



Effect of two biostimulants on some variables of the tomato fruit (*Solanum lycopersicum* L) cultivar Pony Express

Efecto de dos bioestimulantes en algunas variables del fruto de tomate (*Solanum lycopersicum* L) cultivar Pony Express

 Eduardo I. Jerez-Mompie^{1*},  Antonio Gómez-Salazar²,
 Gabriel López Salvador²,  Pedro J. González-Cañizares¹

¹Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700

²Tecnológico Nacional de México. Instituto Tecnológico Nacional de Tecamatlán, México

ABSTRACT: Bioestimulants contribute to increase yield reflected in fruits. In this sense, a study was carried out at the Institute of Tecamatlan, Puebla, Mexico, to evaluate the influence of the Quitomax[®] use and the inoculation with two strains of mycorrhizae in the behavior of some variables of fruit. Seedlings were produced in trays with a commercial substrate, under the same treatments that would later be applied in field conditions, which consisted of a control where seeds were soaked in water three hours, the same time and application form, when mycorrhizae were used, and Quitomax solution. The two mycorrhizal inoculants were applied by coating the seeds. Six treatments were established since both bioestimulants were also used in combination. A randomized block design was used in the field with four replications. At transplantation time, each inoculum was applied at root system and Quitomax[®] was sprayed on the foliage at seven and 28 days. The quantity of fruits per plant was evaluated and in the sixth harvest, 30 fruits were taken at random per replication of each treatments, from which the equatorial and polar diameter were measured. Treatments caused variations in the distribution of fruits by size and in the equatorial diameter, but not in the fruit shape. The number of fruits increased with respect to the control, but not their mass to the same extent.

Key words: chitosan, arbuscular mycorrhizae, mass, diameter, fruit.

RESUMEN: Los bioestimulantes contribuyen al incremento de los rendimientos, lo cual se refleja en los frutos. En este sentido, se realizó este trabajo en el Instituto de Tecamatlán, Puebla, México, para evaluar la influencia del Quitomax[®] y la inoculación con dos cepas de micorrizas en el comportamiento de algunas variables del fruto de tomate. Se produjeron posturas en bandejas con un sustrato comercial, bajo los mismos tratamientos que luego serían aplicados en condiciones de campo, los que consistieron en un control donde las semillas se embebieron en agua por tres horas, el mismo tiempo que se empleó cuando se utilizó micorriza y solución de Quitomax[®]. Los dos inóculos de micorriza se aplicaron mediante el recubrimiento de las semillas. Se conformaron seis tratamientos, pues ambos bioestimulantes también se usaron combinados. Se empleó un diseño de bloques al azar, con cuatro réplicas en campo. En el trasplante se aplicó cada inóculo al sistema radical y el Quitomax[®] se asperjó al follaje a los siete y 28 días. Se evaluó la cantidad de frutos por planta y en la sexta cosecha se tomaron al azar 30 frutos por réplica de cada tratamiento, a los que se les midió el diámetro ecuatorial y polar. Los tratamientos provocaron variaciones en la distribución de los frutos por tamaño y en el diámetro ecuatorial, pero no en la forma del fruto. El número de frutos incrementó respecto al control, pero no en igual medida su masa.

Palabras clave: diámetro, fruto, masa, micorrizas arbusculares, quitosano.

*Author for correspondence: ejerez@inca.edu.cu

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INTRODUCTION

Tomato (*Solanum lycopersicum* L) is one of the most consumed vegetables in the world, due to the different types and forms it can be consumed (1,2), in addition to its high commercial and nutritional value (3). In Mexico it has a very important social importance and its consumption reaches 14 kg per capita (4).

To increase tomato production, the use of agrochemicals has increased, so new alternatives are needed to alleviate the environmental impact that may be created. One of these alternatives is the use of arbuscular mycorrhizal fungi (AMF), which enhances the growth and development of plants, improve their nutritional status, protect them from biotic and abiotic stresses, and increase the nutritional value of the products that will be consumed by humans (5).

Both plants and mycorrhizae have evolved in an intimate relationship since about 460 million years ago (6). In general, fungi form a link between plants and mineral nutrients in the soil and fulfill various functions in terrestrial ecosystems (7) and in the symbiosis they establish with plants, they provide them with nutrients and they receive substances necessary for their life.

On the other hand, other biostimulants have been developed for plants, which, together with the AMF use, contribute to the non-pollution of the environment (8), among them are chitin derivatives, mainly of animal origin, which contain high contents of this compound. A very promising one, due to its proven effect on different crops, including tomato, is Quitomax® (QMax®, to refer to it in the text), a liquid formulation based on chitosan (9).

The use of biostimulants has been done individually, but in recent years it has become increasingly important to use them in combination, which can enhance their action on plants and further increase crop quality.

An aspect of great interest in tomato production is the classification of fruits by size, which is important for farmers because it allows standardization of the product and greater added value, while in academia, grading standards are used to evaluate the effects of crop management on the variables under study (10).

Increasing production through the use of different biostimulants in the case of tomato or another crop, can be given by variations in the size of the fruits or by the increase in the number of fruits, this last aspect, easier to evaluate than knowing the possible changes that can be produced in the fruits, which can modify the form of them, so generating information on the effect on tomato fruits, coming from plants that have been submitted to different treatments with two biostimulants, applied alone and combined, constituted the objective of the present work.

MATERIALS AND METHODS

The work was carried out in the experimental area of the National Technological Institute of Tecomatlán, located in the south of Puebla State, Mexico, between parallels 17°53'18" and 18°07'24" North latitude and meridians 98°12'42" and 98°21'54" West longitude, at 960 m a.s.l.

For the same, tomato seedlings of the commercial cultivar Pony Express (F1) of the Saladette type were produced in a shade house (sowing in September 2019) and the use of polystyrene trays with 200 cells, placing one seed in each one.

A commercial substrate (Peat-Moss Grow-mix) was used and solid inoculants containing INCAM-4 (*Glomus cubense*), DAOM 241198 (11) and INCAM-11 (*Rhizoglyphus irregulare*), DAOM 711363 (12), with a concentration of 30 spore g⁻¹ and abundant fragments of rootlets of the host plant (*Brachiaria decumbens*) were used for the application of AMF. Both certified inocula came from the collection of the National Institute of Agricultural Sciences of Cuba.

Inoculums were applied at the time of sowing by the seed coating method, in an amount equivalent to 10 % of its weight. For this purpose, a fluid paste was prepared with 6 mL of water for each gram of solid inoculant, in which seeds were immersed. Seeds were then dried in the shade and sown.

In the case of QMax®, seeds were soaked in the solution prepared at a concentration of 0.1 g L⁻¹, for three hours and when inoculation was performed with the corresponding mycorrhizal strain only, seeds were first soaked in water for the same time.

Six treatments were prepared with both biostimulants:

T-1. Control (seeds soaked in water)

T-2. QMax® T-2.

T-3. Incam4

T-4. Incam4 + QMax® T-5.

T-5. Incam11

T-6. Incam11 + QMax®.

Transplanting (carried out in October 2019), was performed for a Regosol Eutric soil (13) with a very low level of organic matter, alkaline pH, low level of assimilable phosphorus and low to very low exchangeable cations, except Ca which is classified as medium, according to its content (14).

The same treatments were applied in the field, using a randomized block design with four replicates. The beds covered with black polyethylene were separated at 1 m and the plants were placed at 0.5 m. Each experimental plot had three furrows, two border furrows and a central evaluation furrow, with a plot size of 2 m wide by 11 m long. The staking consisted of stakes placed at the beginning and end of the furrow and in the interior, at a distance of 2 m between each one, which were joined by rows of wires, to which tomato plants were fastened with plastic twine. Prior to transplanting, a chemical fertilization was carried out in which the following doses were applied: 300 kg ha⁻¹ of N, 250 kg ha⁻¹ of P₂O₅ and 600 kg ha⁻¹ of K₂O, in each treatment.

In the treatments with mycorrhizae, this was applied to the root system by submerging it in a mixture of each inoculum for 10 minutes, prepared at a rate of 1 kg of each, in 600 mL of water, depending on the number of plants,

after that time, they were put to dry for a few minutes before planting.

Qmax® (10 g L⁻¹) was applied to the foliage of treatments that required it, at a rate of 300 mL ha⁻¹, 7 and 28 days after transplanting, which coincided with the beginning of flowering.

In six plants per treatment and replicate, the number of fruits was counted before harvesting began, which made it possible to know the average number of fruits per plant. In the sixth collection, 30 fruits were selected from each replicate and treatment, and their equatorial and polar diameters were measured with the aid of a vernier caliper from the polar diameter-equatorial diameter ratio (15), the shape of the fruit was known, being classified by these authors as follows: >1 (long fruits), equal to 1 (round fruits) and <1 (flattened fruits). The mass corresponding to each fruit was also determined individually.

A study of the population in each treatment (n=120) made it possible to define the minimum and maximum values in both diameters evaluated, as well as to establish the frequency of the number of fruits expressed as a percentage, by distributing them in three classes: <49, between 50-69 and >70 mm.

Analysis of variance (Anova) of double classification was carried out to determine the differences between treatments in the variables: equatorial diameter; polar diameter; lower and higher value of equatorial diameter; lower and higher

value of polar diameter; relationship between both diameters; number of fruits per plant and fruit mass.

Means were compared by Tukey's test at a probability of 95 % and the statistical package SPSS v.22 and SigmaPlot v.11 were used for data processing.

RESULTS AND DISCUSSION

The results of population analysis, in terms of minimum and maximum values of each fruit diameter, by treatment, are presented in Table 1.

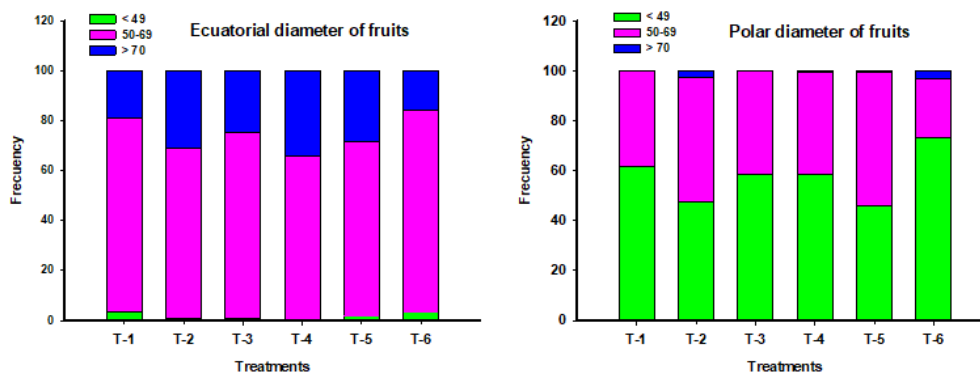
According to the Anova performed, no significant differences were detected between treatments, in terms of the values reached in the minimum and maximum size in each of the diameters, so the treatments did not modify the behavior of these variables. The polar diameter values were lower than the equatorial diameter, both in the minimum and maximum values, but this will be of importance in the relationship established between these variables, which will indicate the shape of the fruit, as will be discussed later.

When the distribution of fruit frequencies for both diameters by classes was carried out (Figure 1), it was found that the greatest quantity of fruit, in the case of the equatorial diameter, was presented in the size from 50 to 69 mm and greater than 70, in this last value, treatments 2 and 4, showed a greater quantity of fruit, than in the rest of the treatments.

Table 1. Minimum and maximum values of equatorial and polar diameters of fruits of tomato plants under the effect of treatments with QMax®, two mycorrhizal strains and a control

Treatments	Equatorial diameter (mm)		Polar diameter (mm)	
	Minor	Greater	Minor	Greater
1	44.53	81.66	33.31	60.85
2	45.28	87.61	33.01	64.69
3	46.88	84.07	36.61	69.62
4	52.45	79.99	36.75	73.90
5	44.07	84.84	30.32	60.41
6	40.80	85.35	33.35	68.00
SE x	2.82ns	2.05ns	1.86ns	2.84ns

(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®)



(T-1: Control, T-2: QMax®, T-3: Incam4, T-4: Incam4 + QMax®, T-5: Incam11 and T-6: Incam11 + QMax®)

Figure 1. Frequency distribution behavior of the equatorial and polar diameter of fruits, under the effect of treatments with QMax®, two mycorrhizal strains and a control

As for the polar diameter, the greatest number of fruits was distributed in the classes below 69 mm, although the greatest number of fruits had diameters below 49 mm, all of which proves the result presented in Table 1, since the value of the equatorial diameter was greater than that of the polar diameter, both in the minimum and maximum values, independently of the treatments.

In the CODEX standard (16) for tomato, it is stated that when size is classified by diameter, this is determined by the maximum diameter of the equatorial section. According to the table provided in the aforementioned document, fruits that were evaluated in this work were in the largest categories, 7, 8 and 9, out of a possible 10.

In a study carried out to check the effect of different production systems on the size of tomato fruits (10), although in this case cherry and grape tomatoes (both of small size), the results indicated that defoliation decreased the number of fruits classified as large for both cultivars under study. All of which indicates that fruit size can be managed, based on the use of one production system or another, although in this case no biostimulant was used.

Saladette tomatoes were classified as large (17) when the diameter of the fruit was greater than 59 mm, while values above 70 mm were considered extra-large, which does not contradict the CODEX Standard (10).

Figure 2 shows the results of the analysis of the average dimensions of the fruit, through the measurements of its equatorial (A) and polar (B) diameters

Treatments applied did not cause changes in the polar diameter, and in the case of the equatorial diameter, only in the treatment in which QMax[®] was applied plus inoculation with the Incam11 strain (T-6), the values were significantly lower than when the plants were inoculated with the same strain and when Qmax[®] was applied, both alone and together with the Incam4 strain.

However, the use of a different mycorrhizal species (Glomeromycota) than the one used in this work (5), caused differences between treatments in the polar diameter, but not in the equatorial diameter, in contrast to what was found here, so it is not possible to establish a similar action,

besides the fact that it was another tomato cultivar and the production conditions were not the same either.

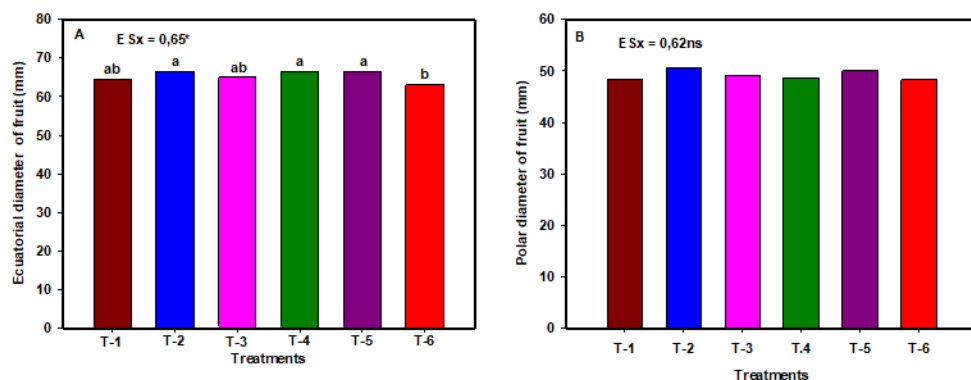
The behavior of both fruit diameters, although not in values, but in the effect of the treatments, was similar to that found in another work in which different substrates were used for the development of plants in greenhouse conditions, since the differences between them were only evidenced in the equatorial diameter, not in the polar diameter (18). On the other hand, in a work carried out in Cuba, when comparing five different tomato lines, the effect of two different water inputs to the soil, did not cause differences between both treatments, regarding the values of fruit diameters (19).

In an investigation carried out under hydroponic conditions with the cultivar Mara, subjected to different water inputs, the variations in the polar diameter were smaller than those found in the equatorial diameter, even when the experimental conditions were not the same as those used in the present work, it is denoted that the influence of different treatments on these fruit variables are less consistent (20). On the other hand, in a study in which fruit thinning was carried out in the bunch to increase fruit diameter and, therefore, fruit mass and yield in general, the behavior was not the same in all the cultivars used, and in some there was no effect at all (21).

The fruit index obtained from the relationship between fruit diameters was similar among treatments (Figure 3), with no significant differences between them, which indicates that the treatments did not modify fruit shape, indicating a varietal effect on these variables.

On the other hand, other authors (15) considered this type of fruit flattened, based on the values of this relation, which was higher than 1, which makes it suitable for fresh consumption and very much appreciated by Mexicans (4).

In a study carried out in Colombia, related to the growth of fruits of three tomato cultivars different from the one used in this work (22), the authors pointed out that the growth in diameter of fruits is an irreversible increase, as a consequence of the increase in mass and number of cells, while the shape of the fruit was established since the fruit



(T-1: Control, T-2: QMax[®], T-3: Incam4, T-4: Incam4 + QMax[®], T-5: Incam11 and T-6: Incam11 + QMax[®])

Different letters above the bars mean differences between treatments

Figure 2. Behavior of equatorial (A) and polar fruit diameter (B) under the effect of QMax[®] treatments, two mycorrhizal strains and a control

set, which is a consequence of the varietal effect in this behavior, independently of treatments.

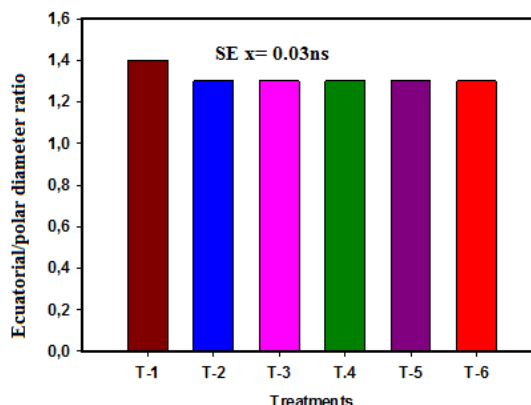
The number of fruits per plant and their mass are presented in Figure 4 A and B, respectively. Regarding the number of fruits (4 A), the control treatment showed the lowest value, as did the treatment in which QMax[®] was applied, but with significant differences between them, which differed from the rest of the treatments by showing the highest values, but without differences between them.

It should be noted that either of the two mycorrhizal strains used, alone or combined with QMax[®], produced the highest values, all of which denotes the positive effect of these strains in stimulating the number of fruits in tomato, which may be related to their action in stimulating the absorption of nutrients and water by the plants, which of course favored the growth and development of the plants in general.

The QMax use alone did not favor the increase in the number of fruits, perhaps because the concentration of the solution used in the imbibition of the seeds was low (0.1 mg L⁻¹), since favorable results were obtained with the concentration of 1 g L⁻¹ (23), even when the same dose was used for foliar spraying.

Recently it has been proved that the active principle of QMax[®] is the chitosan, which has been demonstrated to be a stimulator of the vegetal metabolism (24), that is why in plants in which its nutritional state was favored, its action has been different, but in future works, the use of other concentrations must be evaluated, because in general, in the reviewed works, these have been higher.

When analyzing the average fruit mass (4 B), it was found that it was higher when QMax[®] was used alone (T-2), but without significant differences with the results in the treatments in which it was used combined with the mycorrhizal strain Incam4 and when only the inoculation with both strains was used separately, although it was when the strain Incam11 and the control were used. It was



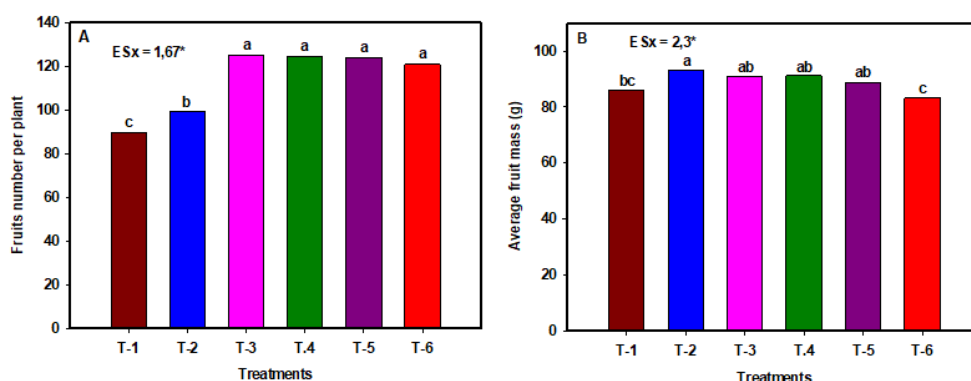
(T-1: Control, T-2: QMax[®], T-3: Incam4, T-4: Incam4 + QMax[®], T-5: Incam11 and T-6: Incam11 + QMax[®]). (SE = 0,03 ns)

Figure 3. Equatorial and polar diameter ratio of tomato fruits under the effect of QMax[®] treatments, two mycorrhizal strains and a control

noteworthy that the lowest fruit mass occurred when inoculation with strain Incam11 was used.

It has been proved that the use of arbuscular mycorrhizae produces different behaviors in tomato at the level of the cellular wall of roots (25), which is the result of different responses, regarding the effect of this biostimulant in plants, from which it is necessary to evaluate the use of other AMF strains, especially for the region where the work was done, where this practice has not been generalized, besides the possible effect that they can cause in the quality of fruits (5).

In relation to QMax[®] applications, and its effect on the variables evaluated in this work, it is necessary to take into account for the future an increase of the dose used, given the results reported when the same were higher than the one used here and this caused an increase in the mass of the fruits, at the same time that increased their quality (26).



(T-1: Control, T-2: QMax[®], T-3: Incam4, T-4: Incam4 + QMax[®], T-5: Incam11 and T-6: Incam11 + QMax[®]).

Different letters above the bars mean differences between treatments

Figure 4. Number of fruits per plant (A) and average fruit mass (B) under QMax[®] effect treatments, two mycorrhizal strains and a control

CONCLUSIONS

- According to the results of the work, it was found that the treatments did not modify fruit shape, but did modify the equatorial diameter of fruits and the fruit size distribution.
- Inoculation with the two mycorrhizal strains and QMax®, especially when used in combination, increased the number of fruits, but not their mass in the same way

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