



Leaf area index and forage productivity indicators of *Lotus corniculatus* L. at different soil moisture contents and seasons of the year



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Abstract:

The objective of the study was to evaluate the response of leaf area index and forage productivity of *Lotus corniculatus* clover genotypes with two different soil moisture contents under shade netting conditions. A randomized experimental block design in a split plot arrangement with three replicates was used. The large plots were soil moisture contents: optimum (OSMC: 26 % ± 1.5) and suboptimal (SSMC: 22 % ± 1.5); and the small plots were *L. corniculatus* accessions: 255301, 255305, 202700, 226792, and the bird's-foot trefoil (*Estanzuela Ganador*) variety. The variables measured were leaf area index (LAI), dry matter biomass production (DM) (g plant⁻¹), dry forage increase rate (DFIR) (g plant⁻¹ d⁻¹), and leaf-to-stem ratio (L/S), plus the climatic variables of temperature (°C) and relative moisture (%) in the shade net. Accession 255305 was the best responder in LAI, DM, and DFIR, with values of 3.2, 94.9 g plant⁻¹, and 0.30 g plant⁻¹ d⁻¹, respectively, with OSMC; while the bird's-

foot trefoil had the best response in LAI with SSMC. There were no differences ($P \leq 0.05$) between the genetic materials evaluated in DM and DFIR, with average values of 82.4 g plant⁻¹ and 0.26 g plant⁻¹ d⁻¹, respectively. Accessions 255301 and 226792 were the best L/S ratio with values of 2.9 and 2.5, respectively. In general, the best productive performance in terms of DM was obtained in spring, summer, and summer-autumn, with values of 17.5, 11.7, and 17.7 g plant⁻¹, respectively.

Keywords: Livestock, Fodder clover, Stress physiology, Leaf area, Arid zones.

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Introduction

In Mexico, 76.3 % of the water volume is used for agricultural and livestock activities⁽¹⁾. This high-water consumption is related to poor water management and the establishment of crops with high water requirements, which aggravates the problem of water scarcity in arid zones. In these regions, droughts are becoming more frequent and more intense, and their effect is causing economic losses in agrifood production, resulting in food shortages, reduced supply of inputs for the industrial sector, and degradation of agroecosystems⁽²⁾. In addition, climate change has increased extreme temperature and rainfall events with a negative effect on the various productive activities, among which forage production stands out⁽³⁾. This economic activity is of great importance in the country. The average national production amounts to 30 million 950 thousand tons⁽⁴⁾, 26.7 % of which corresponds to the cultivation of alfalfa (*Medicago sativa* L.), which is a crop with a high demand for water resources⁽⁵⁾.

In the Comarca Lagunera of the states of Durango and Coahuila, Mexico, there is a serious problem of water scarcity and overexploitation of the aquifer⁽¹⁾. In addition, the establishment of agricultural crops with high water demand is common, having a negative impact from the economic, social, and environmental point of view⁽⁶⁾. This region is the country's main dairy basin, and alfalfa is the main forage crop, established to feed 955,115 head of cattle in the pasture⁽⁷⁾. The traditional irrigation system for this crop generates a demand of approximately 2.0 m of irrigation sheet per year^(8,9).

The high demand for agrifood products such as milk, the low availability of water resources, and the use of forage crops with low water use efficiency make it imperative to explore ways to make water use more efficient for production purposes in the agricultural sector. The use of alternative crops to such traditional crops as alfalfa, which compete with these in quantity

and productive quality with less water requirements, is a viable option that can help mitigate the problem of water scarcity, with the support of other techniques such as the use of vegetative covers, which reduce the high evaporation rate⁽¹⁰⁾.

Among the forage crops with potential in marginal agriculture conditions, various *Lotus* species stand out, the main of which is *L. corniculatus*, used for its stress tolerance to different adverse environmental factors as a way to improve forage production in several countries with dry summers and marked seasonality effect. Certain reports indicate the absence in New Zealand, Uruguay, and Chile of genetic materials of *L. corniculatus*, which have performed well in response to water deficit conditions^(11,12). There are a number of varieties and genetic accessions of *L. corniculatus* that show high flexibility of adaptation to different environments, such as tolerance to drought, flooding, acid soils, and high levels of Al and Mn⁽¹³⁾.

One of the properties of this perennial forage species is its high capacity for regrowth after cutting or grazing, although the regeneration rate varies depending on the variety and the type of stress due to extreme temperatures, soil moisture content, and physical-chemical and fertility characteristics of the soil^(14,15). The objective of this study was to evaluate the response capacity in terms of leaf area and forage productivity of various accessions and a variety of *L. corniculatus* with optimum and suboptimal soil moisture content under shade-mesh conditions in northern Mexico.

Material and methods

Geographical location of the study area

The experiment was established in the experimental field of the Regional University Unit of Arid Zones of the Autonomous University of Chapingo, in Bermejillo, Durango, Mexico, located at the coordinates 25.8° N and 103.6° LW, at an altitude of 1,130 m asl. The region has a dry desert climate with summer rains and cool winters, an average annual rainfall of 258 mm, an average annual evaporation of 2,000 mm, and an average annual temperature of 21 °C, with maximum temperatures of 33.7 °C and minimum temperatures of 7.5 °C⁽¹⁶⁾.

Experimental design and management

A randomized block design in a split plot arrangement with three replications was used. The large plots had two soil moisture contents —optimal OSMC (26 % ± 1.5) and suboptimal SSMC (22 % ± 1.5)— established on the basis of the moisture abatement curve⁽¹⁷⁾, according to the regression equation obtained:

$$\%SM = 26.57 + 13.52x + 4.97X^2$$

Where: SM%= soil moisture content, and X is the negative energy stress in MPa, considering that the field capacity (FC) and the permanent wilting point (PWP) correspond to an energy stress of 0 and -1.5 MPa, respectively. The FC and PWP were calculated based on the above, which were 26.5 % and 17.5 %, respectively. The small plots containing four accessions and one variety of *L. corniculatus* from different regions (Table 1).

Table 1: Relationship, origin, and growth habit of *Lotus corniculatus* genetic materials evaluated in the experiment

Code/ Name of accessions/varieties	Place of origin	Growth habit
255301	France	Semi-erect
255305-	Italy	Semi-erect
202700	Uruguay	Erect
226792	Canada	Semi-erect
Estanzuela Ganador	Uruguay	Erect

The experimental unit was one plant in each 20 kg rigid plastic pot 35 cm in diameter and 31.3 cm in height. Each pot was filled with 18 kg of a substrate mixture with a 50:30:20 ratio of soil:compost:sand. The substrate had a sandy loam texture, with a proportion of 52 % sand, 26 % silt, and 22 % clay, and a pH of 8.69, an EC of 10.76 dS m⁻¹, and a bulk density of 1.46 g cm⁻³. A digital ORIA thermometer/hygrometer placed inside the shade net recorded the daily temperature (°C) and relative moisture (%) during the evaluation period.

Irrigation was applied every four days, and the soil moisture contents were measured by gravimetry, for which purpose the weight of the pots in the OSMC was maintained at 23.9 kg, and that of SSMC, at 23.0 kg. An average of 0.6 L of water per irrigation was added to both moisture contents, restoring the OSMC to 27.5 % and the SSMC to 23.5 % as upper limits of soil moisture, and leaving both values to decrease to 24.5 % and 20.5 % as lower limits, respectively. A margin of 3.5 % (20.5 - 17.5) was considered a usable moisture range so that the plant did not reach PWP.

A total of seven fresh material cuts were made: the first one in July 2021 and the last one in May 2022. They previously had an adaptation period of 60 d after transplanting, and a standardization cut was made 45 d before the first cut. For the cuttings, growth periods were considered according to the seasons and the intermediate periods: spring-summer (Sp-Su), summer (Su), summer-autumn (Su-A), autumn (A), winter (W), winter-spring (W-Sp), and spring (Sp). The time interval between cuts was 45 d, except for W, when it was extended to 90 d due to the slow growth of the plant due to the decrease in temperature.

Measured variables

The leaf area index was calculated. For this purpose, the leaf surface area was first determined by randomly selecting 10 complete stems per plant at each cutting date; the leaves were then separated from the stems and spread and photographed on a white paper surface; the photographs were processed with ImageJ for each treatment and repetition according to the experimental design. Subsequently, equation 1, adapted to the conditions of the experiment, was used to obtain the leaf area index⁽¹⁸⁾.

$$LAI = \frac{LA*NS}{TSA} \quad \text{-----}(1)$$

Where: LA= leaf area of a stem (cm²); NS= number of stems, and TSA= total soil surface area in cm² (pot surface area= 962.11 cm²).

The leaves harvested at each cutting date per treatment were dried in a HAFO[®] (model 1600, USA) forced-air oven at 60 °C for 24 h or until attaining a constant weight; the dry material was weighed on a Shimadzu analytical balance (model AY220M), and the dry matter (DM) production for each cutting was determined.

The dry fodder increase rate (DFIR) was estimated by dividing the dry weight of forage harvested by the number of days of growth elapsed from one cutting period to the next, with the following equation:

$$DFIR = \frac{\text{Amount of fodder (g MS plant}^{-1}\text{)}}{\text{Days of growth elapsed}} \quad \text{-----}(2)$$

The leaf/stem ratio (L/S) was obtained from a representative subsample of 10 stems from each treatment, for which purpose the leaf and stem components were separated and placed separately in a HAFO[®] (model 1600, USA) forced-air oven at 60 °C for 24 h. Subsequently, the leaf/stem ratio was calculated as the quotient between the leaf dry weight (g DM) and the stem dry weight (g DM).

Data analysis

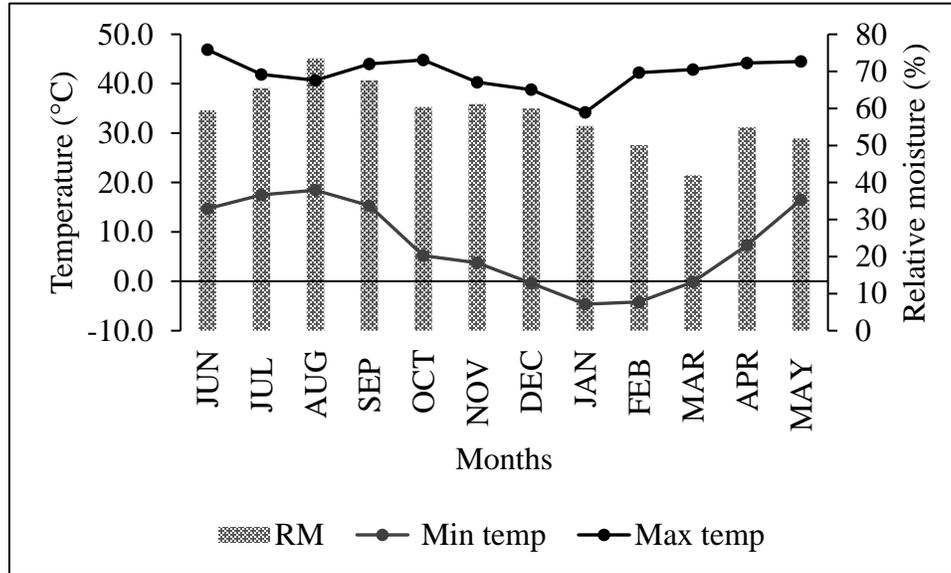
The Statistical Analysis System software⁽¹⁹⁾ was utilized to process the database, performing an analysis of variance and a Tukey multiple range test ($P \leq 0.05$) to identify the effect of each treatment effect. In addition, Excel version 6.0 was used for regression analysis.

Results and discussion

Temperature and relative humidity

During the period from June 2021 to May 2022, a mean maximum temperature of 30 °C and mean minimum of 20 °C, as well as a maximum of 46.9 °C and minimum of -4.6 °C, were recorded inside the shade net (Figure 1), with mean and maximum temperatures per day of 16.6 to 40.1, 19.8 to 32.7, 9.8 to 37 and 4.5 to 30 °C during the spring, summer, fall and winter seasons, respectively. The average relative moisture recorded ranged between 44 and 73 %, with a minimum of 5-10 % in the months of May through July , and a maximum of 100 % during the rainy season, in July, August and September, with a regional historical annual average rainfall of 258 mm⁽¹⁶⁾. In order to avoid alterations of the soil moisture content in the pots due to the effect of rain, the experimental area occupied by the pots was covered with plastic during these periods.

Figure 1: Maximum, and minimum temperatures and monthly relative moisture (RM) recorded during the evaluation of various genetic materials of *L. corniculatus* under shade-mesh conditions from July 2021 to May 2022 in Bermejillo, Dgo.



Leaf area index

The leaf area index (LAI) was significantly higher ($P \leq 0.05$) in accession 255305 at OSMC, with a value of 4.7, followed in importance by accession 255301 and the bird's-foot trefoil variety, with values of 4.1 and 3.7, respectively. In SSMC, the bird's-foot trefoil variety stood out with 3.9 and was followed in importance by the other accessions, except for 226792, which registered the lowest value of 2.6 (Table 2). The leaf surface area achieved by a plant during its development defines the capacity of the plant canopy to intercept photosynthetically active radiation, the primary source for the proper development of organs and tissues⁽²⁰⁾. According to these results, the LAI in general was slightly negatively affected by the suboptimal soil moisture condition, although the bird's-foot trefoil variety showed an above-average performance of the LAI attained under optimal soil moisture conditions.

Dry matter production

The DM production was higher with an optimal soil moisture content, amounting to an average of 98 g plant⁻¹, compared to the suboptimal content (SSMC), which recorded an average of 82 g plant⁻¹ without statistical difference ($P \leq 0.05$) between the genetic materials tested in this study; while with an OSMC, accession 255305 had the best response, with 131.8 g plant⁻¹ (Table 2). The above suggests that biomass productivity is directly dependent on the soil moisture content, and all the genetic materials of *L. corniculatus* are negatively

affected to the same degree by a suboptimal soil moisture content. These results differ from those reported in a clover adaptability study in which 12 materials were evaluated under temperate field conditions⁽⁸⁾, where accession 202700 and the bird's-foot trefoil variety were reportedly the most productive, possibly due to the environmental conditions of temperature, ranging between 5 and 32 °C, which are more favorable for this crop

Dry fodder increase rate

The dry fodder increase rate (DFIR) was consistent with the results shown for DM, with a statistical difference ($P \leq 0.05$) in the OSMC plot corresponding to accession 255305 as the most outstanding, with a DFIR of 0.43 g plant⁻¹ d⁻¹ and no statistical difference between the rest of the genetic materials evaluated. Whereas the SSMC plot had the lowest values, of 0.26 g plant⁻¹ d⁻¹ in average, with no statistical difference between the evaluated accessions and varieties (Table 2). Both the yield and the biomass accumulation of different forage crops develop dynamically⁽²¹⁾ due to the formation of new tissue, which is highly influenced by environmental and management conditions, mainly by the temperature and water availability⁽²²⁾.

Leaf/stem ratio

The leaf-to-stem ratio (L/S) was similar in both moisture contents, with average values of 2.3 and 2.2 in OSMC and SSMC, respectively, with statistical difference between genetic materials in both cases. Accession 255301 excelled in OSMC with an L/S of 2.9 and 226792 in SSMC with a value of 2.5 (Table 2). The results suggest that, in this variable, the genetic materials are not affected when going from an optimal soil moisture condition to a suboptimal one, which makes it possible to save water, without significantly affecting this productivity indicator. It is desirable that this value be as high as possible, as it is determined by the leaf component; this organ is the most digestible part of the forage and has the highest protein content—much higher than the other organs of the plant—and therefore has the highest nutritional value⁽²³⁾. The L/S results obtained for accessions 255305, 202700 and 226792 at both soil moisture contents were similar to those obtained in a temperate region of Mexico⁽⁸⁾ where values of 2.4, 1.7 and 2.3, respectively, were reported. Additionally, the values obtained in accession 255301 and bird's-foot trefoil were higher than those obtained in the aforementioned studies, which reported a ratio of 2.0 and 1.5, compared to the 2.9 and 1.9 obtained in the present study in OSMC. This is relevant, given that the study was carried out in a hot dry climate where, even under a shading mesh, extreme weather events occurred that are regarded as very unfavorable conditions compared to the cold temperate climates from which most of the genetic materials in this study originate.

Table 2: Leaf area index and productivity indicators of *L. corniculatus* accessions/variety under optimal and suboptimal soil moisture contents

Accession/ variety	LAI		DM (g plant ⁻¹)		DFIR (g plant ⁻¹ d ⁻¹)		L/S	
	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC
	255301	4.1 ^{ab}	3.0 ^{ab}	98.8 ^b	74.5 ^a	0.32 ^b	0.24 ^a	2.9 ^a
255305	4.7 ^a	3.3 ^{ab}	131.8 ^a	85.7 ^a	0.43 ^a	0.27 ^a	2.4 ^{ab}	2.3 ^{ab}
202700	3.0 ^b	3.6 ^{ab}	89.3 ^b	94.9 ^a	0.29 ^b	0.30 ^a	1.7 ^b	1.7 ^b
226792	2.7 ^b	2.6 ^b	79.5 ^b	79.3 ^a	0.25 ^b	0.26 ^a	2.5 ^{ab}	2.5 ^a
Bird's-foot trefoil	3.7 ^{ab}	3.9 ^a	90.7 ^b	78.4 ^a	0.28 ^b	0.24 ^a	1.9 ^b	2.2 ^{ab}
Mean	3.6	3.3	98.0	82.4	0.32	0.26	2.3	2.2

OSMC= optimum soil moisture content (26 % ± 1.5); SSMC= suboptimal soil moisture content (22 % ± 1.5);

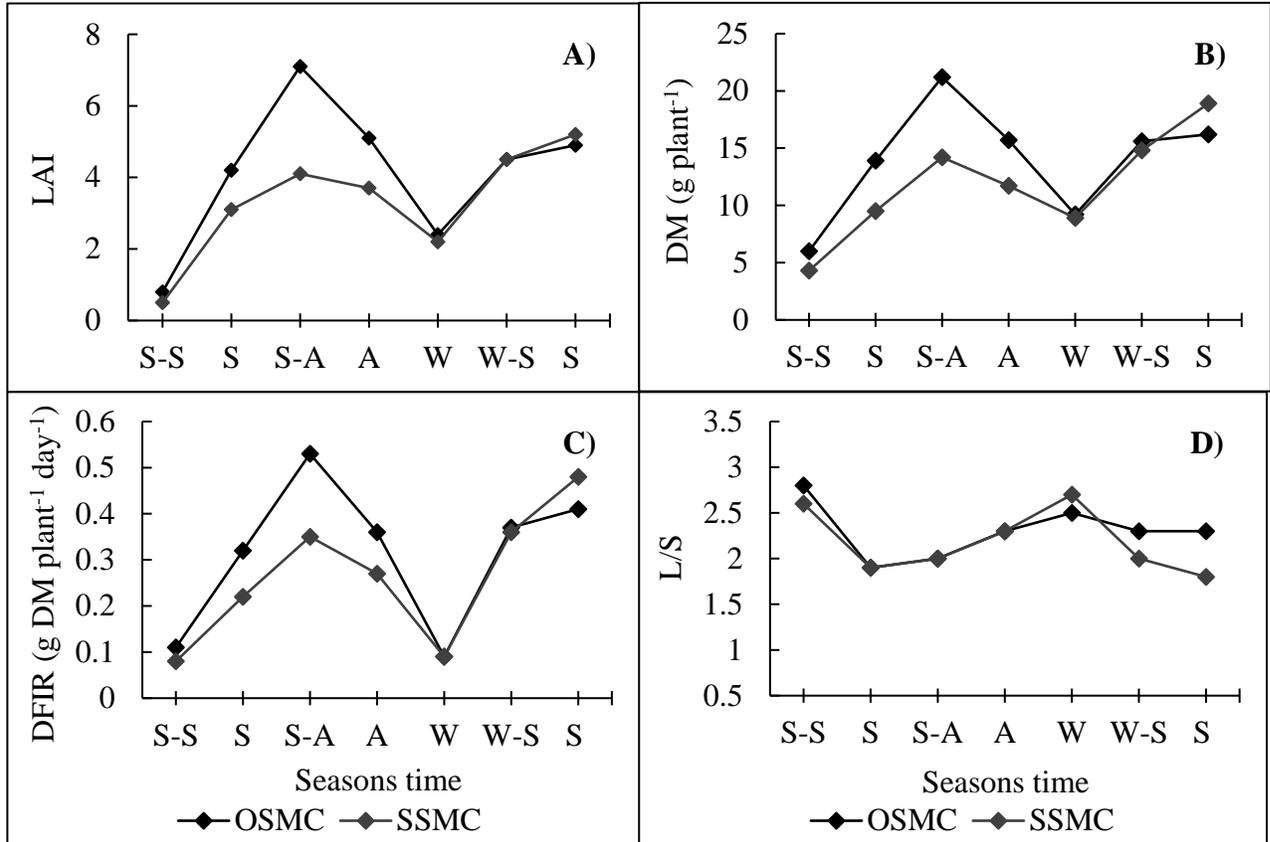
LAI= leaf area index; DM= dry matter; DFIR= dry fodder increase rate; L/S= leaf/stem ratio.

^{ab} Figures with the same letters within the same column are considered equal ($P>0.05$).

Seasonal dynamics of production indicators

In general, the seasonal behavior of *L. corniculatus* exhibited variations in terms of the variables LAI, DM, and DFIR, which were higher during spring, summer and summer-autumn—particularly DM—with a production of 17.5, 11.7, and 17.7 g plant⁻¹, respectively, and a drastic decrease during the winter season (Figure 2A, 2B and 2C); while the L/S remained stable throughout the evaluation period (Figure 2D).

Figure 2: Seasonal dynamics of: A) Leaf area index (LAI); B) Dry matter (DM); C) Dry fodder increase rate (DFIR), and D) Leaf-to-stem (L/S) ratio of *L. corniculatus* in different seasons of the year with optimal (OSMC) and suboptimal (SSMC) soil moisture contents, during the June 2021 to May 2022 period



The LAI showed the highest values in the spring, summer and summer-autumn periods, standing out in the OSMC plot during the summer, and then exhibiting an even behavior between the two soil moisture contents (OSMC and SSMC) in the rest of the year (Figure 2A). A similar behavior was observed for DM (Figure 2B) and DFIR (Figure 2C). L/S showed less variation by soil moisture content during the entire evaluation period (Figure 2D). These results coincide with the seasonal behavior of the temperature, which increases with the beginning of spring and reaches its highest values during the summer, being related to a higher incidence of solar radiation, with the consequent increase in the photosynthetic rate, and then begins to decrease in autumn, due to the beginning of the decrease in temperature⁽²⁴⁾. Higher LAI values translate into higher biomass production⁽²⁵⁾.

The productivity results obtained coincide with those obtained in a temperate region of Mexico⁽²⁶⁾, where the highest yields were obtained in spring and the lowest in autumn; however, they do not coincide with the production obtained in summer, which registered the highest values in the present study. This response behavior suggests that it is related to the

higher temperature regime, with an average of 22 °C, which favors the growth and development of *L. corniculatus*⁽⁸⁾. Although there was a decrease in forage production in the winter period, the DM production increased again in spring, which proves the tolerance of the plants to low temperatures, down to -4 °C, and their ability to recover after the thermal stress⁽²⁷⁾.

Knowledge of *L. corniculatus* forage accumulation per day and its seasonal influence will allow future estimates of forage yield and persistence during the aforementioned periods of the year and will make it possible to establish different management and utilization strategies under field conditions. In this case, the highest DFIR was obtained in the spring, summer and autumn periods for both soil moisture contents, where OSMC exhibited the highest value, of 0.53 g plant⁻¹ d⁻¹.

The response obtained for the L/S variable was most stable between cutting periods in the plots with the two established moisture contents, showing differences only in spring-summer, when the ratio was higher in the OSMC plot (2.8) and in the SSMC plot (2.6), followed by the winter and spring periods. This behavior is similar to that observed in a temperate region⁽²⁶⁾, where the highest L/S values were observed in winter and autumn, followed by spring and summer, with values of 2.4, 2.7, 2.0 and 2.1, respectively. This indicator shows that there are no differences between the genetic materials evaluated for the same phenological stage⁽²⁸⁾. Based on this characteristic, it is possible to implement a sequence of forage utilization in future clover farms for the purpose of improving the production and nutritional quality of the plants^(29,30).

Conclusions and implications

The best productive behavior of the evaluated accessions and varieties of the *L. corniculatus* clover was observed in the spring, summer and summer-autumn seasons; accession 255305 stood out for its leaf area index, dry matter production, and dry fodder increase rate under optimal soil moisture conditions (26 °C ± 1.5), while the bird's-foot trefoil variety exhibited a better leaf area index under water deficit conditions. The evaluation of the genetic materials of *L. corniculatus* based on such variables as leaf area index and production indicators will allow the selection of those with the greatest potential for adaptability as an alternative forage crop in environmental conditions of extreme temperatures and water deficit like those that are prevalent in the arid zones of northern Mexico.

Acknowledgments and conflict of interest

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The authors declare that they have no conflict of interest.

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