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Original article

Definition of the optimal planting spacing for the hybrid H-Ame15 under dry period

Definición del marco óptimo de plantación del híbrido de maíz H-Ame15, en época seca

Pilar Téllez-Rodríguez[1*], Ivis Morán-Bertot[1], Albis Riverón-Hernández[1], Davel Espinoza-Delgado[1], Alberto Martínez-Ramírez[1], Miriam Isidrón-Pérez[2], Yulexis Pino-Blanco[3], Leyenis García-Santos[1], Javier Laguna Lezcano[1]

[1]Centro de Ingeniería Genética y Biotecnología (CIGB), ave. 31 no. 15802 e/158 y 190, Cubanacán, Playa, La Habana, Cuba, P.O. Box 6162, CP 10600

[2]Universidad Agraria de La Habana "Fructuoso Rodríguez Pérez", (UNAH), carretera a Tapaste y Autopista Nacional, San José de las Lajas, Mayabeque, Cuba, CP 32 700

[3]Centro de Producción y Desarrollo Agrofar, Santo Domingo, Villa Clara, Cuba

ABSTRACT: The cultivation of the simple hybrid of corn H-Ame15 on a large scale, constitutes an alternative for an efficient management of corn (*Zea mays*) in the field, as it contains transgenic events, which provide resistance to (*Spodoptera frugiperda* Smith) and tolerance to herbicides based on glufosinate ammonium. The objective of this work was to consolidate the productivity of the hybrid, optimizing its density in the field. For this, the morphological evaluation of the crop was carried out under three plantation spacing: 0.7m x 0.15m; 0.7m x 0.20m and 0.7m x 0.25m, in the period between December 2019 and April 2020 in an experimental plot. The higher population density caused a decrease in the stem diameter; this generated the slenderness pattern of the plants described in the literature. Cobs did not change the number of rows with increasing density, however the number of grains per row decreased, which affected the productive potential of the plants individually. This variation in the morphology of the ear allowed to characterize this hybrid with the flexible pattern of the cob. Despite this, the potential yield increased, due to the contribution of a greater number of plants per hectare: 14.74 t ha^{-1} at 0.7mx 0.15m, 12.83 t ha^{-1} at 0.7m x 0.20m and 10.94 tha^{-1} at 0.7m x 0.25m. It was determined that 0.7mx0.20m (71000 plants ha^{-1}), was the most appropriate plantation framework for this time, since with this distribution the best balance between production and percentage of loss caused by environmental factors such as seasonal diseases was achieved.

Key words: *Zea mays* L., plant density, yield.

RESUMEN: El cultivo del híbrido simple H-Ame15 a gran escala, constituye una alternativa para el manejo eficiente del maíz (*Zea mays* L.) en campo, al contener eventos transgénicos, que aportan resistencia a *Spodoptera frugiperda* Smith y tolerancia a herbicidas a base de glufosinato de amonio. El objetivo de este trabajo es definir el marco de plantación óptimo para este híbrido. Para ello, se evaluó el comportamiento morfoagronómico del cultivo a 0,7m de distancia entre surcos y 0,15 m, 0,20 m y 0,25 m de separación entre plantas. El ensayo se realizó durante el período de seca, comprendido entre 2019 y 2020. Con el aumento de la densidad de plantas disminuyó el diámetro del tallo, esto generó el patrón de esbeltez de las plantas descrito por la literatura. En las mazorcas no varió el número de hileras, sin embargo, disminuyó el número de granos por hilera, lo cual caracteriza al híbrido con el patrón flexible de la mazorca. Esta variación en la morfología de las mazorcas afectó el potencial productivo de las plantas de manera individual, a pesar de

*Author for correspondence: pilar.tellez@cigb.edu.cu

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ello, el rendimiento potencial se incrementó, por el aporte de un mayor número de plantas por hectárea: 14,74 t ha^{-1} a 0,15 m; 12,83 t ha^{-1} a 0,20 m y 10,94 t ha^{-1} a 0,25 m. Se determinó que 0,7 m x 0,20 m (71 000 plantas ha^{-1}), fue el marco de plantación apropiado para esta época y se logró el mejor balance entre producción y porcentaje de pérdida, causada por factores ambientales como enfermedades estacionales.

Palabras clave: densidad de plantación, rendimiento, Zea mays.

INTRODUCTION

Corn (*Zea mays* L.) is a crop in great demand for its various uses. Its worldwide production exceeds 1100 million tons of dry grain per year, with notable productions in countries such as the United States, Brazil and Argentina, supported by yields of 11.6 and 8 t ha^{-1}, respectively. These rates are associated with the use of hybrid technologies and Genetically Modified Organisms (GMOs). In addition, there is a tendency to achieve the maximum productive potential from the efficiency of agro-technical management. The influence of the following elements is currently being studied: climate, nitrogen fertilization, previous crop, stocking density, tillage and growth regulators (1,2).

The National Statistics Office (ONEI) published in 2020 (3), that the national production of dry corn in Cuba was 247 473 tons, with an average yield of 1.93 t ha^{-1} and 873 225 tons of unmilled dry corn were imported, at a cost of $199 040 000 dollars (4). Causes of the low production levels are subject to insufficient availability of inputs, technological indiscipline, seed quality, low field population, among others (5). Another factor to consider is that seed production is mainly directed to the use of traditional varieties, whose potential is inferior to that of hybrids.

The introduction of the simple transgenic hybrid H-Ame15 in the national corn production is a proposal of the Center for Genetic Engineering and Biotechnology (CIGB), as another alternative to increase the production of this crop in Cuba. This hybrid obtained its varietal registration in December 2020. It has high productivity provided by heterosis to which is added the value added by the products of transgenesis: tolerance to glufosinate ammonium herbicides and resistance to the codling moth (*Spodoptera frugiperda* Smith). However, in order to achieve maximum crop yields, it is necessary to make some management adjustments.

The use of an optimal population enhances the efficient use of light and soil resources (6).

Increasing plant density is one of the strategies that has allowed record yields in corn production (7,8). Several authors describe morphological changes, which as a whole allow visualizing slender plants and if the conditions are extreme, plants become unproductive, with a tendency to curl up (9,10). Therefore, it is necessary to evaluate the capacity of each hybrid to face this stress in order not to affect the net yield per area. The hybrids that have greater plasticity to high population density, conserve their morphological pattern (10,11), part of this response is involved in the reproductive flexibility and the capacity to conserve a fixed pattern under these conditions (12).

The objective of this work is to define the optimal planting framework for the simple transgenic maize hybrid H-Ame15, based on its agronomic behavior during the dry period in Cuba.

MATERIALS AND METHODS

Plant material: seed of the H-Ame15 hybrid was used, product of the cross between the transgenic line L-Moltó and CT9, with a harvest date of April 2019, at the Credit and Service Cooperative (CCS) "Juan Darias" in Jarahueca, Sancti Spíritus. Seed of the CT9 line was also required, for use as Refuge (10 % of planted area). The use of Refuge is one of the most recommended strategies to prevent the appearance of insect resistance in transgenic plants carrying toxins of *Bacillus turingiensis* (Bt) (13). All the seed was treated with Celestop at a dose of 3 mL kg-1 of seed, as recommended by the commercial company (14).

A completely randomized design was carried out with three planting frame schemes: 1) 0.7 m x 0.15 m (95000 plants ha^{-1}); 2) 0.7 m x 0.20 m (71000 plants ha^{-1}); 3) 0.7 m x 0.25 m (57000 plants ha^{-1}). Each treatment was represented by three plots (replicates), composed of four 10 m long furrows. Planting was manual and two furrows were planted on each side of the experimental area with the conventional line CT9 as refuge. This trial was conducted in the dry period from December 2019 to April 2020.

Agrotechnical Management: The experiment was conducted on a Ferrallitic Red soil with background fertilization (9-13-17) at a dose of 600 kg ha^{-1} (15). Two foliar fertilizations with Byfolan were applied at a rate of 2 L ha^{-1}, with an interval of one week from the V1 vegetative stage of plants. Urea (200 kg ha^{-1}) was applied when the plants reached the V6 vegetative stage. No product was used to control codling moth and SphereMax (250 mL ha^{-1}) was used at V8 to control phytopathogenic fungi. Weed control was carried out in V3, with Lifeline herbicide at a single dose of 1.5 L ha^{-1}. Drip irrigation was used for 12 hours, with a frequency of four days, to ensure 80 % of the field capacity. Harvesting was done manually when the ears had 25 % of grain moisture. Drying was carried out in the sun. Shelling of the ears was carried out manually at 13 % grain moisture.

Agronomic evaluations: to evaluate the performance of the agronomic traits of the hybrid in each planting frame, 20 plants were selected from the central furrows of each plot. The selected plants should not have any missing plants in their vicinity, to guarantee the planting frame under study. Measurements were made according to the CIMMYT manual for monitoring trials (16). In R1, plant height (cm), cob height (cm), angle of insertion of the leaf to the stem (°)

and stem diameter (cm) were evaluated. Subsequently, at harvest, each ear was measured for closure (category 1 to 5); length (cm); diameter (cm); number of rows; number of kernels per row; harvest weight (ear and kernels) (g) and grain weight (g).

From the above data, calculations were made for the following characteristics:

Shelling percentage = [kernel weight (g)/ harvest weight (g)] * 100

Potential Yield (t ha^{-1}) = (average grain weight (g)) * (# plants in 1 ha)

The impact of the environment on the productivity of the hybrid was evaluated by counting the number of productive and unproductive plants in each plot (28 m^2) at the time of harvest, and from these data the estimated actual yield was calculated.

Actual yield (t ha^{-1}) = [production per plot (kg) / Plot area (m^2)] * 10

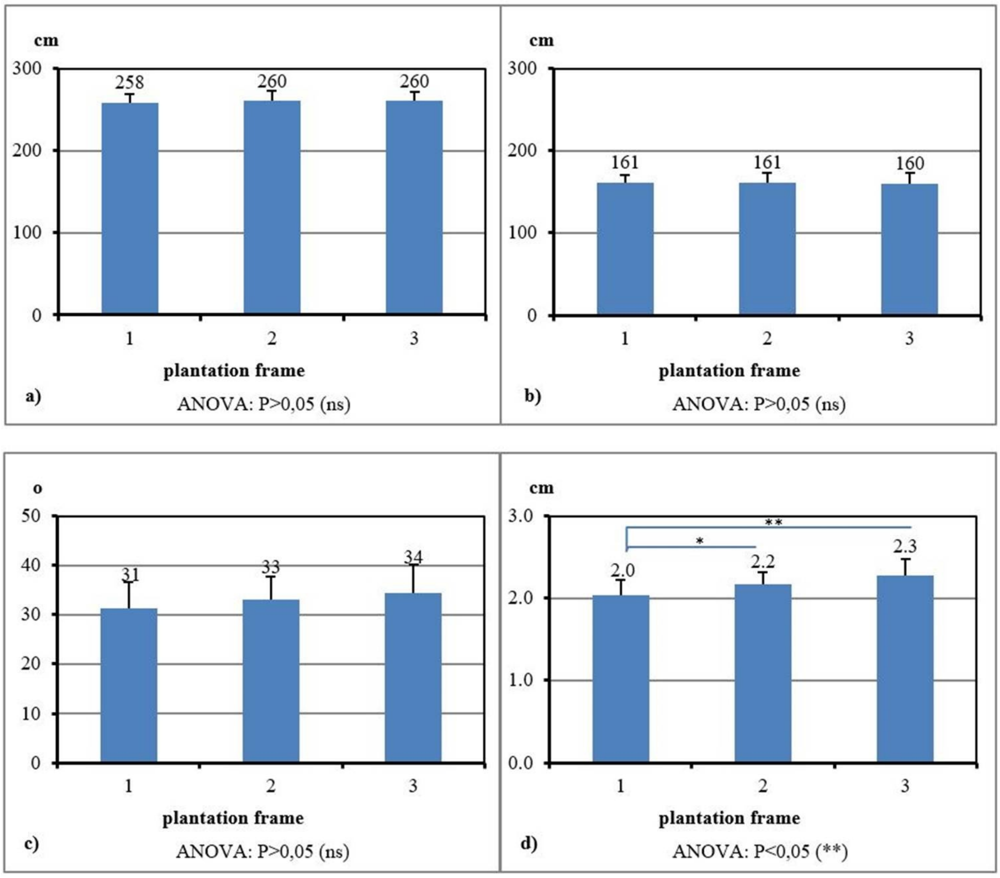
Statistical Analysis: For the statistical processing of the data corresponding to the morphological characters, the statistical package GraphPad Prism version 6.01 was used. To evaluate the similarity or difference between the means obtained between treatments, an ANOVA was performed with a non-parametric Kruskall Wallis test, followed by Dunn's multiple comparison test. In the particular case of potential yield, in order to characterize yield behavior as a function of population density, a linear regression was also performed using Microsoft Excel.

RESULTS AND DISCUSSION

Morphoagronomic characterization of plants in R1

As a result of the measurements at the R1 stage of the crop for the three planting frames, there were no significant differences in plant height, cob height and the angle of insertion of the leaf to the stem (Figure 1); however, there were significant differences in stem diameter (Figure 1d), where thinner plants were found in the narrowest planting frame.

The reduction of the stalk diameter with the increase of planting density in corn has been reported by several authors (17-19), as for the height of the plant and of the cob there are differences in the behavior, according to different authors (17,19-21). In this sense, it is suggested that this behavior is related to the plasticity of the specific cultivar, as is the case of the Colombian hybrid Impacto, which at densities higher than 100 000 plants conserves its pattern for height (22).



a) Height of the plant b) height of the cob c) angle of insertion of the leaf to the stem d) stem diameter

Figure 1. Morphological appearance of plants after flowering for the three planting frames

The morphological pattern of the hybrid H-Ame15 with high plant density agreed with the generality observed for several cultivars (9). These authors emphasize that the increase in plant density causes a decrease in stem diameter and leaf biomass, where the presence of plants with a slender appearance stands out. Competition for light is one of the main factors to consider when establishing high densities. The H-Ame-15 hybrid is characterized by its great height and the angle of insertion of the leaf to the stem is not completely narrow, which can make it vulnerable to very high densities, as it generates more shade between neighboring plants. Several authors suggest that plants are sensitive to the decrease in the ratio between the red spectrum and the far red (R/RL), the main component of the shade caused by neighboring plants (23). To perform photosynthesis, chloroplasts require the absorption of light, mainly from the red and blue part of the spectrum and very little from the green and far-red part. The selection of genotypes with characteristics of lower height and narrowing of the angle of insertion of the leaf to the stem, generate less shade on the neighboring plant and, therefore, have a better adaptive response to high densities.

Morphoagronomic characterization of plants at harvest

As a result of the morphological characterization (Figures 2 and 3), the H-Ame15 hybrid did not show significant variation in the following ear characters: ear closure, diameter, number of rows and shelling percentage. Some studies highlight that the number of rows is marked by strong genetic factors (12,17,21,24) (Figures 2 and 3). The productive result is in correspondence with the interaction effect of several factors throughout the crop cycle, among which density plays a fundamental role.

As shown in Figures 2 and 4, for the number of grains per row and ear length there were significant differences between the 0.7 m x 0.15 m treatment (95000 plants ha^{-1}) and the other two treatments. These cobs traits were the most sensitive to high plant density, thus characterizing H-Ame15 as a flexible ear hybrid. Cultivars capable of varying ear number and length in response to density changes and compensating for population variations in the field are called flexible (11). Those that conserve the number and dimensions of the cob under any condition are called fixed, which gives them an advantage in high plant density (12). This characterization of H-Ame15 was also reaffirmed by previous experiences in production areas, where under low population conditions, between 30.000 and 40.000 plants, more than one commercial ear developed and the size of the cob increased notably.

The number of grains per cob row is defined during the V10 to V12 vegetative stages of corn plant development (25). These stages are characterized by the elongation of the stem and the reduction of the time between one vegetative stage and the next, which are accompanied by physiological changes that demand greater availability of water, nitrogen and potassium (25). Therefore, supplying the correct nutrition, in correspondence with the field population, ensures the availability of these elements in the plant at the time of ear formation and filling (26).

Figure 2. Appearance of average ears at harvest, for the three planting frames

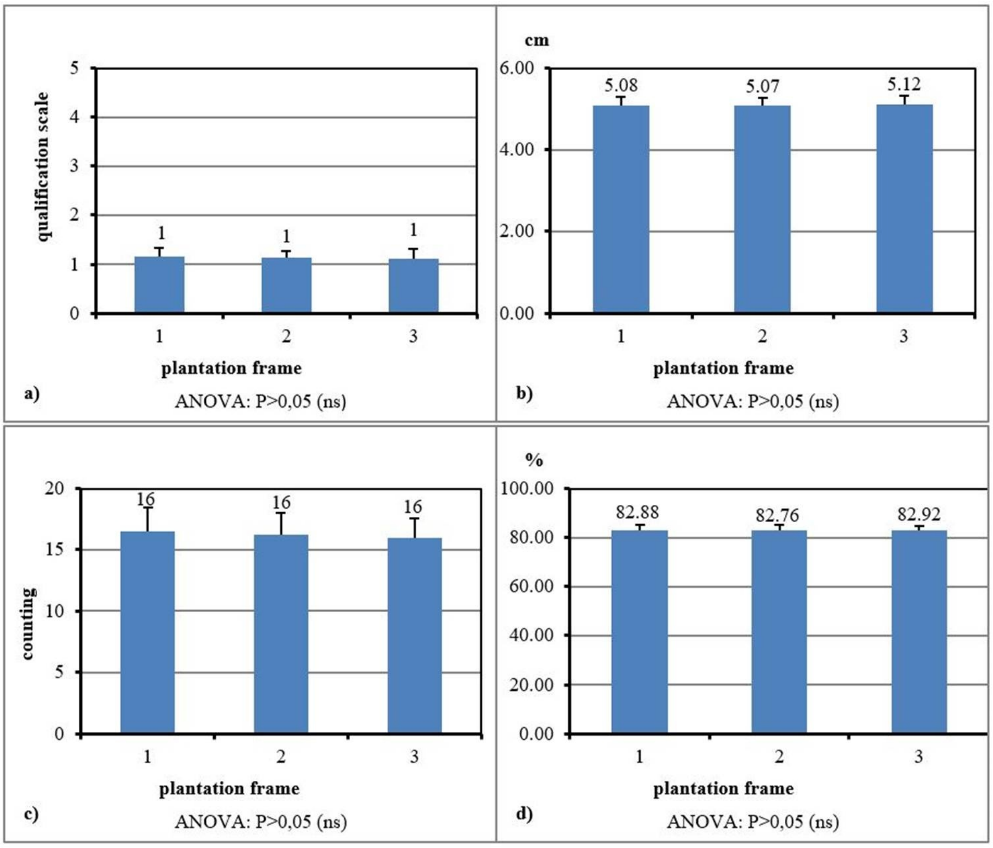
The reduction in the number of grains per ear row had an impact on the individual production of these plants. As shown in Table 1, there was a marked difference in the weight of grains per plant obtained for the three planting frames. Despite this, there was a positive balance in the potential yield in the field, as the number of plants per hectare increased.

Analysis of productive potential

In the analysis of productive potential (Table 1), the highest potential yield corresponded to the 0.7 m x 0.15 m planting frame, with a value of 14.74 t ha^{-1}, and a difference of approximately 1.9 t ha^{-1} was determined between one planting frame and the one before it.

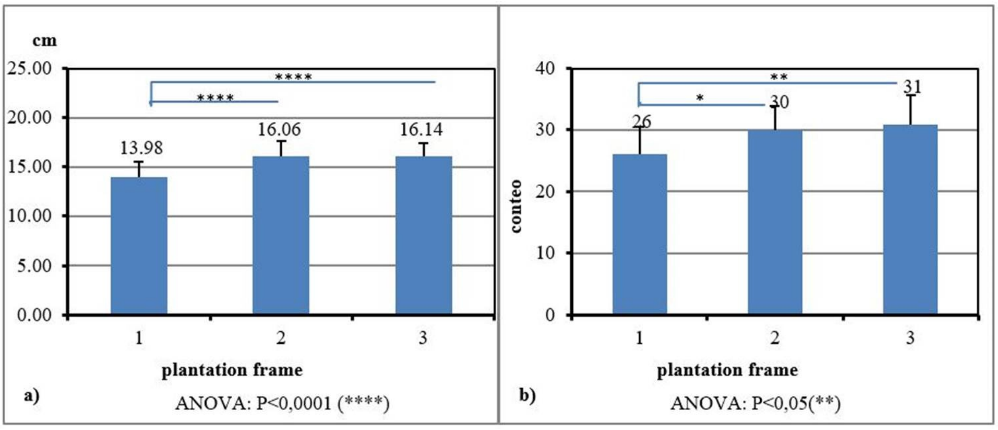
When analyzing the behavior of grain weight per plant and potential yield of the H-Ame15 hybrid as a function of plant density, it was observed that for both cases this behavior conforms to a linear model (R^2=1 and R^2=0.9774) (Figure 5). Other authors described a logarithmic behavior for grain weight per plant and a quadratic one for yield in two modern hybrids (27). The differences observed between the results of these authors and the present work could be due to the fact that they evaluated a narrower range of populations for H-Ame15, between 57 000 and 95 000 plants ha^{-1}, while the one used by these authors was more comprehensive, covering the range between 15 000 and 180 000 plants ha^{-1}. The equations obtained in Figure 5 are a useful tool for yield estimation, depending on the hybrid populations established in the field.

Despite the high productive potential of the H-Ame15 hybrid in the 0.7 m x 0.15 m planting frame, the best yield estimate value corresponded to 0.7 m x 0.20 m with 9.42 t ha^{-1}(Table 2). This indicates that there are environmental factors that affected, to a greater extent, the production under conditions of high plant density.



a) cob closure b) diameter c) number of rows d) shelling percentage

Figure 3. Morphological aspect of the ears at harvest, for the three planting frames



a) length of cob b) number of kernels per row

Figure 4. Morphology of cobs at harvest, for the three planting frames

Tabla 1. Comparación del peso promedio de granos por planta del rendimiento potencial para los tresmarcos de plantación

Plantation frames	Weight of grains per plant (g)	Potential Yield (tha^{-1})
0.7 m x 0.15 m (95000 plants ha^{-1})	151.1	14.74
0.7 m x 0.20 m (71000 plants ha^{-1})	180.9	12.83
0.7 m x 0.25 m (57000 plants ha^{-1})	195.1	10.94
	ANOVA - Test of Dunn/alpha 0.05	
0.7 m x 0.15m vs. 0.7 m x 0.20 m	**	***
0.7 m x 0.15 m vs. 0.7 m x 0.25 m	****	****
0.7 m x 0.20 m vs. 0.7 m x 0.25 m	ns	***

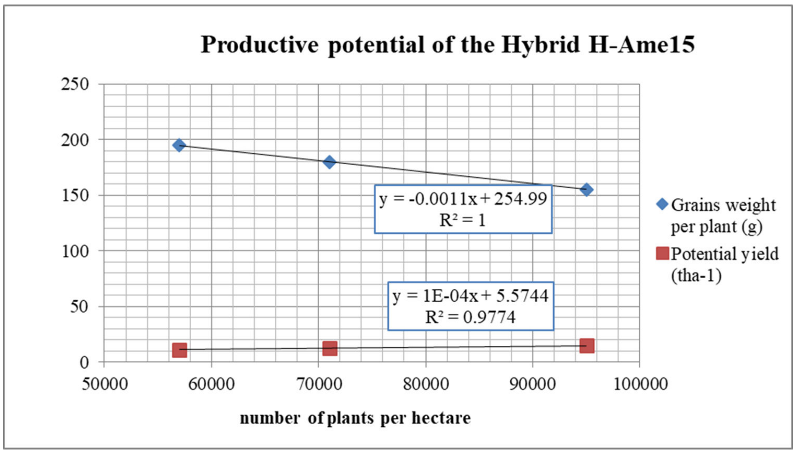


Figure 5. Behavior of the productive potential of hybrid H-Ame15, as a function of the number of plants per hectare

Table 2. Estimated average actual yield for the three planting frames

Planting frames	Estimated True Yield (tha-1)
0.7 m x 0.15 m (95000 plants ha^{-1})	9.21
0.7 m x 0.20 m (71000 plants ha^{-1})	9.42
0.7 m x 0.25 m (57000 plants ha^{-1})	8.13
ANOVA - Test of Dunn/alpha 0.05	
0.7 m x 0.15m vs. 0.7 m x 0.20 m	ns
0.7 m x 0.15 m vs. 0.7 m x 0.25 m	ns
0.7 m x 0.20 m vs. 0.7 m x 0.25 m	*

There are references with other simple hybrids, in which the highest productive potential was obtained with populations similar to those of the present work, between 70000 and 80000 plants per hectare, such is the case of studies carried out in Brazil (17,26) and Serbia (21). While other authors report the best yields in densities between 80 000 and 100000 plants h^{-1} (27-29). This behavior is associated with the particularities of each hybrid and the effect of the environment where they are grown.

A data that illustrates the incidence of this last aspect is the number of unproductive plants found at harvest; an indicator of the competition between neighboring plants and the incidence of diseases in the plantation. Figure 6 shows a comparative analysis of the percentage of unproductive plants for the three planting frames. As could be seen, the 0.7 m x 0.15 m planting frame was the most sensitive variant to this factor. Under the conditions of this planting frame, there was an increase in the percentage of unproductive plants, which caused a decrease in the actual yield, with respect to the potential yield (Tables 1 and 2).

In spite of the proximity between the plants, in the 0.7 m x 0.15 m frame, no nutritional affectation was observed at the visual level, according to the deficiency patterns described for corn (30). This is important if we take into account that in this work the same fertilization was used for the three planting frames. Therefore, we consider that the production of these plants was mainly defined by the morphological pattern generated in the competition for light. We add to this

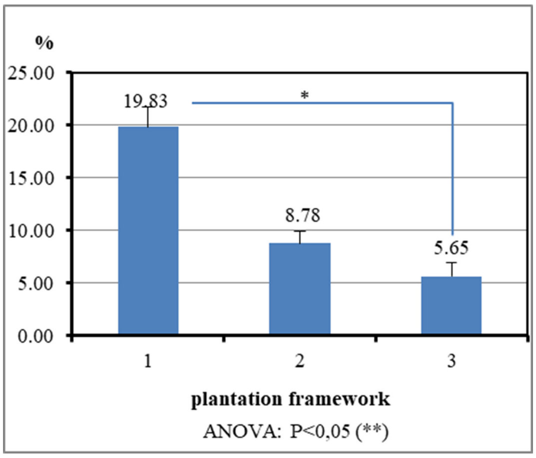


Figure 6. Percentage of unproductive plants at harvest

the effect of a higher incidence of stunt disease, caused by *Spiroplasma kunkelii* and transmitted by the leafhopper *Dalbulus maidis*, in the 0.7 m x 0.15 m planting frame (31). Although the disease did not reach severity, it did restrict the productivity of a significant group of plants. This result is similar to that observed by other authors in Brazil (16). It should also be considered the experience of greater spread of diseases such as asphalt spot caused by the association of three fungal microorganisms (*Phyllachora maydis*, *Monographella maydis* and *Coniothyrium phyllachorae*), in tropical environments similar to that of Cuba with high plant density (32).

CONCLUSIONS

- The simple maize hybrid H-Ame15 retains its plant height, ear height and leaf-to-stem insertion angle pattern when grown at a row spacing of 0.7 m and a plant spacing range of 0.15 to 0.25 m. At higher densities, plants with smaller stalk diameter and a slender stalk pattern previously described in the literature are produced. Higher plant density results in plants with smaller stem diameter and a slender pattern previously described in the literature.
- The increase in plant density did not affect the number of rows per ear, but did affect the number of grains per row and the length of the cob. This behavior allows classifying this hybrid with a flexible ear pattern.
- The increase in the number of plants per hectare affects the productive potential of individual plants; however, it compensates the result per hectare. Despite the high potential of the hybrid in the narrower planting frame, the percentage of losses is higher when compared to the other treatments. As a consequence, the estimated actual yield for the 0.7 m x 0.15 m and 0.7 m x 0.20 m planting frames was similar; therefore, we recommend the use of 0.7 m x 0.20 m as the most appropriate planting frame for the hybrid H-Ame15 in the dry period. With this distribution, a good balance between production and loss due to environmental factors such as the incidence of seasonal diseases is achieved.

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