Article

Morphological variation in *Moringa oleifera* Lam. at different population densities

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Abstract

The tree species are used to improve the nutrition of ruminants and *Moringa oleifera*, can be a feeding alternative, for forage of high nutritional value. However, topological arrangements modify the structure of the plant and consequently the nutritional value. Therefore, the effect of different population densities (D50, D100 and D200 for 50, 100 and 200 thousand plants ha^{-1}, respectively) and 5 cuts every 28 days on the morphological characteristics of *Moringa oleifera* was evaluated, under a design of complete random blocks with arrangement in divided plots and three repetitions, in storm conditions and during the period from July to November 2017. The variables evaluated were height regrowth (HR), basal plant diameter (BPD), number of branches (NBP), relative content of chlorophyll (RCC), leaf area index (LAI) and specific leaf area (SLA). The results indicated that there was no interaction ($p > 0.05$) between population densities and cuts. It was observed that the HR, BPD and NBP values decreased ($p > 0.05$) with increasing plant density; the opposite case happened with LAI and SLA. The RCC was similar ($p > 0.05$) in all the evaluated densities. In conclusion, increasing the moringa population density from D50 to D200 negatively affected the aerial morphological components of the plants, mainly the stem, which influenced the number and size of branches.

Keywords: agronomic management, forage trees, population densities.

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Introduction

Livestock farming in Northeast Mexico presents difficulties, mainly because most of the grassland used have forage yields of 2 to 5 t DM ha^{-1} and low nutritional value, around 40% digestibility and crude protein content between 3 and 7% (Ávila, 2013). Likewise, during the dry season forage production decreases considerably, up to 90% (Garay-Martínez *et al*., 2018). To cope with the above, the inclusion of tree species with high nutritional value in the diet for ruminants has been proposed, with the aim of increasing the nutritional value of the diet and, therefore, improving the productive parameters in animals (Lombo *et al*., 2013).

In this sense, the use of *Moringa oleifera* Lam., commonly known as moringa, is an arboreal species (García *et al*., 2006), native to the tropical forests of northeast India (Ramachandran *et al*., 1980). It stands out for its nutritional value, mainly for the amount of protein (from 23 to 30%) in its leaves (Moyo *et al*., 2011; Zheng *et al*., 2016; Álvarado-Ramírez *et al*., 2018), which favors animal feed (Roman-Miranda *et al*., 2013), since protein is the most expensive nutrient (Lugo *et al*., 2012).

In addition to this, moringa leaves contain 321 to 521 g kg^{-1} DM of neutral detergent fiber, 224 to 361 g kg^{-1} DM of acid detergent fiber (Makkar and Becker, 1996; Reyes *et al*., 2006; Mendieta -Araica *et al*., 2012), approximately 2.27 Mcal kg^{-1} DM of metabolizable energy and 79% *in vitro* digestibility of dry matter (Makkar and Becker, 1997; Reyes, 2004). Likewise, the presence of 19 amino acids has been reported, of which 10 are essential in animal nutrition; in greater and lesser proportion are leucine and methionine with 19.6 and 2.9 mg g^{-1} of DM, respectively (Moyo *et al*., 2011).

On the other hand, moringa presents remarkable ecological plasticity to adapt to its environment, tolerates soils from acidic to alkaline (Padilla *et al*., 2012), but without problems of prolonged puddling; likewise, it subsists at extreme temperatures from -1 to 48 °C (Troup, 1921), prolonged periods of drought and requires a minimum rainfall of 250 mm distributed throughout the year to survive (Abdulkarim *et al*., 2007). As a forage species, its regrowth capacity after cutting is efficient (Nouman *et al*., 2014) and produces large amounts of biomass, 15 t DM ha^{-1} yr^{-1} (Reyes, 2004).

However, the factor that significantly influences the performance of a species is the population density of plants (Goss, 2012; Noda-Leyva and Martin-Martin, 2017). Being optimal, it provides adequate foliar coverage (Sadeghi *et al*., 2009) and this allows intercepting photosynthetically active radiation (Tinoco *et al*., 2008), which is used by the plant in the photosynthesis process (Monteith, 1977) for biomass production (Strieder *et al*., 2008).

However, it has also been reported that high densities can cause loss of plants (Foidl *et al*., 2001) and alterations in their morphological and chemical composition (De Carvalho *et al*., 2012), this as a consequence of intra and interspecific competition for radiation, space, nutrients and water (Pérez *et al*., 2010). Therefore, the objective of this research was to evaluate the effect of different population densities on the morphological characteristics of *Moringa oleifera* Lam. in semi-arid conditions and during the rainy season.

Materials and methods

The study was carried out under temporary conditions, from July to November 2017, at the Zootechnical Post ‘Ing. Herminio García González’, owned by the Faculty of Engineering and Sciences of the Autonomous University of Tamaulipas, located in the municipality of Güemez, Tamaulipas, Mexico (23° 56’ 26’’ north latitude and 99° 05’ 59’’ west longitude, at 193 masl). The soil is clayey in texture, with a pH of 8.4, electrical conductivity of 0.84 dS m^{-1}, content of organic matter and nitrogen of 4.43 and 0.26%, respectively. The climate is classified as semi-arid [BS_1 (h’) hw; Vargas *et al*., 2007] and is characterized by a rainfall and mean annual temperature of 884 mm and 24.3 °C, respectively. The accumulated temperature and rainfall recorded during the evaluation period is shown in Table 1.

Table 1. Accumulated precipitation, minimum and maximum temperature recorded in each of the samplings (5 cuts) during the evaluation period (July to November 2017).

Climate variable	Cut				
	1	2	3	4	5
Minimum temperature (°C)	21	21	17	10	7
Maximum temperature (°C)	42	42	39	35	35
Precipitation (mm)	10.1	60.9	97.6	127.9	22.2

Three population densities (D50: 50 000; D100: 10 000 and D200: 200 000 plants ha^{-1}) of *Moringa oleifera* were evaluated. The sowing was in rows 0.5 m apart and a distance between plants of 0.1, 0.2 and 0.4 m, to obtain the densities of D200, D100 and D50, respectively. The size of the large plot was 20 × 20 m and the girls 4 × 4 m. A 2 × 2 m area located in the central part of each small plot was considered as a useful plot.

Soil preparation consisted of aerating and loosening the soil by means of two crossed harrow passes and one with a lump harrow (Rotovator). The sowing was carried out on February 5, 2017 manually with botanical seed, without pre-germination treatment and two seeds were deposited per blow. 30 days after sowing (dds), thinning was carried out, leaving the plant more robust. Likewise, in the stage of establishment of the crop, aid irrigation was applied to the field capacity with an interval of 14 days from sowing until the uniform cut, and from this moment the crop was managed under temporary conditions.

On July 5, 2017, the uniformity cut (120 dds) was made at 25 cm above ground level and fertilized with 100, 50 and 50 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Urea (46% N), triple calcium superphosphate (46% P_2O_5) and potassium oxide (60% K_2O) were used as fertilizer sources. Weed control was carried out manually each month between plants and furrows.

Measurements were made every 28 days for five months from the uniformity cut; for this, in the useful plot of each repetition in each treatment, five representative plants were selected and labeled and the following was measured: the basal diameter of the plant (BPD, mm), height regrowth (HR, cm), number of branches (NBP) were measured, relative content of chlorophyll (RCC, SPAD units), leaf area index (LAI) and specific leaf area (SLA, cm^2 g^{-1}).

BPD was measured 5 cm above ground level using a Mitotuyo® brand digital caliper (model 500, AOS Absolute Digimatic), RA with a flexometer (Truper®) from the level of the uniformity cut to the apical bud of a representative branch per plant. For the estimation of RCC, LAI and SLA, a fully developed leaf was selected in the middle third of the plant. The RCC was obtained with the SPAD-502 Plus portable chlorophyll meter (Konica Minolta®, Inc.), with which the reading of 10 apical leaflets per leaf was taken and averaged.

For LAI and SLA, the leaflets of the main spine present in the leaf that was used in the RCC measurement were separated and the area was determined (leaf area meter model Cl-202, CID Bio-Science®, lnc.), with these values were estimated by the LAI and the SLA. All samples were placed in brown paper bags and dried in a forced air circulation oven (Thermo Scientific™ model Heratherm ™ OGS100) at 65 °C until a constant weight was maintained. Once dried, they were weighed on an ADAM® brand analytical balance (Model NBL 254 e/i, Nimbus®, Analytical Balances ™). The NBP was obtained by quantifying the branches that had more than one leaf.

Variables were analyzed using the GLM procedure (SAS, 2002), in a randomized complete block design with four replications, in an arrangement of divided plots; where the large plot was the density and the small the cuts. Where a significant difference was found between treatments, the Tukey mean comparison test (p= 0.05) was applied. In addition, a regression analysis was performed to determine the effect of density on the evaluated variables.

Results and discussion

The analyzes of variance carried out showed statistical differences ($p\leq 0.05$) between population densities in all the variables evaluated, with the exception of the relative content of chlorophyll (Table 2). In the cut variation source, it was found that only in the variables HR, BPD and NBP there was a significant effect ($p\leq 0.05$), as expected, since the environmental conditions varied during the evaluation period (Table 1). Regarding the effect of the population density interaction by cut, no significant differences were detected ($p\leq 0.05$; Table 2).

Table 2. Analysis of variance of population density, cuts and interactions (D×C) of each variable evaluated in *Moringa oleifera* Lam.

Variable	Effects		
	Density (D)	Cut (C)	D × C
Height regrowth (cm)	**	**	ns
Basal diameter of plant (mm)	**	**	ns
Number of branches per plant	**	**	ns
Specific leaf area (cm^2 g^{-1})	*	ns	ns
Foliar area index	**	ns	ns
Relative content of chlorophyll (SPAD units)	ns	ns	ns

*= ($p\leq 0.05$); **= ($p\leq 0.01$); NS= not significant.

The RA decreased ($p \leq 0.05$) 31% when the density was from 50 to 100 thousand plants ha^{-1} (19.05 vs. 13.13 cm; Table 3). It was observed that at higher plant density, the values of BPD and NBP were lower ($p \leq 0.05$). In this sense, when the density was from 50 to 200 thousand plants ha^{-1}, the BPD and NBP decreased 34 and 36% respectively (Table 3).

Table 3. Means of the morphological characteristics of *Moringa oleifera*, evaluated at different population densities, during the period from July to November 2017.

Variable	Population density (plants ha^{-1})						MSD
	D50		D100		D200		
Height regrowth (cm)	19.05	a	13.13	b	11.75	b	2.2
Basal diameter of plant (mm)	20.5	a	14.9	b	13.6	c	1.12
Number of branches per plant	5.3	a	4	b	3.4	c	0.54

Different literals (a, b, c) within the same row they indicate a significant difference between population densities (Tukey; p= 0.05). MSD= minimal significant difference.

It was observed that in sections one, two and five, as population density increased, RA decreased ($p \leq 0.05$). In this sense, the values obtained in the D100 and D200 in sections one (7.3 and 5.3 cm, respectively) and five (3 and 2.7 cm, respectively) were statistically similar ($p > 0.5$) and lower than those obtained in the section two (18.7 and 14.6 cm, respectively), while in the D50 all their values differ significantly from each other. The highest RA was recorded in section two in D50 (29.7 cm), followed by those recorded in sections one (18.3 cm) and five (4.6 cm; Figure 1A). Significant differences ($p \leq 0.05$) were observed between the cuts for the BPD variable (Figure 1B). Likewise, when the population density increased from D50 to D200, the BPD was reduced by 22% and the lowest and highest values were presented in section one (19.8 and 15.1 mm) and five (23 and 18.3 mm), in D50 and D200, respectively (Figure 1B).

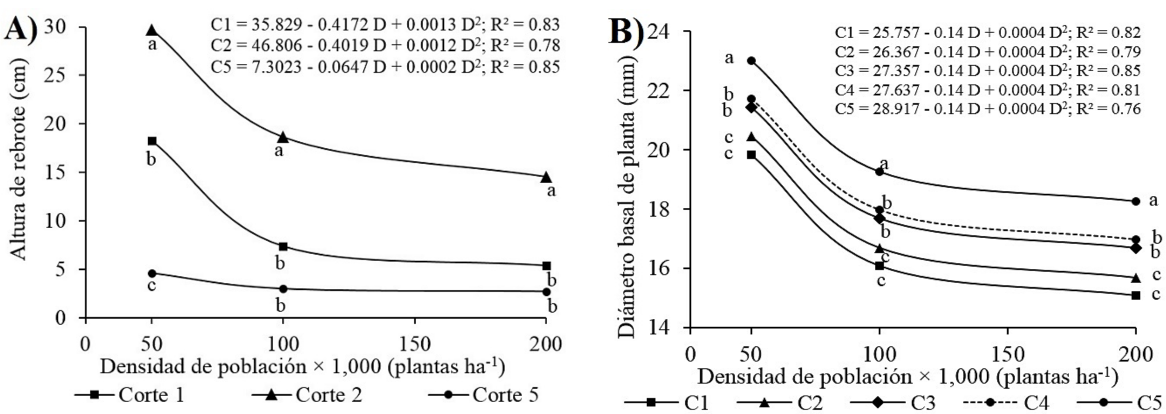

Figure 1. Trend of the regrowth height (A) and the basal diameter of the plant (B) in relation to the population density (D) and sections (C) of *Moringa oleifera*. Means in the same density with different literals (a, b, c) indicate a significant difference (Tukey; p= 0.05).

The results found in this study indicate that the increase in the density of plants per unit surface area, had a significant effect on the aerial structure of moringa. In this sense, the main stem decreased in thickness in the densest population (D200), while in the least dense population (D50) it increased. This is consistent with what was observed by other researchers, who, when evaluating different densities of the moringa population, observed that morphological development was negatively affected by increasing population density and that the stem was the aerial organ that underwent the most changes, as the density increased, the diameter decreased (Goss, 2012; Longo *et al*., 2017).

This is due to the intraspecific competition that is generated between plants, mainly for underground and air space, available nutrients and water, when the populations are very dense (Ella *et al*., 1989; Foidl *et al*., 2001). Contrary to these results, in a study carried out in Cuba it was observed that in densities of 250 000 to 1 000 000 plants ha^{-1} there was no difference in stem thickness, but there was an elongation (Sosa-Rodríguez *et al*., 2017), which leads us to suppose that the crop responded to the management it was subjected to (Toral *et al*., 2006).

In this work, the height of regrowth decreased with increasing population density from D50 to D100, this contrasts with a study where they evaluated from 12 to 197 thousand plants ha^{-1} and found no differences in plant height (Goss, 2012); however, the sprouts were thin and flimsy (Sosa-Rodríguez *et al*., 2017), which indicates that the plants competed for the available sunlight (Amaglo *et al*., 2006). Given this, it can be inferred that the difference in regrowth height between densities was due to the remaining leaf area, which contributed to the reduction of the recovery period, therefore, in the rapid formation of new aerial organs to subsist (Stür *et al*., 1994).

Also, it was observed that when the environmental temperature was maintained in a range of 21 to 42 °C and the accumulated pluvial precipitation increased from 10.1 to 60.9 mm, the plants responded positively in all densities; while at higher thermal amplitude (7 - 35 °C), the response was negative, even when there was a cumulative precipitation of 22.2 mm. In this regard, it has been mentioned that in this species the environmental temperature is the factor that significantly influences its development (Meza-Carranco *et al*., 2016), temperatures that are in a range of 26 to 29 °C are favorable to Olson and Alvarado-Cárdenas (2016); while those below 20 °C affect growth and development (Paliwal *et al*., 2011; Ferreira *et al*., 2015).

Similarly, other authors indicate that plant growth is determined by the amount of water available in the soil (Ledea *et al*., 2017) and in the case of forage species, it has been shown to influence the morphogenesis of plants (Patel *et al*., 2014). Likewise, the humidity in the soil is necessary so that the available nutrients are absorbed and translocated towards the different organs of the plant and when using dense populations, the demand for them is greater (Firbank and Watkinson, 1990).

Regarding the NBP, it was observed that the density had a significant effect ($p \leq 0.05$) in sections one and three. Where, increasing the density caused a decrease in the number of branches and leaves per plant (Figure 2A). In this sense, the NBP obtained in both cuts, D50 and D100 differed significantly from each other ($p \leq 0.05$) and it was in the third cut that the highest values were presented (7.0 and 4.2, respectively) and in the first cut the lowest (3.8 and 2.1, respectively), while in D200 the values were similar ($p > 0.05$) in both sections (2.3 and 3.6, respectively, Figure 2A).

It was observed that the highest values ($p > 0.05$) in the LAI and SLA were obtained in the D200 density (1.8 and 245 cm^2 g^{-1}, respectively), while in the D50 and D100, the values for these variables were similar (Figure 2B). On the other hand, no differences were observed in the RCC in the three densities evaluated and the values were between 48 and 50 SPAD units (Figure 2B).

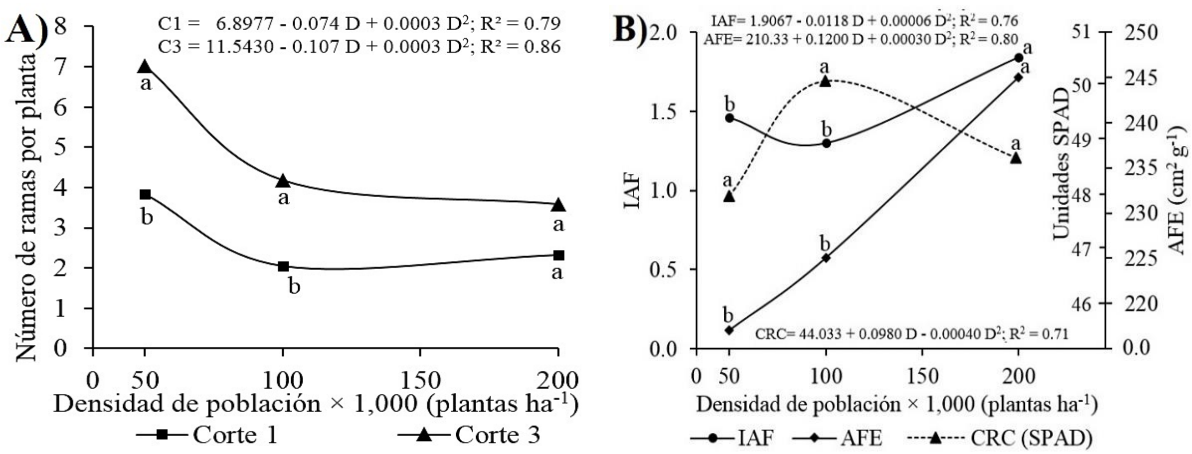

Figure 2. Trend in the number of branches per plant (A) and the relative content of chlorophyll, specific leaf area and leaf area index (RCC, SLA and LAI, respectively (B) in relation to population density (D); and cuts (C) in *Moringa oleifera*. Means in the same density (A) and variable (B) with different literals (a, b) indicate significant difference (Tukey; $p = 0.05$).

The behavior in the NBP coincides with that reported by other researchers, who observed that in low populations the number of branches is greater (Sosa-Rodríguez *et al*., 2017) and therefore the number and size of the leaves tends to increase (Dos Santos *et al*., 2017). In this regard, it has been mentioned that pruning is used in forage tree species as a mechanism to stimulate the emission of new branches (Ramachandran *et al*., 1980).

However, it has been reported that when pruning the plant, an inhibition is generated in the assimilation of available nutrients in the soil (Reyes *et al*., 2006) and that the regrowth capacity is limited by the thickness of the stem (Foidl *et al*., 2001), because it acts as a reserve carbohydrate accumulation organ and that these in turn are used by plants in the formation of sprouts and photosynthetic tissue (Sadeghi *et al*., 2009).

In this study, the plants that presented stems with greater thickness (D50), the accumulation of reserves was greater, which was reflected in a more vigorous regrowth, in terms of quantity and dimensions of branches. Also, it was observed that in the D50 and D100 populations the emergence of new branches per plant was greater when the rainfall was increased, while in the D200 population the behavior of this variable was the same, regardless of the ambient temperature or rainfall.

In this sense, climatic conditions affect the morphology of the plant's organs (Ledea-Rodríguez *et al*., 2018). An important factor is the availability of water in the soil, since it directly influences foliar development, which is governed by stomata, which regulate water expenditure (transpiration) depending on environmental conditions and photosynthetic activities (Medrano *et al*., 2007).

Regarding the effect of plant density on the leaf area, these results agree with those of other authors, who pointed out that the population density is positively associated with the leaf area index (Damtew *et al*., 2011), since the increasing the plant population increases foliar coverage on the ground (Sánchez-Hernández *et al*., 2011), which happened when the population increased to D200. It has been mentioned that high leaf area indices increase the interception of photosynthetically active radiation (Tinoco *et al*., 2008), which in turn is used by the crop (Strieder *et al*., 2008) and increases photosynthesis (Goss, 2012).

The specific leaf area values were higher in the highest density (D200), therefore, it can be assumed that the plant responded physiologically to competition between plants and consequently the thickness of the leaf was less; which is favorable, since it has been mentioned that in forage species there is a positive correlation between the SLA and the nitrogen concentration, and this in turn, with the crude protein content (Pérez *et al*., 2004; Garay *et al*., 2017).

Conclusions

The morphology of the organs of the moringa plant was modified by increasing the density of plants; mainly in the vigor of the regrowth, which was reflected in the size and number of branches per plant after cutting. With D200 densities, land use is optimized, since the highest values in the LAI are obtained.

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