

## Planting density in verdolaga growth

Erika Lagunes-Fortiz<sup>1§</sup>  
Clemente Villanueva-Verduzco<sup>1</sup>  
Edgar Ricardo Lagunes-Fortiz<sup>1</sup>  
Erika Janet Zamora-Macorra<sup>1</sup>  
Norma Ávila-Alistac<sup>1</sup>  
Evert Villanueva-Sánchez<sup>2</sup>

<sup>1</sup>Chapingo Autonomous University. Highway México-Texcoco km 38.5, Chapingo, Texcoco, State of Mexico. ZC. 56230. Tel. 595 9521500, ext. 6133. <sup>2</sup>CONACYT-Chapingo Autonomous University. National Laboratory for Research and Food and Forestry Service. Highway México-Texcoco km 38.5, Chapingo, State of Mexico, Mexico. ZC. 56230.

§Corresponding author: elagunesf@chapingo.mx.

### Abstract

In Mexico there are more than 358 species of plants, of which only tender leaves and stems are consumed, known as ‘quelites’. Given the productive, economic and nutritional potential of these species, including verdolaga, factors that affect their growth and development need to be determined. Verdolaga is an alternative as a vegetable due to its nutritional, productive and economic potential; its yield will be determined mainly by the development of stems and leaves, so the knowledge generated about the factors that affect its growth is of great agronomic importance. This research assessed the effect of three planting densities on verdolaga growth. Data for three phenological stages of verdolaga development (vegetative development, vegetative maturity and flowering) were considered to determine whether there were significant differences between the mean treatments evaluated by an Anova and to determine whether variance homogeneity existed, the Levene method was carried out. Plants planted at a high planting density (295 plants m<sup>-1</sup> linear) had higher height, smaller stem diameter, fewer leaves (large and small) and lower branch development (branch length) compared to those planted at medium and low planting densities (250 and 134 plants m<sup>-1</sup> linear); however, the number of leaves on the branches depends on the level of the canopy where they are located. Planting density is a contributing factor in determining the growth of the ‘Chapingo’ verdolaga.

**Keywords:** phenological stages, quelites, verdolaga.

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## Introduction

In Mexico there are more than 500 species of wild edible plants, whose use dates back to pre-Hispanic times given its great cultural, culinary and nutritional relevance, including quelites, nahuatl 'quiltl', of which only tender leaves, stems and flowers are consumed (Bye and Linares, 2002; Balcázar-Quiñones *et al.*, 2020), as they are an important source of nutrition (Ca, K, Mg, Fe, Mn, Zn, P), protein, dietary fiber, vitamins A, C and bioactive compounds, such as phenols, betalains, flavonoids, alkaloids, omega 3, omega 6, carotenes, ascorbic acid,  $\alpha$ -tocopherol, glutathione, etc., which give these plants a high antioxidant capacity, in addition to providing dishes with different fragrance, colors and flavors (Bye and Linares, 2002; Santiago-Saenz *et al.*, 2019; Balcázar-Quiñones *et al.*, 2020).

Given their high productive potential and the existence of an ancestral culture of consumption of some of these crops (verdolagas, quintoniles, papalos, epazotes, etc.) in the Mexican population of the center of the country, where there are 25 million inhabitants in The City and The State of Mexico alone (INEGI, 2015), the Educational and Research Institutions must develop technology, varieties, intensive production systems and agronomic practices that provide a comprehensive knowledge of the different species of quelites and that help to promote the optimal use of their economic potential and as a food plant.

In Mexico, commercial verdolaga production is concentrated in the states of Morelos (171 ha), Mexico City (158 ha), State of Mexico (45 ha) and Baja California (46 ha) with yields ranging from 12 to 14.86 t ha<sup>-1</sup> and a production of 5 474.15 t, with a production value of just over 26 361.30 thousand pesos (SIAP, 2019).

In our country, there are two well-defined producing regions (contrasting in climate) with local creole varieties domesticated under cultivation: the region of Xochimilco-San Gregorio-Mixquic (temperate climate) and that of Cuautla, Morelos (warm climate) (Villanueva and Ramírez, 2003). The active and continuous domestication of verdolaga in the different producing regions has allowed the obtaining of genotypes with thicker leaves, habits of erect growth, greater precocity and resistance to diseases, such as the crop 'Chapingo' of verdolaga, whose bromatological analyses have shown that it also contains high levels of iron, magnesium, omega 3 and omega 6 (Solís *et al.*, 2016).

Studies conducted under greenhouse (without heating) during the winter cycle indicate that it is possible to produce up to 20 kg m<sup>-2</sup> of vegetables in 50 days (200 t ha<sup>-1</sup>), with a population density of 500 m<sup>-2</sup> (Villanueva and Ramirez, 2003). The value of the winter verdolaga harvest can reach up to \$8.00 per kilogram to the producer, although it drops dramatically at times of the year of its natural production (Villanueva and Ramirez, 2003; Mera *et al.*, 2010).

The study of the growth of verdolaga and the factors that affect it is of great importance, since its yield will be determined mostly by the development of the vegetative part of the plant, which in turn will depend on multiple factors, some of them typical of the genotype, others of the environment and various conditions of crop management.

Recently Montoya-García *et al.* (2017) assessed the effect of N, P and K fertilization on verdolaga production, found that the height and yield of verdolaga increase in response to the application of applied nitrogen and the harvest date. Montoya-García *et al.* (2018) report that it is possible to maximize the nutraceutical content, as well as the concentration of fatty acids and antioxidants with fertilizations with nitrogen, phosphorus and potassium, so factors such as fertilization not only influence the growth of the plant, but also on the potentiation of its properties for the benefit of health.

Knowledge about crop growth forms the basis for the optimal planning and execution of cultural work to be carried out during the production process, in order to obtain the best yields. The genotype, planting date, planting density, locality and climate in which plants develop are factors that can influence the amount of foliar tissue, growth habit, part of the plant and development of other structures.

The most important factors affecting the duration of the stages of verdolaga development include the genotype (inside and between populations), the environment (climate and soil) and its interactions, which can vary the growth habit and precocity of the plant. Some other characteristics of the plant such as the habit of growth, flavor, phyllotaxis and stem, may vary depending on whether the plant develops as weeds or cultivated (Villanueva and Ramírez, 2003). Plant growth involves at the physiological level a series of changes and irreversible reactions in the plant, on which the final agronomic characteristics (phenotype) and the potential yield of the different genotypes will depend.

The evaluation of the growth of a plant can be carried out through direct measures (plant height, stem diameter, number of leaves, number of branches, foliar area, fresh or dry mass, etc.) and indirect (net assimilation rate, crop growth rate, relative growth rate, etc.) (Rodríguez, 2000).

Planting density is a factor that can influence crop yield such as verdolaga, as it interferes with plant physiological events and directly with accumulation of dry matter from the different organs of the plant, and also determines competition between plants by light, water and nutrition (Rodríguez, 2000; Ayala *et al.*, 2004). Based on the above, the objective of this research was to evaluate the effect of three planting densities on the growth of the crop 'Chapingo' of verdolaga, as well as characterize its dynamics and habit of growth.

## Materials and methods

The experiment was established in a batch of the Experimental Agricultural Field of the Chapingo Autonomous University, Mexico at 19° 29' 27" north latitude and 98° 52' 23" west longitude, at an altitude of 2 240 meters above sea level (msnm). The modifications made by Garcia (1988) to the Köppen climate classification system describe the site of the establishment of the experiment as a C(W)(w)b(i)g, which means that it is the driest of temperate subhumid with rains in summer, an average annual temperature of 15.6 °C and -3° to 18 °C in the coldest month (January), a cool summer, the temperature of the warmest month is less than 22 °C (may), with little annual thermal oscillation ranging from 5° to 7 °C. The soil is classified as Mollic Ustifluent, with a depth of 150 cm and a slimy clay loam texture on the Horizon Ap (Survey Soil Staff, 2010).

40 furrows  $\pm 1$  of 30 m long and a distance between furrows 0.6 m with verdolaga of the crop 'Chapingo' were planting. The sampling sites were established at random, so that each experimental unit had a different planting density, it is delimited 1 m for each experimental unit and it is counted the number of plants in each experimental unit, leaving three different planting densities (treatments): high (295 plants. linear meter), medium (250 plants  $m^{-1}$  linear) and low (134 plants  $m^{-1}$  linear). In each experimental unit, data were taken to 20 plants (repeats).

The following variables were evaluated every 4 days from planting and up to 48 days after planting (dds). Variables studied were total plant height (AT), was measured in centimeters (cm), from ground level to the end of the main stem; number of branches (NR), the branches that were inserted directly into the main stem were counted; stem diameter (DT), was measured in millimeters (mm) with the help of a Vernier, in the second internode of the plant, just below the third knot; number of large leaves and small leaves (NHG and NHCH, respectively), the large and small leaves of the plant were counted, considering as large leaves those between 1.5 to 2 cm wide and 2.5 to 3 cm long. Average length of the branches of the n knot (LPRn), was taken the average, in centimeters, of the length of the two branches that were inserted into the n knot of the main stem, numbering the knots from the base to the tip of the plant. The average number of leaves of the n knot branches (NHPn), refers to the average number of leaves of each of the branches that were inserted into the n knot of the main stem, numbering the knots of the base to the tip of the plant.

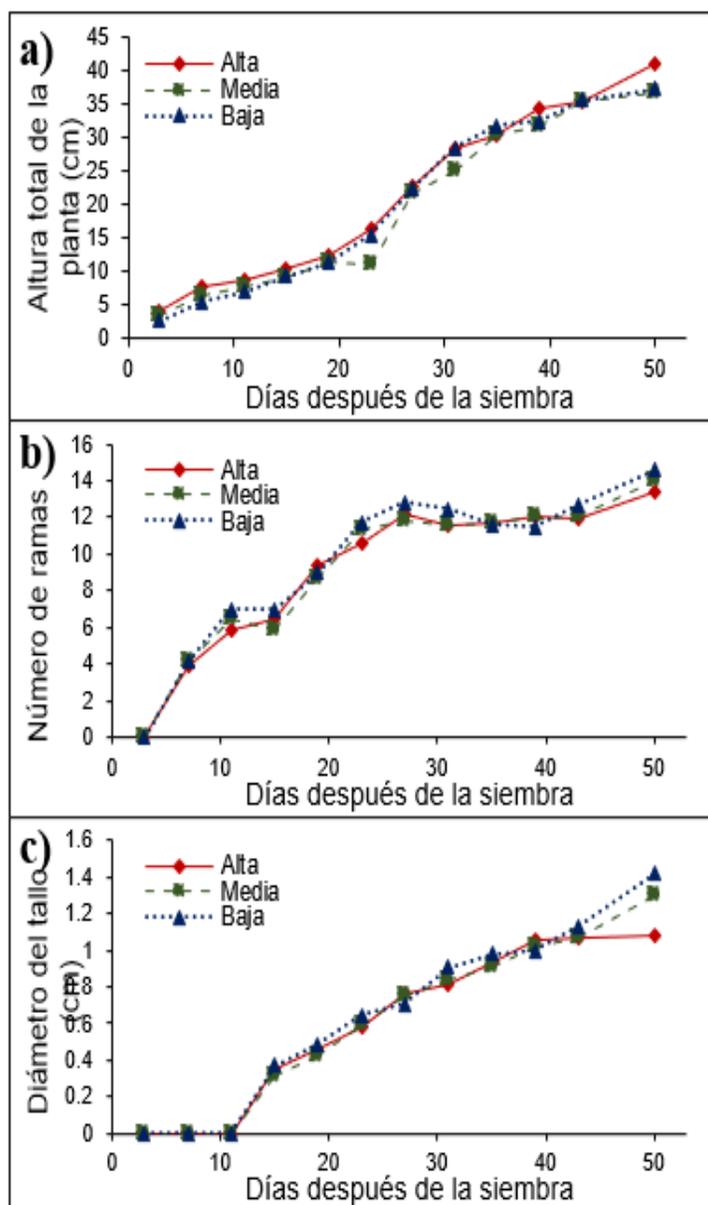
For statistical analysis, data were taken for three phenological stages of verdolaga development: vegetative development (19 dds; 52.1 Units Heat, UC), vegetative maturity (31 dds, 100.65 UC) and flowering (39 dds, 39 142.55 UC) of verdolaga of the crop 'Chapingo'. The data was analyzed with the SAS System for Windows 9.1 (SAS Institute, 2002) package, to determine whether there were statistically significant differences between the averages of the three planting densities evaluated an Anova was performed and to determine whether there was equality of its variances the Levene method was used, both with a significance level of  $p \leq 0.05$ .

## Results and discussion

Figure 1 shows the growth of the verdolaga in plant height (AT), number of branches (NR) and stem diameter (DT); in Figure 2, the number of large leaves (NHG) and number of small leaves (NHCH), while in Figure 3, the growth of the branches of the main stem at the height of the 1<sup>st</sup> and 4<sup>th</sup> knot, counting from the base to the tip of the plant. The growth dynamics represented in these figures show that it is between 20 and 40 dds where the growth of the verdolaga is accelerated so, although the variables were evaluated every 4 days, the Anova and the Levene test of variance comparison was performed with the data obtained in the phenological stages covering that period (vegetative development, vegetative maturity and flowering).

## Plant height

Throughout the life cycle of the plant, the height increases constantly and exponentially, particularly between 20 and 40 dds, which match with the beginning of the vegetative development stage and until the beginning of flowering (Figure 1a). The AT mean comparison test shows that only during the vegetative maturity stage there were statistically significant differences between the means of the three planting densities ( $p < 0.05$ , Table 1). The highest AT was obtained in the high planting density (295 plants m linear) relative to the mean and low densities (250 and 134 plants m linear, respectively).



**Figure 1.** Growth dynamics of a) total plant height (cm), b) number of branches and c) stem diameter (in cm) of the verdolaga in three planting densities (high: 295; medium: 250 and low: 134 plants m linear, respectively).

**Table 1. Anova of total height of the plant (AT, in cm), number of branches (NR), stem diameter (DT, in cm), number of large leaves (NHG) and number of small leaves (NHCH) at 19 dds (vegetative development), 31 dds (vegetative maturity) and 39 dds (start of flowering) of verdolaga of the variety ‘Chapingo’ sown in three planting densities (high: 295; medium: 250 and low: 134 plants m linear, respectively).**

Planting density	AT		NR		DT		NHG		NHCH	
	M	Anova (F/Pr value)	M	Anova (F/Pr value)	M	Anova (F/Pr value)	M	Anova (F/Pr value)	M	Anova (F/Pr value)
Vegetative development										
High	12.29		9.4		0.45		10.75		41	
Medium	11.6	0.20/0.8197	8.65	0.45/0.6419	0.42	0.64/0.5335	10.55	2.02/0.1416	38.4	4.39/0.0169
Low	11.4		9		0.48		12.25		54.3	
Vegetative maturity										
High	28.35		11.55		0.81		11.5		107.15	
Medium	25.26	3.68/0.0313	11.6	2.68/0.077	0.84	2.62/0.0813	12.15	1.31/0.2768	111.45	0.45/0.6376
Low	28.6		12.45		0.9		13.75		114.3	
Flowering										
High	34.51		12.05		1		10.15		227.1	
Medium	31.69	2.54/0.0877	12	0.66/0.5205	1.02	0.64/0.5335	12.35	2.79/0.07	224.8	0.04/0.9633
Low	32.48		11.5		1.05		11.5		222.5	

M= average. Bold data group indicate statistically significant differences between them ( $p < 0.05$ ).

A preliminary analysis of the data revealed that the dispersion of observations at the three planting densities might be different, so a Levene test was performed. It was concluded that the variances are statistically different so planting density is a contributing factor in determining the plant height of the verdolaga (Table 2).

In this sense, Rodríguez (2000) reports that the increase in population density generates the production of longer plants, while Favaro and Pilatti (1997) indicate that tomato plants planted in high planting density, originate a higher proportion of far-red light (730 nm) in their lower strata, affecting phytochrome activity, which is expressed by an increase in the length of internodes and consequently in plant height.

### Number of branches

Noda and Gildardo (2008) when studying the growth of *Morus alba* in different planting densities, report that, plants develop more branches in low planting densities, since little competition by light and the arrangement of direct solar radiation favors the development of lateral branches; however, in this research, no statistically significant differences were found in the number of branches (NR, Table 1) determined by planting density, probably because verdolaga ‘Chapingo’ being a previously selected material, has well-defined structural characteristics, such as erect growth habit, opposite phyllotaxis, long stem, thick stem, succulent green stem and arrangement of its opposite lateral branches, which it preserves very stable.

Between 20 and 30 dds the development of branches is accelerated (period that coincide with the vegetative development stage) and subsequently increases, but to a lesser extent, remaining stable from 30 dds until the end of its life cycle. The verdolaga ‘Chapingo’ develops 8 to 9 branches in the vegetative development stage, 12 branches at the beginning of flowering (Table 2) and up to 14 branches at the end of its cycle (48 dds, Figure 1).

**Table 2. Standard deviation of total plant height (AT, in cm), number of branches (NR), stem diameter (DT, in cm), number of large leaves (NHG) and number of small leaves (NHCH) at 19 dds (vegetative development), 31 dds (vegetative maturity) and 39 dds (flowering) of verdolaga of the variety ‘Chapingo’ sown in three planting densities (high: 295; medium: 250 and low: 134 plants m linear, respectively).**

Planting density	AT		NR		DT		NHG		NHCH	
	DS	Levene (F/Pr value)	DS	Levene(F/Pr value)	DS	Levene(F/Pr value)	DS	Levene (F/Pr value)	DS	Levene (F/Pr value)
Vegetative development										
High	4.94		2.6		0.2		2.27		16.62	
Medium	4.77	0.06/0.9373	2.46	0.05/0.9536	0.17	0.09/0.9142	2.61	1.64/0.2024	18.64	0.37/0.693
Low	4.42		2.47		0.18		3.7		19.27	
Vegetative maturity										
High	4.28		1.5		0.19		2.37		25.4	
Medium	3.17	2.94/0.061	1.43	0.53/0.5896	0.1	0.77/0.465	6.96	1.8/0.1741	25.26	0.27/0.768
Low	5.28		1.19		0.1		2.68		20.75	
Flowering										
High	5.35		1.68		0.2		2.77		34.13	
Medium	3.49	3.48/0.0377	1.84	0.46/0.6364	0.17	0.09/0.9142	2.91	1.67/0.1978	62.67	0.99/0.3786
Low	3.79		1.47		0.18		3.65		60.59	

M= average. Bold data group indicate group of variances with statistically significant differences between them ( $p < 0.05$ ).

Authors mention that one of the most sensitive factors to density variations is the number of branches per plant, an increase in the population of plants produces a decrease in the number of branches (Ayala *et al.*, 2004; Kakiuchi and Kobata, 2004) which coincides with the results obtained in this research as the number of branches was lower in verdolaga plants sown in high planting density compared to medium and low planting densities.

### Stem diameter

From 10 dds the diameter of the stem increases exponentially, from 20 and 40 dds there is a similar accelerated growth in the three planting densities, the growth stops from the 43 dds (capsule development stage) in the high planting density (295 plants m<sup>-1</sup> linear) (Figure 1c). No highly significant differences in stem diameter were found between the verdolagas sown in the three planting densities (Table 1).

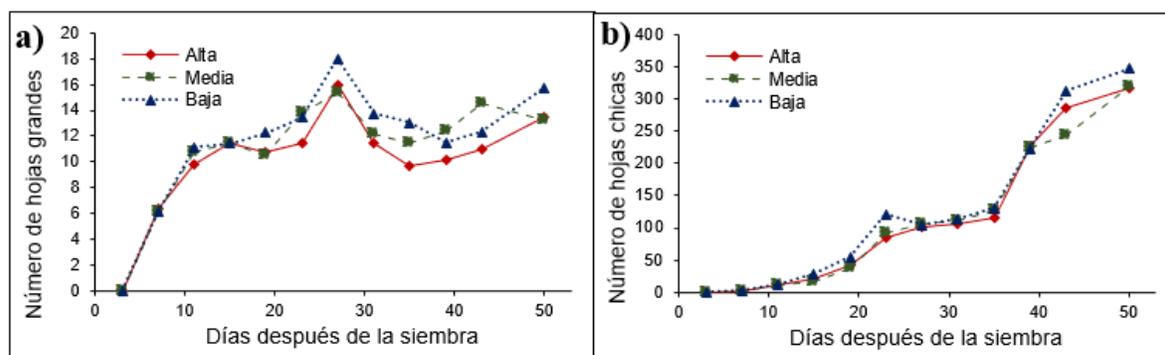
According to García and Watson (2003), the thickness of the stem depends on the genotype, the environmental and nutritional conditions of the soil, states that in maize the resistance that the plant presents to the acame, depends to a large extent on the stem diameter and that in addition the stem diameter tends to decrease when the planting density is increased, due to competition between the plants.

High planting densities and light competition with weeds cause elongation of stems, longer internodes and taller plants, reducing the thickness of the stems and increasing the chances of acame of the plants, this coincides by observing the results obtained in this research at 48 dds of verdolaga sown in three different planting densities. The diameter of the stem is a very important parameter since in addition to containing the ducts that channel the transport of water and nutrients to the different organs of the plant, it will also influence the resistance to bending of the plant when affected by external factors such as wind or rain. The verdolaga crop 'Chapingo', has an erect growth, so an optimal thickening of the stem will favor the development of all the structural organs of the plant.

### Number of leaves

In the three planting densities evaluated, from 10 dds to the end of the verdolaga life cycle, the number of large leaves (NHG) is maintained between 10 and 18 large leaves, being at 27 dds where there are the most large leaves (18 leaves) which coincides with the end of the vegetative development stage, then some leaves fall (mainly those located at the base of the lower branches), an event that coincides with the insinuation stage (development of visible floral buttons on the terminal part of the main stem) and flowering, finally towards the end of the plant's life cycle, the number of large leaves is maintained between 10 and 14 leaves according to planting density (Figure 2a).

The dynamics of the development of the small leaves (NCH) is different (Figure 2b) because from the beginning of the vegetative development stage (18 dds) and until the insinuation stage (35 dds) the number of small leaves is constant in the three planting densities, however from 35 to 48 dds (seed maturation stage) the number of small leaves increases, perhaps due to the demand that represents the production of photo-assimilated for the development of plant reproductive structures during the hint and maturation stages of the seed.



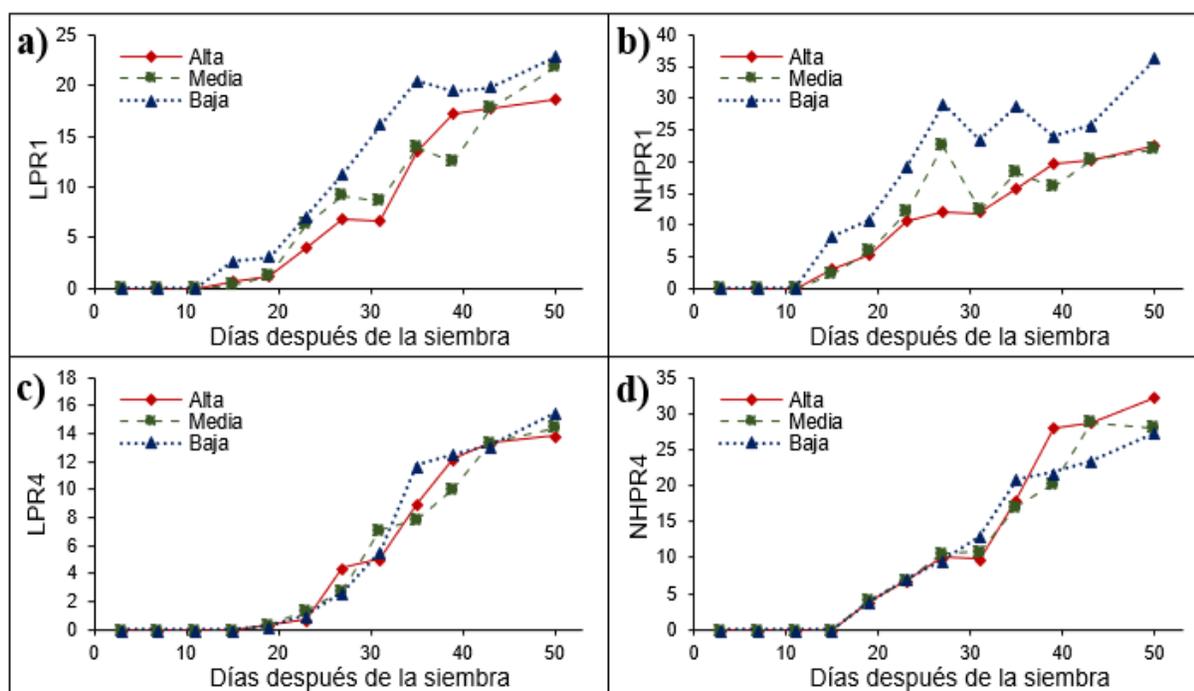
**Figure 2. Growth dynamics of a) number of large leaves and c) number of small leaves in three planting densities (high: 295; medium: 250 and low: 134 plants m<sup>-1</sup> linear, respectively).**

The verdolaga plants planted in the low planting density (134 plants m linear) are the ones that reach the greatest number of large and small leaves, followed by those planted in medium and high planting densities, behavior similar to what was reported by Díaz *et al.* (1999), which point out that biomass production decreases as density increases, due to increased competition between plants by light, CO<sub>2</sub>, water and minerals.

### Length and number of branch leaves

During the development of the verdolaga a total of 8 knots were developed on the main stem, on which two lateral branches were developed, as well as new leaves on these branches. In this study only those branches located in the 1<sup>st</sup> and 4<sup>th</sup> knot were measured (numbering from the base to the tip of the plant) because they are the ones that develop in greater magnitude compared to the branches of the upper knots.

The growth of branches and leaves in the 1<sup>st</sup> knot (LPR<sub>1</sub> and NHP<sub>1</sub>, respectively) begins to be noticeable from 10 dds (Figure 3a and 3b). On the other hand, the growth of the branches and leaves of the 4<sup>th</sup> knot (LPR<sub>4</sub> and NHP<sub>4</sub>, respectively) is presented between 15 and 20 dds (Figure 3c and d); that is, during the vegetative development stage.



**Figure 3.** Growth dynamics of the average length of the two branches of knot a) 1 (LPR<sub>1</sub>) and c) 4 (LPR<sub>4</sub>) and number of average leaves of the two branches of knot a) 1 (NHP<sub>1</sub>) and d) 4 (NHP<sub>4</sub>) of the verdolaga in three planting densities (high: 295; medium: 250 and low: 134 plants m linear, respectively).

In the LPR<sub>1</sub> variable, statistically significant differences were found between the three planting densities ( $p < 0.05$ ), in the vegetative development, vegetative maturity and flowering phases, while for the NHP<sub>1</sub> statistically significant differences were found ( $p < 0.05$ ) between the sowing

densities evaluated in the vegetative development stages, vegetative maturity, but not in the flowering stage. For the LPR<sub>4</sub> and NHP<sub>4</sub> variables, no statistically significant differences were found between the mean of the three planting densities evaluated with the exception of the LPR<sub>4</sub> variable in the vegetative maturity stage ( $p < 0.05$ , Table 3).

**Table 3. Length and number of leaves of the branches at 19 dds (vegetative development), 31 dds (vegetative maturity) and 39 dds (flowering) of verdolaga of the 'Chapingo' variety sown in three planting densities (high: 295; medium: 250 and low: 134 plants m linear, respectively).**

Planting density	LPR <sub>1</sub>		NHP <sub>1</sub>		LPR <sub>4</sub>		NHP <sub>4</sub>	
	M	Anova (F/Pr value)						
Vegetative development								
High	1.13		5.35		0.3		3.85	
Medium	1.28	8.17/0.0008	5.9	14.187/<0.0001	0.33	0.44/0.6488	4.1	0.1/0.9083
Low	3.1		10.8		0.26		3.8	
Vegetative maturity								
High	6.6		12.1		5.06		9.9	
Medium	8.54	17.34/<.0001	12.35	7.86/0.001	7.15	3.29/0.0444	10.75	3.06/0.0546
Low	16.1		23.35		5.63		13	
Flowering								
High	17.22		19.75		12.23		28	
Medium	12.49	4.4/0.0169	16.1	2.49/0.0916	10.1	3.04/0.0558	20.4	2.59/0.0836
Low	19.46		24		12.6		21.8	

LPR<sub>n</sub>= average length of the two branches of the n knot; NHP<sub>n</sub>= number of average leaves of the two branches of the n knot. Bold data group indicate statistically significant differences between them ( $p < 0.05$ ).

According to the variance comparison test for the variables LPR<sub>1</sub>, NHP<sub>1</sub>, LPR<sub>4</sub> and NHP<sub>4</sub>, only in a few cases there were statistically significant differences, so the planting density influences the number of branches and leaves that grow on the plant (Table 4). Poulain (1984) notes that high planting densities generate higher plants, but with fewer branches, as happened in the growth of the branches in the 1<sup>st</sup> and 4<sup>th</sup> knot (LPR<sub>1</sub> and LPR<sub>4</sub>, respectively) where plants sown in high planting density reached lower length compared to those sown in medium and low densities.

The branches of the first knot of those plants planted in low density contained the largest number of leaves (NHP<sub>1</sub>) compared to those of the medium and low densities, possibly because their length and availability of space allowed them to produce more leaves. On the other hand, the number of leaves of the branches of the 4<sup>th</sup> knot (NHP<sub>4</sub>) was higher in the verdolaga plants sown in the high planting density, compared to the medium and low densities, probably because the level of the canopy where they were located influenced the development of the leaves to supply the demand for photo-assimilated necessary for the formation of structures typical of the subsequent phenological stages.

**Table 4. Standard deviation of branch length (in cm) and number of branch leaves at 19 dds (vegetative development), 31 dds (vegetative maturity) and 39 dds (flowering) of verdolaga of the ‘Chapingo’ variety sown in three planting densities (high: 295; medium: 250 and low: 134 plants m linear, respectively).**

Planting density	LPR <sub>1</sub>		NHP <sub>1</sub>		LPR <sub>4</sub>		NHP <sub>4</sub>	
	DS	Levene (F/Pr value)						
Vegetative development								
High	1.01		2.6		0.2		2.3	
Medium	1.86	1.62/0.2072	3.6	2.76/0.0721	0.21	0.21/0.8091	2.51	0.41/0.6638
Low	2.09		4.29		0.25		2.12	
Vegetative maturity								
High	5.92		8.96		2.03		3.31	
Medium	5.82	0.61/0.5485	14.15	6.74/0.0023	1.96	3.61/0.0334	5.6	2.69/0.0767
Low	4.28		5.86		3.66		2.83	
Flowering								
High	4.67		8.41		3.06		13.01	
Medium	7.18	5.24/0.0082	9.1	2.83/0.0675	3.41	0.3/0.7406	10.76	0.54/0.5857
Low	10.01		14.83		3.57		11.02	

LPR<sub>n</sub>= average length of the two branches of the n knot; NHP<sub>n</sub>= number of average leaves of the two branches of the n knot. Bold data group indicate group of variances with statistically significant differences between them ( $p < 0.05$ ).

## Conclusions

There are significant differences between planting densities for total height (AT), number of small leaves (NHCH), average length of the two branches of knot 1 and 4 (LPR<sub>1</sub> and LPR<sub>4</sub>, respectively) and number of average leaves of the two branches of knot 1 (NHP<sub>1</sub>). High planting densities generate taller plants, smaller stem diameter, fewer leaves (large and small) and less branch development (branch length); however, the number of leaves developed in the branches depends on the level of the canopy where they are located.

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