

Agronomic performance and incidence of diseases in tomato grafted plants (*Solanum lycopersicum* L.)

Comportamiento agronómico e incidencia de enfermedades en plantas injertadas de tomate (*Solanum lycopersicum* L.)

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Abstract

To determine the performance of tomato grafted on native tomato from México, a study in Valle de Apatzingán Michoacán, México in 2008 was established. As rootstock ecotypes of tomato *Solanum lycopersicum* L. variety *cerasiforme* (Tinguaraque) were used, and as graft the cultivar Toro[®] was used. A randomized complete block design with 13 treatments was performed: six grafts of tomato on Tinguaraque, six Tinguaraque and one control (tomato). Specifically in grafts and tomato (six treatments) the following characteristics were evaluated: plant height, stem diameter, number of flower clusters, height to the first floral cluster, weight, size and fruit yield per plant; moreover in all treatments (13 treatments) pH, soluble solids and moisture were registered in fruits; also the plant disease incidence was evaluated. Significant differences were found for height, diameter, flower clusters and fruit size, grafts showed higher values in contrast to tomato. Among the three registered diseases (damping off, *Alternaria-Fusarium* complex and geminivirus), only damping off had statistical differences, the grafts presented lower incidence. By comparing the values of fruits characteristics, Tinguaraque and tomato presented particular values, and these did not influence the graft fruit. It was concluded that the tomato graft showed potential benefit in crop management, without devaluing fruit and plant characteristics, or yield; for grafting, the incidence of "damping off" with respect to the tomato was reduced.

Key words: Disease resistance, fruits characteristics, fungal diseases, grafting, México, *Solanum lycopersicum* L. variety *cerasiforme*.

Resumen

En el Valle de Apatzingán, Michoacán, México, se estudió el comportamiento del tomate injertado sobre tomate nativo de México. Como porta-injerto se utilizaron ecotipos de tomate *Solanum lycopersicum* L. variedad *cerasiforme* ('tinguaraque') y como injerto el cultivar (cv.) Toro[®]. En un diseño experimental de bloques completos al azar se dispusieron 13 tratamientos: seis injertos de tomate sobre Tinguaraque, seis Tinguaragues y un testigo (tomate). Específicamente en los injertos y tomate (seis tratamientos) se midieron las siguientes características: altura de planta, diámetro de tallo, número de racimos florales, altura al primer racimo floral, peso, tamaño y rendimiento de frutos por planta; además, en los frutos de todos los tratamientos se midieron el pH, los sólidos solubles y humedad; y en las plantas la incidencia de enfermedades. Se encontraron diferencias para altura, diámetro, racimos florales y tamaño de fruto. En los injertos se presentaron los mayores valores de estos parámetros, a diferencia del tomate. Entre las enfermedades registradas ('damping-off', complejo *Alternaria-Fusarium* y geminivirus) sólo el damping off fue significativo, siendo menor la incidencia en plantas de injerto. Las características de frutos fueron

diferentes en Tinguaraques y tomate que no afectaron las características de fruto del injerto. Los resultados mostraron que el injerto de tomate tiene beneficios en el manejo del cultivo, debido a buenas características de planta y rendimiento de frutos, así como la reducción en la incidencia de damping-off con respecto a tomate.

Palabras clave: Características de fruta, enfermedades fungosas, injerto, México, resistencia a enfermedades, *Solanum lycopersicum* L. variedad *cerasiforme*.

Introduction

Tomato, also known as 'jitomate' in some regions of Mexico, and its more related plants have their center of origin in the mountainous, narrow and elongated region of los Andes comprising Peru, Ecuador and Chile (Peralta and Spooner, 2000). The probable ancestor of the cultivated tomato is *Solanum lycopersicum* L. var. *cerasiforme* (Esquinas-Alcázar and Nuez, 1995; Sánchez *et al.*, 2006) characterized for producing rounded fruits between 1 and 2.5 cm diameter (Martínez, 1979; Rick and Holle, 1990). It grows in a wide variety of habitats and responds to biotic and abiotic factors that cause death (Hoyt, 1992).

In Mexico, native tomato is widely distributed in ecological reserve areas, associated with crop fields where, commonly, it becomes a weed (Sánchez *et al.*, 2006). In Michoacán, this tomato is known as 'tinguaraque' (Martínez, 1979), grows under adverse humidity conditions and resists high incidence of pests and diseases (Eigenbrode *et al.*, 1993; Pérez *et al.*, 1997), which are important agronomic characteristics for genetic breeding of this crop.

Tomato is one of the vegetables with the highest phytosanitary problems, since diseases are a limiting factor in its production. Pathogenic diseases are presented in seedlings (damping-off), leaves (early and late blight), in stem (*Fusarium* spp.) and in fruits (apical rot and rot by *Alternaria*) (Tigchelaar, 2001; González-Chávez *et al.*, 2003; Quiroga *et al.*, 2007). In this crop, like in so many others, disease control is based on the use of organo-synthetic fungicides, carrying the already documented problems (Esquinas-Alcázar and Nuez, 1995; Zavaleta-Mejía, 1999).

Conventional breeding by plant hybridization is a fundamental method to transmit characteristics of adaptation to adverse biotic

and abiotic conditions (Nuez, 1995; Pérez *et al.*, 1997; Restrepo *et al.*, 2008); however grafting, although less common, was one of the first techniques used with that aim (Kogan, 1990). In the last years, this technique have awaken the interest in vegetable crops like cucumber, melon, watermelon, chili, eggplant and tomato in order to obtain resistance to diverse root diseases (Santos *et al.*, 2000; Lee, 2003). In crops like tomato, eggplant and watermelon grafting causes better plant development and yield (Choi *et al.*, 2002; Cürük *et al.*, 2005; Khah *et al.*, 2006). Specifically, the tomato rootstock Hawaii 7996 has been used as resistance source to reduce wilting by *Fusarium* (Lee, 1994) and to control vascular wilting caused by *Rals-tonia solanacearum* (Nakaho *et al.*, 2004; Coutinho *et al.*, 2006). Tomato cv. Monika (SC 6301) is a rootstock resistant to nodular nematodes attacks, resistance that is given by the Mi-1.2 gen (Williamson, 1998; Verdejo-Lucas and Sorribas, 2008), graft use improves quality and physico-chemical characteristics of fruits (Coutinho *et al.*, 2006), enhances tolerance to soil salinity (Fernández-García *et al.*, 2004; Estañ *et al.*, 2005), and to high temperatures and altitudes (Venema *et al.*, 2008).

In Mexico few works document vegetable grafts (González-Chávez *et al.*, 2003); therefore, the aims of the present research were to evaluate the agronomical performance and register the incidence of the main diseases in tomato grafted on its wild relative *S. lycopersicum* var. *cerasiforme*.

Materials and methods

Field work was done in 2008 in Morelos, Paracuaro (19° 00' 53" N and 102° 16' 44" O, 300 MASL), Michoacán, Mexico. The predominant weather is warm semi-dry (Bs₁) and soil is vertosol pelico (clayey) (García, 1988).

It was used as graft the commercial tomato cv. Toro® and as rootstock native tomato cultivars (tinguaraque), collected in three regions of Michoacán (Álvarez-Hernández *et al.*, 2009). Rootstocks were: Chico Apatzingán (ChAp), Grande Apatzingán (GAp), Acahuato (Ac), Los Reyes (LR) and Jiquilpan (Jiq); plus the wild ecotype Tabasco (Tab) from La Chontalpa region, Tabasco, Mexico.

Seeds were sown in polystyrene containers with a moss substrate base previously humidify (Sunshine® SunGro Horticulture, Canada). The tinguaraques were established first with a time interval of 30 days in order to homogenize stem diameters (rootstock-graft). Rootstock seedlings with 5 – 6 leaves and graft with 3 – 4 leaves, and similar diameter, were selected. In both stems and above the cotyledon leaves a horizontal cut was done and a pencil tip (0.5 mm) was inserted in the rootstock; next, the graft was inserted until it touched the rootstock. Immediately after, grafted plants were placed on a humid chamber (relative humidity 100%), at 25 ± 1 °C and 12:12 h (light-darkness). After seven days, plants were kept at room temperature for 5 days to acclimate and later transplant to the field. Distances between plant and furrow were 1 x 0.6 m.

Plants were drip irrigated each 3 – 4 days for 30 min using 2 lt/plant of water keeping a lamina of around 8 cm. As fertilizer 60 g/plant of triple 17 (N-P-K) were added in a manual way in the days 20 and 45 after transplant. Pesticides were not used during the crop cycle and the few emerged weeds were manually controlled. 13 materials were conformed (each one is a treatment): five tinguaraques (ChAp, GAp, Ac, LR and Jiq) plus Tabasco (Tab) and their corresponding grafts (I-ChAp, I-GAp, I-Ac, I-LR, I-Jiq and I-Tab) and tomato (Jit) as control. Treatments were organized on a complete randomized experimental design with three replicates, each experimental unit was composed of 20 plants (12 m²), for a density of 16,666 plants/ha.

Both, in grafts as in tomato alone, it was evaluated stem height from the base of the plant till main apex tip, stem diameter (1 cm over graft callus) and number of floral stems, 75 days after transplanting (dat). Height to

the first floral stem from the base till inflorescence rachis was registered at 50 dat.

Production was measured based on fruit weight per treatment in six established categories with 15 g differences between them (2.2 - 17.2, 17.3 - 32.2, 32.3 - 47.2, 47.3 - 62.2, 62.3 - 77.2 and 77.3 - 92.2 g), this criterion was defined taking into account the weight of the harvested fruits. It was measured, as well, the fruit size according to the polar and equatorial diameters and the yield per plant in one cut.

In all the treatments (grafts, tomato and tinguaraque tomatoes) were measured fruit pH (manual potentiometer Hanna®), soluble solids (manual refractometer Atago® model HSR-500) and humidity content (%) by differences between initial and final weight after having the fractionated fruits at room temperature for 10 days.

Disease incidence was registered by weekly samplings of leaves, wilting or death plants during the crop cycle. In the fungal diseases, besides their symptoms description in sick plants, the causal agent was isolated for its identification. For viral diseases it was considered incidence and severity (Table 1) with a harm scale designed for this evaluation, additionally, leaves samples with viral symptoms were sent to the specialized lab GISENA, Texcoco, Mexic, to determine the causal agent.

Data analysis for agronomic characteristics of grafts and tomato, excepting fruit weight, was done by variance on a completely randomized block design and, mean separation was performed by Tukey's test ($P \leq 0.05$). For fruit weight the value limits were taken and classified in six categories of 15 g weight and by percentage it was determined the amount per weight interval. Fruit physico-chemical characteristics of grafts, tomato and tinguaraques, were analyzed by descriptive analytics (mean and standard deviation). In this type of tomatoes, the accumulated disease incidence (%) was transformed to the arcsine of the square root ratio for its processing by analysis of variance and mean separation by Tukey's test ($P \leq 0.05$). For all the cases the statistical software SAS (SAS, 1997) was used.

Table 1. Scale used to evaluate viral disease damage level, Michoacán México.

Level	Damage characteristics
1	Without damage; leaves extended, normal plants with buds, flowers and fruits.
2	Initial damage: some leaves extended, others rolled, normal plant with buds, flowers and fruits.
3	Medium damage: leaves rolled, reduced apical buds, fanleaf foliage, reduced bud and flower development, with fruits.
4	Total damage: fanleaf in all foliage, dwarf and slim plants, no flowers, buds and fruits.

Results and discussion

Agronomical characteristics

Seventy-five days after transplanting, plant height and stem diameter showed significant differences ($P < 0.05$). The highest plants were presented in the grafts I-Ac, I-Jiq and I-LR, while the control Jit (tomato) had 41.75 cm; the shortest plants were observed in the graft I-GAp (Table 2). The largest stem diameter was observed in Jit, but it was similar to the graft I-LR (Table 2).

Height to the first flower stem varied between 25.16 and 26.58 cm in the grafts and tomato (Table 2). The highest number of floral stems per plant ($P < 0.05$) happened in the I-Jiq, I-LR, I-ChAp and I-Tab grafts, and the lowest number was found in I-Gap graft (Table 2).

Graft and tomato fruits had weights bet-

ween 2.2 and 92.2 g, and were grouped in six categories (Figure 1). In general, most of the fruits were in the weight range between 17.3 and 32.2 g with fluctuation between 41% in the I-LR graft and 63% in tomato (Jit). Contrary, in the ranges with highest fruit weight there were lower variation percentages (0 – 5% and 0 – 0.75%). In important to highlight that tomato did not produce fruits over 47.2 g whereas all the grafts had fruits heavier than that weight, even, some grafts like I-LR, I-Jiq and I-Tab had fruits in all the categories above 47.2 g (Figure 1).

Fruit size, determined by polar and equatorial diameters, was different ($P < 0.05$), bigger fruits were found in I-LR, I-Jiq, I-Ac and I-ChAp (Table 3). To the contrary, fruit yield/plant was similar (Table 3).

Grafts did not affect negatively the evaluated characteristics in tomato and fruits. Thereby, the value of plant height was low

Table 2. Agronomic characteristics of grafts and jitomate plants,, Michoacan México.

Treatments	Plant height (cm)	Stem diameter (cm)	Height to first floral stem (cm)	Floral stem number
I-ChAp	40.21 b†	0.76 c	26.45 a	4.45 a
I-GAp	31.86 c	0.80 bc	25.94 a	3.58 b
I-Ac	44.15 a	0.81 bc	25.94 a	4.44 a
I-LR	43.12 a	0.91 ab	26.58 a	4.47 a
I-Jiq	43.41 a	0.88 bc	25.16 a	4.70 a
I-Tab	39.41 b	0.74 c	26.50 a	4.45 a
Jit (tomate)	41.75 ab	1.04 a	26.25 a	4.25 ab
DMS	2.47	0.15	6.92	1.04
CV (%)	2.18	6.45	6.16	9.36

† Means with the same letter within columns are not statistically different (Tukey, $P \leq 0.05$). DMS: Minimal significant difference. CV: coefficient of variation.

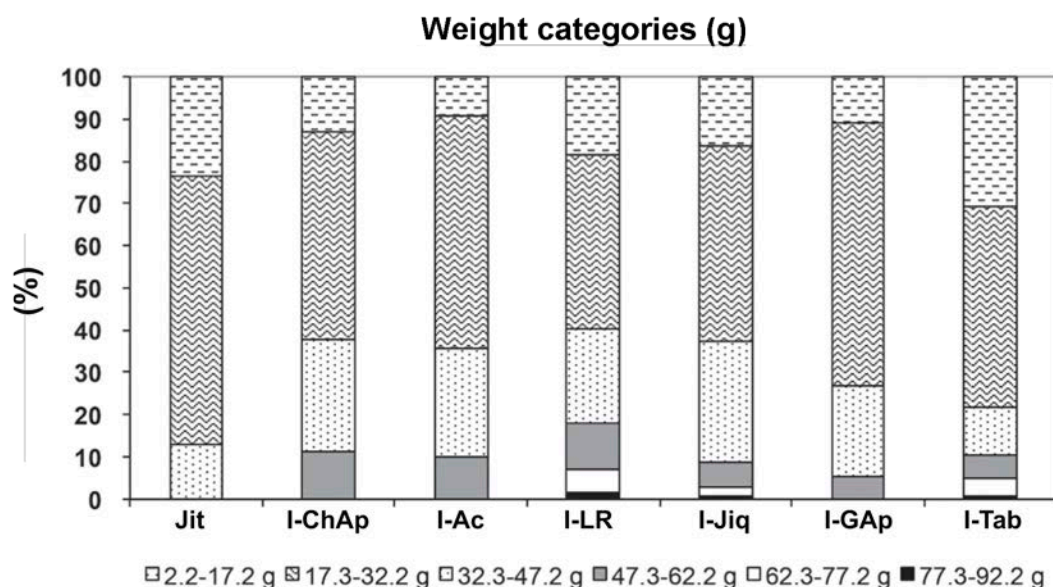


Figure 1. Weight fruit frequency (%) in six intervals in grafts and tomato, Michoacan México.

Table 3. Size and yield of grafts and jitomate fruits, Michoacan México.

Treatments	Polar diameter (cm)	Equatorial diameter (cm)	Fruit yield/plant (g)
I-ChAp	3.93 ab [†]	3.53 a	125.16 a
I-GAp	3.77 b	3.52 a	107.33 a
I-Ac	3.95 ab	3.51 a	112.99 a
I-LR	4.07 a	3.59 a	167.79 a
I-Jiq	3.96 ab	3.58 a	141.42 a
I-Tab	3.71 b	3.32 ab	128.61 a
Jit (tomate)	3.72 b	3.17 b	128.54 a
DMS	0.29	0.29	80.53
CV (%)	17.51	19.17	22.17

[†] Means with the same letter within columns are not statistically different (Tukey, $P \leq 0.05$).

DMS: Minimal significant difference. CV: coefficient of variation.

(31.86 – 44.15 cm) if compared to results of other studies (Khah *et al.*, 2006) where it was found heights between 69.31 and 75.31 cm. However, in this study there were no disease and pest controls, which could affect plant development. For the variable height to the first floral stem there were no differences between grafts and tomato. Number of bunches/plant showed a tendency to be higher in I-LR and I-Jiq grafts (Table 2). Similarly, the biggest fruits (62.3 - 77.2 g and 77.3 - 92.2 g categories) belonged to I-LR, I-Jiq and I-Tab grafts despite of their higher

variability when compared to tomato (Figure 1). Although, there were no detectable differences in yield per plant (Table 3), it is important to notice that grafts and control reached all the phenological phases without any pesticide application, even so, LR (167.79 g) and I-Jiq (141.42 g) produced more than tomato (128.54 g), which is a desirable characteristic from the agronomical point of view.

Fruit physico-chemical characteristics: pH, soluble solids and humidity percentage showed variable values; for instance, pH was higher in tinguaraques than in tomato and

grafts since, ChAp, GAp, Ac, LR, Jiq and Tab tinguarques showed higher acidity, and the grafts showed an acidity similar to tomato (Table 4). Tinguarques with more soluble solids were Ac, LR and Jiq, whereas Tab had the lowest value. In grafts the soluble solids value varied from 5.5 for I-Jiq to 6.75 (I-Gap and I-Tab), and Jit (tomato) showed 7.0 °Brix (Table 4). Fruit humidity was lower in tinguarques tomatoes with values varying from 88.39 to 91.43% compared to grafts and tomato that were on 93.99 – 97.44% range (Table 4).

Fruit physico-chemical characteristics in the grafts were similar to the ones of tomato, however tinguarques were different according to the cultivar used. The less acidic pH of tinguarques fruits (4.77 – 5.37) did not affect the graft fruits. In this sense, it has been observed that in grafted tomato plants pH is more acidic (4.04 – 4.30) than in plants without grafting (4.35 – 4.47) (Coutinho *et al.*, 2006). However, this variation is minimal since in commercial varieties pH is between 4.2 and 4.4 (Nuez, 1995); on the other hand, Khah *et al.* (2006) did not find statistical differences in pH of grafts (4.33 – 4.41) and control (4.34). Soluble solids, except for the materials ChAp, GAp, Tab, I-Ac and I-Jiq, tended to be more concentrated in tinguarques (7.5 to 7.75 °Brix) in comparison to grafts (6.25 –

6.75 °Brix), while tomato had 7.0 °Brix. The range of soluble solids in commercial tomatoes is between 4.5 and 5.5 °Brix, however more than the variety character, the agroecological factors affect the soluble solids state since they can make changes between 4 and 7 in fruits of the same variety (Nuez, 1995). In other studies there have not been found differences in soluble solids between grafted and no-grafted plants, for instance, Coutinho *et al.* (2006) found values between 3.95 and 4.7 °Brix in grafted plants and between 3.95 and 4.95 °Brix in no-grafted plants. Similarly, Khah *et al.* (2006) found values between 3.1 and 4 °Brix in grafts and 3.68 °Brix in the control. In relation to fruit humidity, tinguarques showed less humidity (88.39 – 91.33%) than grafts and tomato, which have values of 93.99 and 97.44%, respectively. These differences could be due to the wild origin of tinguarques. Humidity values for tomato were 94 and 95% (Nuez, 1995), very similar to the grafts used in this study.

Disease incidence

Registered disease during the crop development were damping-off caused by the complex of soil fungi *Pythium* sp., *Fusarium* sp. and *Rhizoctonia solani*, which cause base stem constriction. Another disease observed was

Table 4. Physico-chemical characteristics of grafts, jitomate and tinguarques fruits, Michoacan México.

Treatments	pH	°Brix	Humidity (%)
ChAp	5.07 ± 0.05 [†]	6.0 ± 0.0	90.73
GAp	5.02 ± 0.05	6.0 ± 0.0	91.43
Ac	5.35 ± 0.1	7.75 ± 0.5	89.94
LR	4.77 ± 0.05	7.75 ± 0.5	89.05
Jiq	4.87 ± 0.05	7.5 ± 0.57	90.13
Tab	5.37 ± 0.05	5.25 ± 0.5	88.39
I-ChAp	4.67 ± 0.05	6.25 ± 0.5	97.37
I-GAp	4.55 ± 0.05	6.75 ± 0.5	93.99
I-Ac	4.45 ± 0.05	6.0 ± 0.0	97.44
I-LR	4.45 ± 0.05	6.5 ± 0.57	96.52
I-Jiq	4.5 ± 0.0	5.5 ± 0.57	96.41
I-Tab	4.5 ± 0.0	6.75 ± 0.5	97.44
Jit	4.52 ± 0.09	7.0 ± 0.0	94.28

[†] n = 5.

the late blight *Alternaria solani*) followed by general plant wilting and death in adult plants. In that stage the causal agent was isolated and identified as an unknown *Fusarium* sp. For the leaf analysis in plants with viral symptoms, positive results were found, being identified a Geminivirus as causal agent.

Analysis of variance for damping-off attack showed highly significant differences. I-LR graft was not affected by this disease whereas tinguaraque Ac was the most susceptible. With exception of I-AGp, all the other grafts showed to be less susceptible, although only I-LR showed differences with the control (tomato-Jit) (Table 5). For the affected plants variable in the case of the complex *Alternaria solani-Fusarium* sp. there were no differences, however, LR, I-AGp and I-ChAp were not affected by these fungi opposite to Jit (tomato) and Tab, which had the highest percentage of incidence (Table 5). Finally, Geminivirus incidence did not show statistical differences ($P > 0.05$) and the values varied between 12.93% and 52.67% (Table 5).

When Geminivirus incidence was evaluated based on damage level, results were diffe-

rent, for instance, materials with damage level 3 (medium damage) were I-Gap, Tab, Gap, I-ChAp, I-Tab and tomato (Jit) with infected plants percentage from 5.26% and 27.27%. Only GAP and Tab showed plants with damage level 4 (total damage) with 8.82% and 15.38%, respectively (Figure 2).

In relation to disease incidence is important to highlight the behavior of I-LR graft, which was not affected by damping-off. However, the result was not the same for LR, ChAp, Gap, Ac, Jiq and Tab tinguaraques and Jit (tomato), because they were all susceptible to the disease (Table 5). Even though, low incidence was observed (6.56 – 16.66%) in I-Tab, I-Jiq, I-Ac and I-ChAp grafts. Independently of the lack of statistical significance between the evaluated materials for early blight and wilting by *Fusarium*, as well as for the incidence of the virus from Geminivirus group, different response trends were observed between the materials. Highlighted materials against *Alternaria solani-Fusarium* sp were I-ChAp, I-Gap and LR because they did not show any symptoms.

Although it was expected that wild plants showed higher disease tolerance, apparently,

Table 5. Disease (damping-off, *Alternaria solani-Fusarium* sp. complex and Geminivirus) incidence in grafts, tomato and tinguaraques tomato, Michoacan México.

Treatments	Damping off (%)	<i>Alternaria solani-Fusarium</i> sp. complex (%)	Geminivirus (%)
ChAp	22.33 bc †	5.55 a	36.00 a
GAp	29.66 bc	3.92 a	33.33 a
Ac	76.66 a	3.33 a	20.00 a
LR	10.00 cd	0.00 a	19.33 a
Jiq	18.33 bc	5.00 a	18.33 a
Tab	18.33 bc	5.00 a	32.33 a
I-ChAp	16.66 bc	0.00 a	30.33 a
I-GAp	46.00 ab	0.00 a	52.67 a
I-Ac	10.23 cd	5.12 a	12.93 a
I-LR	0.00 d	4.44 a	15.07 a
I-Jiq	8.33 cd	1.66 a	19.33 a
I-Tab	6.56 cd	6.66 a	21.00 a
Jit (tomate)	25.00 bc	16.66 a	17.03 a
DMS	12.20	5.75	8.37
CV (%)	30.71	87.34	42.26

† Means with the same letter within columns are not statistically different (Tukey, $P \leq 0.05$). DMS: Minimal significant difference. CV: coefficient of variation.

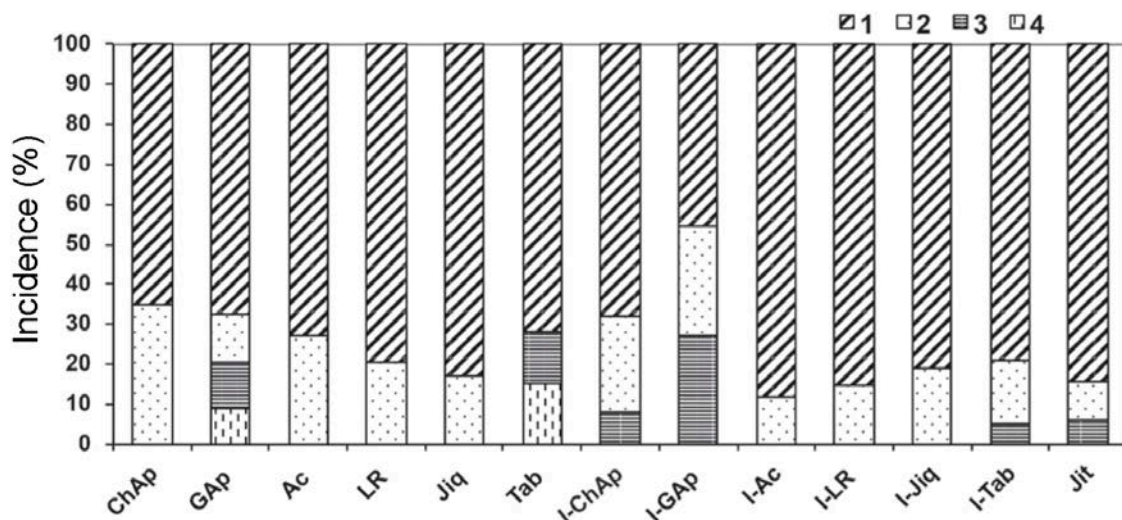


Figure 2. Distribution of Geminivirus damage level in different outbreaks in grafts, tomato and Tinguaraque tomato, Michoacan México.

the graft technique favored plant development and their adaptation to the environmental conditions in the location of the study. Finally, it was observed that viral disease affected tinguaraques, grafts and tomato in levels 2, 3 and 4 (initial damage, medium damage and total damage), however, plants were able to develop.

Conclusions

Tomato graft in its wild relative (*S. lycopersicon* var. *cerasiforme*) showed potential for crop management. By grafting disease incidence was reduce without devalue fruits agronomical and physico-chemical characteristics, or plant yield in the cultivated tomato cariety. I-LR graft was not affected by damping-off, however, the result was not the same as in tinguaraques LR, ChAp, GAp, Ac, Jiq and Tab and, in tomato (Jit), because all of them showed high suceptibility to the disease.

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