.

Site (Bioclimate)	Sebgag (Semi-arid)	Houita (Arid)
pH	$7.85^b \pm 0.33$	$9.31^a \pm 0.15$
CE (ds/m)	$0.23^b \pm 0.10$	$0.55^a \pm 0.25$
CEC (méq/100g)	$6.86^a \pm 4.48$	$5.08^b \pm 2.50$
MO (%)	$0.30^a \pm 0.13$	$0.29^a \pm 0.10$
Total CaCO3 (%)	$6.91^a \pm 3.15$	$7.98^a \pm 4.76$
Active CaCO3 (%)	$5.01^b \pm 2.73$	$7.00^a \pm 2.75$
Clay (%)	$10.91^a \pm 7.79$	$7.87^b \pm 4.04$
Silt (%)	$25.14^a \pm 9.65$	$21.50^a \pm 7.87$
Sand (%)	$63.93^a \pm 16.70$	$69.42^a \pm 14.43$

Values represent mean ± standard error (n = 20). Different letters in the same line indicate significant difference at 5% level according to the Tukey's test.

Biometric and physiological parameters of L. spartum

Pant clump volume

The clumps of *L. spartum* (Fig. 5) are larger in the semi-arid rangeland, the maximum average clump volume 0.313(m^3) is observed in the spring and the smaller one 0.013 (m^3) is observed in the same season in the arid rangeland. The clump volume of *L. spartum* was significantly affected by the bioclimate only (P<0.001).

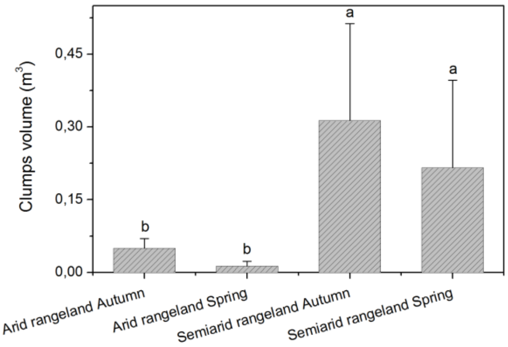

Fig. 5. Seasonal variation of the clump size (m3) of *Lygeum spartum* grown at Laghouat Province in Algeria at Sebgag (semiarid bioclimate) and at Houita (arid bioclimate).

Water content in the leaves of the plant

L. spartum leaves, the highest water content of 24.16% was observed in the spring in the semi-arid rangeland, and the lowest value of 8.20% was measured in the arid rangeland during the autumn, while intermediate values were measured in autumn in the arid rangeland and in the spring in the semi-arid rangeland (Table 3). The effect of bioclimate and season on the water contents in *L. spartum* leaves is significant (p<0.001).

Table 3. Physiological parameters contents in fresh leaves of *Lygeum spartum* growing at Sebgag (Semiarid bioclimate) et at Houita (Arid bioclimate) (Laghouat Province, Algeria), in spring and in autumn.

Bioclimate	Season	Water content (%)	Total chlorophyll (mg/g FM)	Soluble sugars (mg/g FM)	Accumulated proline (mmol/g FM)
Semiarid	Autumn	$11.43^b \pm 1.91$	$0.87^b \pm 0.17$	$76.92^b \pm 8.77$	$(9.60^b \pm 5.26)10^{-5}$
Semiarid	Spring	$24.16^a \pm 4.89$	$0.71^b \pm 0.14$	$71.53^b \pm 10.25$	$(2.69^b \pm 1.25)10^{-8}$
Arid	Autumn	$08.20^c \pm 3.51$	$1.31^a \pm 0.45$	$89.9^a \pm 10.88$	$(4.03^a \pm 2.20)10^{-3}$
Arid	Spring	$12.21^b \pm 3.50$	$0.85^b \pm 0.16$	$17.45^c \pm 3.44$	$(1.00^b \pm 0.06)10^{-3}$

Values represent mean ± standard error (n = 20). Different letters in the same column indicate significant difference at 5% level according to the Tukey's test.

Table 4. Pearson Correlation Matrix.

Parameter	Climate	Season	CV	WC	T Chl	S S	Pro
Clump volume	Arid	Autumn	1	+0.450**	NC	NC	NC
	Semi-arid	Autumn	1	+0.632*	NC	NC	NC
		Spring	1	0.098	NC	NC	+0.717*
Water content	Arid	Spring		1	NC	NC	+0.567*
	Semi-arid	Spring		1	NC	-0.590*	NC

The sign (+) indicates a significant correlation in a positive sense. The sign (-) reflects a significant correlation in a negative sense. NC indicated no correlation. Significance is indicated by superscripts (**: p <0,01; *: p <0,05).

CV: Clump volume, LWC : Leaf water content, T chl: Total chlorophyll, S S: Soluble sugars, Pro: Proline.

Accumulated proline content in the leaves of the plant

L. spartum accumulated more proline in the arid rangeland, the maximum average 4.03×10^{-3}(mmol/gMF) was observed in autumn, in the spring, a relatively small decrease of 3×10^{-3}(mmol/gMF) was observed (Table 3). The minimum of accumulated proline in *L. spartum* leaves 2.69×10^{-8}(mmol/gFM) was observed in the spring in a semi-arid rangeland, registering a reduction of almost a thousand times compared to the value accumulated under the same bioclimate in autumn. ANOVA revealed a significant difference on proline accumulation in *L. spartum* leaves according to bioclimate and season forming four statistical groups (P <0.001).

Soluble sugars content in the leaves of the plant

The highest content of soluble sugars in *L. spartum* leaves has been observed in autumn in an arid rangeland 89.90 (µg/gFM) followed by that observed in the same season in a semi-arid rangeland 76.92 (µg/gFM). The lowest soluble sugars content 17.45 (µg/gFM) in the leaves of *L. spartum* was observed in the spring in the arid rangeland. The effect of bioclimate and season on the soluble sugar content in *L. spartum* leaves is significant (P<0.001), which formed three statistical groups (Table 3).

Total chlorophyll content in the fresh leaves of the plant

In the leaves of *L. spartum*, the highest average content of chlorophyll 1.31 (mg/g FM) was observed in the arid rangeland in autumn and dropped to 0.85 (mg/g FM) in spring (Table 3). The behavior of this species is similar in the semi-arid rangeland, with an average chlorophyll content of 0.87 (mg/g FM) observed in autumn, decreasing to 0.71 (mg/g FM) in the spring. The bioclimate and season significantly affect the total chlorophyll content in *L. spartum* leaves, which formed two statistical groups (P<0.001).

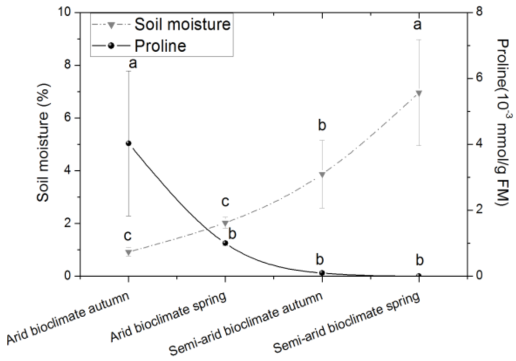

Fig. 6. Seasonal change in soil moisture (%) and, seasonal variation of accumulated proline content (10^{-3} mmol/g FM) in the fresh leaves of *Lygeum spartum* grown at Laghouat Province in Algeria at Sebgag (semiarid bioclimate and at Houita (arid bioclimate).

Discussion

Soil physicochemical parameters in the study sites

Edaphic conditions greatly affect the growth and the physiological behavior of plants (Araujo *et al.,* 2015; Ashraf and Harris, 2013). The soils of both rangelands have a light texture, with sand content greater than 63%, as consequence, they would be very permeable, their water retention capacity could be very low. Alkalinity is a characteristic of soils from Algerian arid regions (Bock, 1984), and the pH observed in this study was similar to those reported by Amghar *et al.,* (2016) and Djili and Hamdi-Aissa (2017) for the non-landscaping steppe at Laghouat Province and other soils in southern Algeria near our study sites. This can be explained by the calcareous nature of their parent rocks (Bock, 1984), which gives them this characteristic. The pH of the soil in arid rangeland exceeds 9, which can be at the origin of the degradation of the soil structure (Aubert, 1978); consequently, the circulation of rainwater and soil solutions would be strongly affected.

The electrical conductivity of both soils (Table 2) is well below 0.6 (dS/m); therefore, they cannot be described as saline (Aubert, 1978). The soil electrical conductivity values observed in this study are in agreement with those reported by (Amghar *et al.,* 2016; Djili and Hamdi-Aissa, 2017) for soils in Laghouat Province. The organic matter content of the studied soils is very low (<1.4%), in northern Laghouat Province and at Wadi Zegrir in the south, similar grades were reported by Amghar *et al.,* (2016) and Djili and Hamdi-Aissa (2017). These organic matter quantities do not allow for a good aggregation of soil sediments, generating skeletal soils of low fertility, and additionally contain very little clay (Table 2), which prevents good water retention (Aubert, 1978).

Limestone is a common feature of soils in arid Algerian regions. Its presence has been reported by Bock (1984), who observed a component inherited from the parent rock that has resulted in the soil formation. The active CaCO3 content observed in this work are in agreement with those reported by Djili and Hamdi Aissa (2017), active limestone causes in soils a decrease in the availability of some elements such as manganese and iron, as well as difficulties in nitrogen nutrition of plants (Le Tacon 1978), this suggests that *L. spartum* is affected by these influences by active limestone in the two soils of this study. The presence of limestone in both soils explain also their alkalinity (pH> 7.8). The CEC, affects soil fertility, and plant growth, it is the strength of the soil tank to reversibly store of some nutrients. According (Aubert 1978) the two soils of this study have a low CEC. The nutrient cations are susceptible to leaching in soils with a low CEC, and as a result, plants are most likely to develop nutrient deficiencies (Ashraf and Harris, 2013), thus affects in the case of this study the physiological behavior and the size of the clumps of *L. spartum.*

Biometric and physiological parameters of Lygeum spartum

Plant clumps volume

The volume of the canopy of the plant greatly affects the pastoral biomass that it can offer to grazing animals. *L. spartum,* seems to grow better in a semiarid rangeland. In the spring, its clump sizes exhibited a difference of 16 times under the two bioclimates (Fig. 2). This can be explained by a rainfall effect, the relative availability of rainwater and its presence in soil which are better under the semi-arid rangeland (Fig. 3b and Fig. 4), the length of the dry period in the arid rangeland (Fig. 2), combined with the relatively high temperature causing rising in Evapotranspiration at Houita (Table 1). In fact, *L. spartum* clumps can form mounds of organic debris and fine soil particles that retain more moisture and nutrients than the soil between these mounds (Nedjmi, 2016), as a result, the mineral nutrition of *L. spartum* is better in the semi-arid rangeland because of the annual rainwater distribution (Fig. 3b and Fig. 4) causing more soil moisture (Fig. 4). A better availability of mineral elements under the semi arid bioclimate can also be explained by soil moisture and CEC which are higher at Sebgag (Table 2). The relative dwarfing of *L. spartum* clumps observed at the arid rangeland can be attributed to the soil and climate conditions that prevail there, that influence also the plant biomass (Araujo *et al.,* 2015). The relatively water deficit in soil (Fig. 4) combined to the light soil texture and the limestone soil content under arid bioclimate (Table 3), and the high temperatures at Houita (Fig. 3a), are in favor of high rate of soil evaporation, induce precipitation of $CaCO_3$, biological activity is quite affect: the degradation of the organic matter is slowed, lowering its fertility. However, pH and dynamics of trace elements in the soil solution are also affected by low levels of soil moisture (Aubert, 1978). The $CaCO_3$ precipitation has been already reported by Pouget (1980), who noticed a pulverulent calcareous crust impervious to the roots of plants in soils of steppic rangelands in North Laghouat Province nearby our study sites. Because of these Calcareous crust this author noted that the development of plant root system could be hindered and slow down. Consequently, The small size of *L. spartum* clumps measured at Laghouat under arid bioclimate, would be related to the mineral nutrition of the plant, which is hindered, by a poor circulation of the soil solution, and generating an alkalinity, marked by a pH >7.85; which allows to assume that pH of > 7.85 hampers the growth of *L. spartum* at Houita.

Water contents in the leaves of the plant

Our results show seasonal variability of the water contents in the leaves of *Lygeum spartum* in the two rangelands (Table 3). The organ that suffers the first effects of dehydration is the leaf. Adaptation to water stress results in reduction of leaf area and limb rolling (Turner *et al.,* 1986), and this strategy is a morphological trait previously exhibited by *Lygeum spartum* (Harche *et al.,* 1990). The observed water contents (Table 3) are ranged in intervals from those measured by Filek *et al.,* (2014) on barley leaves under water stress. In our case, the highest values recorded in the spring reflect a clear climatic effect explained mainly by the rainfall distribution in the two study sites (Fig. 3b and Fig. 4). More than 77% of the annual rainfall accumulations in the two rangelands occur between October and April (Fig. 3b), combined with the low temperatures marking winter and early spring (Fig. 3a), which would limit plant transpiration (Pugnaire and Haase, 1996). In addition, the very low rainfall that marks the summer season (Fig. 3b), the water deficit would be at its maximum in the two rangelands, affecting the water reserves in soils (Fig. 4), the plant would be also subject to a weakened root absorption until autumn, which affects the water contents observed in the leaves of *L. spartum* during autumn (Table 3). However, an osmotic adjustment allows the maintenance of their water potential observed during this season and would also maintain their growth metabolism.

Soluble sugars content in the leaves of the plant

Soluble sugars content increased considerably in plants subjected to different types of abiotic stress (El Sayed *et al.,* 2013; Thalmann and Santelia 2017): indeed, this has been verified by Shi *et al.,* (2015) in *Arabidopsis thaliana* and in *Dendrobium officinal* by Yu *et al.,* (2017). Soluble sugars contents observed in this work are ranked in the ranges reported by Mutava *et al.,* (2015) in soybeans under water stress. In this study, in both rangelands, soluble sugar content in leaves of *L. spartum* in autumn is affected by the seasons, the values observed in autumn are higher than those observed in the spring (Table 3). Under the two bioclimate, the low water contents in the leaves of *L. spartum* measured in autumn (Table 3) would explain the increase of the soluble sugars content which would be an osmotic adjustment (Kumar *et al.,* 2017) to avoid plant dehydration because of the lack of water in summer that recurrences in autumn. In *L. spartum* leaves, soluble sugars content observed in autumn, could also be at the origin of its clump dwarfism measured in the arid rangelands, because the plant biomass is greatly affected by sugars content (Fareen *et al.,* 2016). The relationship between the water and sugar content observed in the leaves of *L. spartum* at Sebgag and Houita (Algeria), are in agreement with the assumed role of sugars as protectors of cell membranes during water stress to reduce rates of photosynthesis and transpiration (El Sayed *et al.,* 2013) to avoid dehydration of plants. This implies an osmotic adjustment that *L. spartum* develops to keep its water potential under water deficit at Laghouat.

Total chlorophyll content in the leaves of the plant

The effect of bioclimate and season on the chlorophyll content observed in this study is highlighted (Table 3). Total chlorophyll content of *L. spartum* leaves in this study are ranged near the lower bounds of those observed by Hu *et al.,* (2016) and Khayyat *et al.,* (2014) in the leaves of other plants grown under stress. These low values observed at Laghouat case are probably due to the typology of cultivated and spontaneous species and to the anatomy of their organs, especially their leaves and their respective surfaces. Chlorophyll content in *L. spartum* at Laghouat can be attributed to the narrowness of leaf limb in *L. spartum*. Indeed, in extreme environmental conditions, plants form structures that constitute a basic strategy for their adaptation to specific conditions (Johnson *et al.,* 2005). In addition, plant functions depend on particular anatomical, morphological or physiological characteristics such as leaves during photosynthetic processes to resist stress (Schurr *et al.,* 2006). Ben Mabrouk *et al.,* (2012) have shown the regular presence of many stomata on the surface of the leaf blade of a typical Poaceae like *L. spartum*. The relatively low chlorophyll content observed at

Laghouat (Table 3) can also be explained by the reduction of leaf stomata opening to limit water losses due to their low contents regulated by sugars content observed on *L. spartum*: this would be a reaction to a water deficit constraint at Laghouat reinforced by an osmotic adjustment (ratios of water and sugars content in the leaves of *L. spartum* Table 3) which were already confirmed in other plant species by Armbruster *et al.,* (2017); Derks *et al.,* (2015) and Kaliji *et al.,* (2016), whose observed deterioration of photosynthetic pigments in leaves of these plants subject to a water deficit.

Accumulated proline in the leaves of the plant

At Laghouat, a seasonal and bioclimatic effect of the accumulation of proline in *Lygeum spartum* is highlighted (Table 3). The accumulated proline in the leaves of *Lygeum spartum* observed in autumn in both rangelands is similar to that observed in other plants subjected to various abiotic stresses by Mu *et al.,* (2016); Noushina *et al.,* (2015), the accumulated proline measured at Laghouat in *L. spartum* could explain also a reaction to maintain the clump development subjected to a summer water deficit (Table 1), that persists until autumn, which is reflected by the low water contents in leaves observed during this season (Table 3). The accumulated proline would be a drought tolerance reaction that *Lygeum spartum* demonstrate to maintain an osmotic potential (Per *et al.,* 2017) that protects the structure of their cell membranes (Maggio *et al.,* 2002). *L. spartum* responses in the arid rangeland were manifested as, a higher proline accumulation (Table 3) with a relative dwarfism of its clumps (Fig. 5), suggesting that it is constrained.

Responses of *L. spartum* at Laghouat are the results of influences of conditions in which it grows, they are an obvious combination of bioclimate and soils, which are reflected in physiological and growth behavior regulated by proline accumulation, as describe in another plants (Choudhury *et al.,* 2017; Pandey Ramegowda and Senthil-Kumar, 2015).

Behavior of L. spartum under arid and semiarid bioclimate in the Algerian steppe

To grow, plants have to absorb atmospheric CO_2 through wide-open stomata. However, these causes water to evaporate, especially if the air is hot and dry (Katul *et al.,* 2010). At Laghouat, an increase in clump volume of *L. spartum* in autumn induces an accumulation of proline and total sugars, with an increase in photosynthetic activity and, generated by a low water contents in the leaves of the plant. This implies an osmotic adjustment under drought stress. At Sebgag and at Houita, the salt stress that can constrain *L. spartum* is not implicated, because of the non-saline nature of soils at Laghouat (Table 3).

It seems that the growth of *L. spartum* is slowed in the arid rangeland , this can be explain by the high rate of Evapotranspiration at Houita and the prolonged rainwater deficit there, that lasts approximately 11 months per year inducing a lack of soil moisture. This climatic effect associated with that of soil conditions: probable poor circulation of the soil solution induced by the high relative content of active $CaCO_3$ and pH> 7.85, resulting in a negative effect on the mineral nutrition of the plant. *L. spartum* seems to have a low pH tolerance above 7.85, and active $CaCO_3$ content that exceeds 6%. The plant would be a true Gypsophyt (gypsum-loving) as reported by Aidoud *et al.,* (2006) and by Nedjimi (2016), our findings have shown that *Lygeum spartum* is sensitive to soils with Active limestone contents greater than 5%, this soil condition was reflected by the dwarfism of *L. spartum*'s clumps size at Houita.

Correlations between Physiological parameters measured on L. spartum

As shown in Table 4, the measured variables with the greatest number of significant correlations with the others one include the following:

1) The volume of plant clumps, two significantly correlated variables: a) The leaves water content in autumn in the arid rangeland, and in the spring in the semiarid rangeland; b) Accumulated proline in the spring in the semiarid rangeland.

2) The leaves water content, one significantly correlated variable: a) the accumulated proline in the spring in the arid rangeland.

Conclusions

Soils at Laghouat are slightly textured, nonsaline, alkaline, calcareous with a low cation exchange capacity. Combined with the low rainfall under arid bioclimate, these soil conditions would be unfavorable to the circulation of the soil solution. The biometric measures of *L. spartum* at Laghouat, revealed that its grows is better in the semiarid rangeland, this implies that the plant is sensitive to soils and climatic conditions in the arid rangeland.

The soil water deficit imposed by the weak annual rainfalls, the length of the annual dry period, and the slight soil texture in the arid rangeland, would be at the origin of a high rate of soil evaporation and against a good circulation of the soil solution. These would have complicated the root absorption of *L. spartum*, that was reflected by dwarf clumps of *Lygeum spartum*. With such conditions, total plant biomass would be low, rangelands could not provide a significant livestock carrying capacity.

Under the conditions of the arid bioclimate *L. spartum*'s behavior characteristics were: a high proline accumulation, an osmotic regulation, and a reduced chlorophyll content. The nonsaline soils characters at Laghouat, discard the fact that the plant is subjected to salt stress. The behavior of *L. spartum* in the Algerian steppe at Laghouat is linked to a water deficit. In the case of planning to combat desertification or, the rehabilitation of degraded rangelands, the use of *L. spartum* under arid bioclimate should be well thought out. Climatic variations threatening Africa with decreases in annual rainfall (more drought), and increases in aridity imply that conditions will be more restrictive for the significant growth of *L. spartum*.

A rational use of pastures of groupings *L. spartum*, whose pastoral production is currently significant under the semi-arid bioclimate of North African steppes is a task that would contribute to preservation of biodiversity and to maintain equilibrium of steppic ecosystem threatened with a decrease of precipitations and a aridity increase.

This work is an initial attempt at understanding *L. spartum*'s reactions to environments threatened by desertification. Measurements of other physiological parameters during other times of the year and at other sites across the twenty million hectares of Algerian arid and semiarid rangelands can reinforce this work's ability to help decision-makers for the sustainable management of steppes.

Conflicts of interest

The authors declare no conflicts of interest.

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