HORTSCIENCE 54(6):982–987. 2019. https://doi.org/10.21273/HORTSCI13800-18

Adaptation of a Grafting Method for *Carica papaya* Based on Seedling Behavior

Juan Carlos Álvarez-Hernández[1]
Valley of Apatzingan Experimental Field, Pacific-Center Regional Research Center, National Institute of Forestry, Agriculture and Livestock Researches, Highway Apatzingan-Cuatro Caminos, km 17.5, C.P. 60781, Antunez, Paracuaro, Michoacan, Mexico

Javier Zaragoza Castellanos-Ramos and Cesar Leobardo Aguirre-Mancilla
Technological Institute of Roque, National Technological of Mexico, Highway Celaya-Juventino Rosas, km 8, C.P. 38110, Celaya, Guanajuato, Mexico

Additional index words. cleft grafting, graft survival, papaya, tongue approach grafting, rootstock, vigor

***Abstract*. Grafting *Carica papaya* plants can have several benefits for productive, phytosanitary, and sexing purposes. However, the literature on the subject of papaya grafting is limited. The tongue approach and cleft grafting techniques seem to be the most adequate for *C. papaya*, but the quality of grafts depends on several factors. With the objective of developing and adapting a grafting method for papaya, experimental assays were carried out in the Valley of Apatzingan, Michoacan, Mexico. The physical condition of the seedlings was assessed, and the most advantageous time for grafting was determined based on the size and thickness of the stems. Three assays were then carried out. The first assay was a test of the tongue approach and cleft grafting techniques using two clamping devices. The second assay involved the same techniques with modifications and the addition of another treatment. In the third assay, the modified tongue approach grafting method was tested on three containers with papaya plants. Seedling vigor, graft survival, and graft quality were the recorded variables. The results indicated that unwanted tissue should be cut 6 days after grafting. The tongue approach grafting method using tape as the fastening device (T-T) yielded a graft survival of 80%. The modified tongue approach grafting method, in which the tongues were formed just below the stem-site cut and tape was used as the fastening device (M-T-Bc-T), yielded a graft survival of 90%. In the third assay, the previously described modified method, but with seedlings grown in plastic bags (M-T-Bc-T-B), yielded a graft survival of 92.5%. It can be concluded that the modified tongue approach grafting method with seedlings grown in plastic bags (M-T-Bc-T-B), is a reliable grafting method for papaya that does not require special handling conditions.**

In Mexico, *Carica papaya* L. is cultivated in 19 states, with a harvested area of 16,684 ha, concentrated mainly in Veracruz (3196 ha), Colima (2850 ha), Oaxaca (2702 ha), Michoacan (2368 ha), and Guerrero (1124 ha) (SIAP-SAGARPA, 2017). Despite its economic and nutritional importance, the papaya production chain faces several difficulties associated with a low capacity for innovation and development. This could be resolved or diminished through the implementation of technical innovations that increase productivity and resolve existing phytosanitary problems (Feitó and Portal, 2013). Agronomic management and breeding techniques have been the main focus of research associated with the papaya crop. The selection of parents for hybridization and crosses (Mirafuentes and Azpeitia, 2008; Mirafuentes and Santamaria, 2014), genetic engineering (Song et al., 2015), and explant multiplication (Kumar and Kumari, 2013; Singh et al., 2010) are all complex processes in which the time and costs involved must be taken into account. In contrast, micropropagation and production of seedlings from cuttings and grafts are alternatives for improving the commercial production of papaya and have been little studied (Teixeira et al., 2007; Schmildt et al., 2016; Senthilkumar et al., 2014).

Grafting *C. papaya* plants can have several benefits for productive, phytosanitary, and sexing purposes. Grafting could be used to improve fruiting and yield in papaya plants, as well as to reduce the height of the varieties and hybrids of interest (Allan, 2007; de Lima et al., 2010; Lange, 1969). Thus, grafting could generate improvements throughout the entire production chain of papaya (Senthilkumar et al., 2014). Several grafting techniques have been used in herbaceous species (Alvarez-Hernández et al., 2009; Davis et al., 2008; Honma, 1977; Kumar and Sanket, 2017; Lee, 1994; Oda, 1995); most of them agree on the following general guidelines: the grafting must be carried out during the first stages of plant development, the seedlings must be kept under controlled conditions of temperature and relative humidity during the period of formation of the graft union callus, and the grafted plants should be acclimated to the prevailing environmental conditions before transplantation. Although few studies have focused on papaya grafting (Allan et al., 2010; Van-Hong and Chung-Ruey, 2018), it is generally agreed that the tongue approach and cleft grafting methods are the most appropriate grafting techniques (Lee and Oda, 2003); however, several factors can influence the quality of the grafts, including the grafting method used; the compatibility, vigor, and health of the plants involved; and postgrafting handling (Kawaguchi et al., 2008). In papaya, a very important factor is bacterial contamination (Allan et al., 2010). In the absence of studies aimed at the development of an effective and efficient grafting method for papaya, the objective of our study was to develop and standardize a grafting method for papaya that can be used in the main papaya-producing region of Michoacan, Mexico.

Materials and Methods

Study area. When the environmental conditions are more favorable for the papaya grafting in the region (Sept.–Dec. 2016 and Jan.–Apr. 2017), different trials were carried out in the nursery of the Valley of Apatzingan Experimental Field, located in Antunez, municipality of Paracuaro, Michoacan, Mexico (Fig. 1). The area's climate is subtype BS_1 [a semidry warm climate (Garcia, 1988], the predominant vegetation of the area consists of species that predominate in low deciduous forests (García and Linares, 2012), and the type of soil corresponds to Pelic Vertisol (clay) (INEGI, 2016).

Seedling development. Papaya seeds from 'Maradol' were used to obtain seedlings for use as rootstock and scion. The seeds were moistened by immersing them in water for 24 h, and only those that stayed on the bottom of the container were seeded in polystyrene trays with either 128 or 200 cavities, and in plastic bags (7.8 × 12.4 cm). The cavities contained wet commercial substrate (Canadian sphagnum moss).

After germination, the development of seedlings was sustained by daily irrigation, and preventive applications of cypermethrin

Received for publication 5 Dec. 2018. Accepted for publication 19 Mar. 2019.
We express our gratitude to the National Technological of Mexico for the support granted to the development of this investigation (project 5841.16-P) and to the National Institute of Forestry, Agriculture and Livestock Researches for the facilities granted to carry out this investigation.
[1]Corresponding author. E-mail: alvarez.juan@inifap.gob.mx.

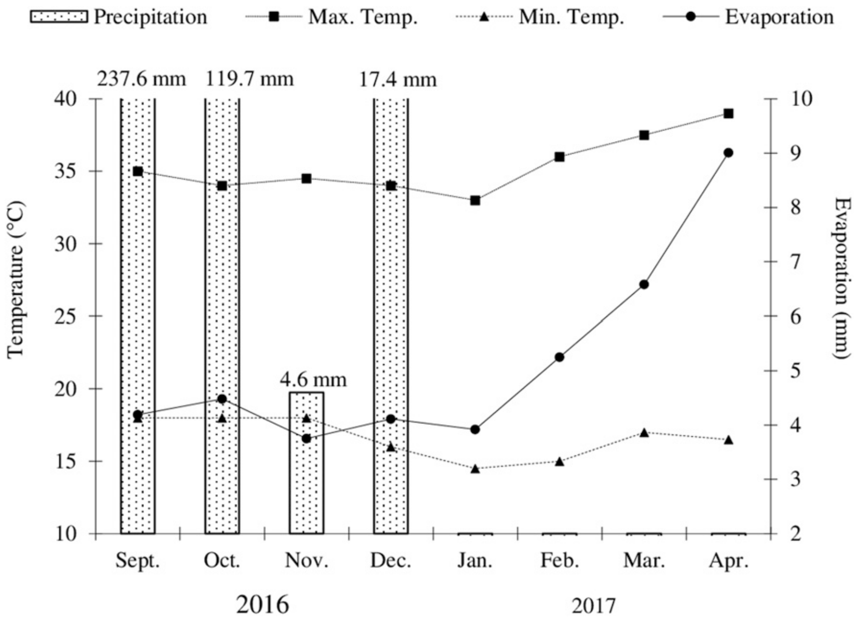

Fig. 1. Climatic variation during the assays. *Source*: Department of Hydrometry. Irrigation District 097. CONAGUA. Mexico.

(1 ml·L^{-1}) and a fungicide mixture containing Captan (1 g·L^{-1}) (Lucaptan®, Celaya, Mexico), supplemented with nitrogen fertilizer (1 g·L^{-1}) (Urea New Green®, Naucalpan, Mexico), every 5 days. This stage lasted ≈40 d, and the seedlings developed under normal environmental conditions. The nursery was protected by covering it with an antiaphid mesh and a shade mesh.

Description of treatments. The seedlings were evaluated before and after grafting. Before grafting, seedling development was recorded 30 d after emergence. The evaluated seedlings were grown under three different treatments: 1) in trays with 128 cavities (46 mL/cavity), 2) in trays with 200 cavities (22 mL/cavity), and 3) and in plastic bags (7.8 × 12.4 cm). All seedlings were measured for the following parameters: height, using a graduated ruler, from the base to the apical meristem; stem diameter, with a Vernier caliper at the base of the stem; number of leaves, by visual counting; and vigor, using an ordinal scale based on the work of Maiti et al. (1981) and Barchuk and Diaz (2000) (Table 1).

The grafting procedure was initiated when the seedlings presented adequate physical conditions in terms of size and stem thickness. This occurred ≈30 d after emergence. To determine the time at which grafting was most likely to succeed using the tongue grafting method with grafting tape laminated as the fastening device, six treatments were evaluated 3 to 8 d after grafting. Tissue fragments were removed from the grafted seedlings every day, so they would function as single plants. The percentage of graft survival and seedling vigor were recorded.

Another three evaluations were developed after grafting (Table 2); however, it is important to note that temperature and humidity were not regulated. The first evaluation involved four treatments: 1) tongue approach grafting with grafting tape as the fastening device, 2) tongue approach grafting with a clip as the fastening device, 3) cleft grafting with grafting tape as the fastening device, and 4) cleft grafting with a clip as the fastening device. In the second evaluation, the tongue grafting method was modified as follows: The meristem of the seedling to be used as rootstock was removed before fixing the tongues. The treatments consisted on the place of formation of the tongue in rootstock seedling, one was formed above the cut site of stem, and other below the cut site of stem. In both cases, the tongues formed of rootstock and scion were combined with the two fastening devices: tape and clip. In the fifth treatment, cleft grafting, transversal cuts were made on both stems, and the tissue of both plants was fastened using a graphite pin. In the third evaluation, a modified tongue approach grafting method (tongues formed below the site cut and fastened with grafting tape) was used, but the grafted seedlings were grown under three treatments: in polystyrene trays with 128 or 200 cavities, or in plastic bags (7.8 × 12.4 cm). The percentage of graft survival was determined 6 d after grafting whereas seedling vigor was assessed 9 and 12 d after grafting, according to the evaluation table (Table 1). The three evaluations are specified in Table 2.

In all treatments, graft quality was assessed based on the physical and visual conditions of the graft site. Four characteristics were evaluated: superficial adherence, compatibility, callus formation, and absence of necrotic tissue. The graft quality was classified as bad regular, or good.

Experimental design. A completely randomized block design was used in all evaluations. Because of the continuity of each experiment and according to the evaluation, the number of repetitions varied. In the pregrafting stage, 10 seedlings were used. After grafting, to evaluate grafting survival, four replications were performed, each with 10 seedlings. To evaluate seedling vigor, seven replications (seedlings) were used—the plants derived from the graft survival variable. An analysis of variance (ANOVA) was performed on most of the variables, with the exception of the seedling vigor variable, which was analyzed with the Kruskal-Wallis nonparametric test. According to the analysis, means were compared using Tukey's Studentized range test and the Mann-Whitney test ($P \leq 0.05$). The statistical software used was SAS (ver. 9; SAS Institute, Cary, NC) and PAST [ver. 3.2 (Hammer, 2018)].

Results and Discussion

ANOVA results showed statistically significant differences only in seedling height and stem diameter ($P \leq 0.01$ and $P \leq 0.001$, respectively). Treatments 1 and 3, which had statistically similar means, yielded better results than treatment 2. The variables number of leaves and vigor of seedlings did not show differences (Table 3).

Recording seedling development parameters is always important, regardless of whether grafting is intended, because seedlings should always be of the highest quality. The use of quality growth media during sowing had a positive effect on germination, development, and rooting (Meena et al., 2017). There was a report that the addition of gibberellic acid, cow urine, and coconut fiber substrate affected these parameters positively (Desaid et al., 2017). In our study, we followed some general guidelines for the production of seedlings, but the response of the plants was different in different containers. The containers with more substrate (treatments 1 and 3) showed better seedling development (Table 3). Larger containers provided better anchoring and support for the seedlings, in addition to serving as a reservoir of nutrients and water, and facilitating gas exchange (Abad et al., 2002). The space between plants was decisive. When producing seedlings for grafting, the most important parameters were height and stem diameter, so that it was possible to cut and fasten into place the rootstock and the scion (Kumar and Sanket, 2017). The better characteristics, in terms of height and stem diameter, as well as seedling vigor, were shown by the seedlings significantly in treatment 3.

The tongue approach grafting method (Lee and Oda, 2003) was used to determine the best moment to cut off the unwanted parts of the plants to keep only the functional parts of a grafted plant. After the grafting procedure, the percentage of graft survival increased as more days passed, before cutting the unwanted tissue between days 3 and 8 after grafting. But, no grafts survived when the cuts were made on days 3 and 4, which is why the data from those days are not shown in Fig. 2. When the cuts were made between

Table 1. Ordinal scale used to assess seedling quality.

Value	Qualifying	Visual characteristics
1	Vigorous plant	Seedling has normal bearing, all leaves erect, uniform foliage with green color, active apical meristem.
2	Plant with good vigor	Seedling has normal bearing, leaves erect, green foliage with tonality slightly variable, active apical meristem.
3	Plant with little vigor	Seedling has reduced bearing, some decayed leaves, variable foliage color with light-green tonality in the majority, active apical meristem.
4	Less vigorous plant	Seedling has compact bearing, decayed leaves, light-green foliage with yellow tonality on lower leaves, passive apical meristem.
5	Rickety plant	Compact seedlings, generally stressed, with tendency to wilt.

Table 2. Description of the grafting treatments.

Evaluation	Treatment	Description	Abbreviation
1	1	Tongue approach grafting fastened with tape	T-T
	2	Tongue approach grafting fastened with pin	T-P
	3	Cleft grafting fastened with tape	C-T
	4	Cleft grafting fastened with pin	C-P
2	1	Modified tongue approach grafting method; tongues formed above the stem-site cut; fastened with tape	M-T-Ac-T
	2	Modified tongue approach grafting method; tongues formed below the stem-site cut; fastened with tape	M-T-Bc-T
	3	Modified tongue approach grafting method; tongues formed above the stem-site cut; fastened with pin	M-T-Ac-P
	4	Modified tongue approach grafting method; tongues formed below the stem-site cut; fastened with pin	M-T-Bc-P
	5	Modified cleft grafting method; fastened with lace	M-C-L
3	1	Modified tongue approach grafting method; tongues formed below the stem-site cut; fastened with tape; 128-cavity tray	M-T-Bc-T-128
	2	Modified tongue approach grafting method; tongues formed below the stem-site cut; fastened with tape; 200- cavity tray	M-T-Bc-T-200
	3	Modified tongue approach grafting method; tongues formed below the stem-site cut; fastened with tape; plastic bag	M-T-Bc-T-B

Table 3. Physical state of papaya seedlings without grafting with 30 d of growth in different containers.

No.	Treatments	Seedling ht (cm)	Stem diam. (cm)	No. of leaves	Vigor[y]
1	Seedling grown in tray with 128 cavities	15.27 ± 0.45 a[z]	0.35 ± 0.07 a	5.50 ± 0.52 a	1.80
2	Seedling grown in tray with 200 cavities	14.16 ± 0.37 b	0.26 ± 0.05 b	5.00 ± 0.66 a	1.80
3	Seedling grown in plastic bag	15.51 ± 0.57 a	0.41 ± 0.05 a	5.60 ± 0.51 a	1.40
SMD		0.44	0.07	0.61	
cv		2.62	19.52	10.07	
H					3.09
HC					4.64
Significance		***	**	NS	NS

[z]Mean ± SD with different letters in the same column indicate significant differences (Tukey).
[y]Kruskal-Wallis nonparametric test.
NS, **, ***Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.
SMD = significant minimum difference; cv = coefficient of variation; H = statistical H value calculated; HC = critical value.

days 5 and 8. All results were positive, with graft survival of 55% to 95% (Fig. 2).

Seedling vigor was least favorable when the unwanted tissue was cut off on day 5 after grafting. At days 6 and 8, vigor values were 5 and 4, respectively; from days 8 to 11, the vigor ranking changed to 3, which corresponded to plants with little vigor (Table 1). On day 12, seedling vigor had a value of 2. When the cuts of unwanted tissue were done on days 6, 7, and 8 after grafting, all seedlings showed a vigor value of 3. Nine to 12 d into the observation, with the exception of day 9 d, which corresponded to unwanted tissue cut at a previous day after grafting, the vigor of seedlings improved until reaching a value of 2 (Table 1), which corresponds to plants with good vigor (Fig. 2).

Greater precocity is the desired result in grafted papaya plants. The problems related to the rapid production of grafted seedlings depend on the grafting techniques used, temperature, and the skill with which the grafting procedure is performed. The grafting process should last between 7 and 10 d (Lee and Oda, 2003). In our work, graft survival was evaluated based on the time at which unwanted tissue could be removed without preventing grafted plants from functioning as single plants. When the unwanted tissue was removed 6, 7, or 8 d after the grafting procedure, the percentage of graft survival was more than 90% (Fig. 2). Therefore, the time at which the unwanted tissue was removed was standardized to 6 d after the grafting procedure. This ensured the highest

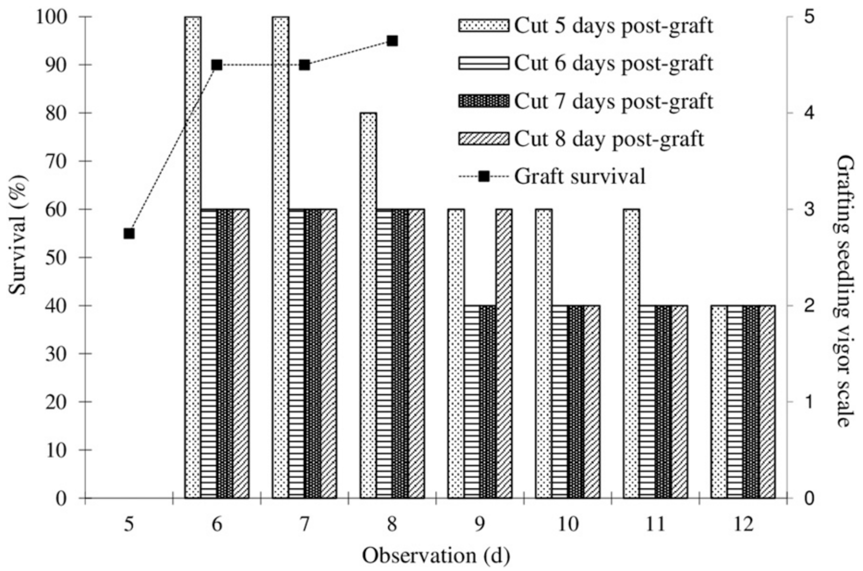

Fig. 2. The most appropriate moment for the selection of grafted seedlings, based on graft survival and vigor. The observations were made after removing unwanted tissue from grafted seedlings.

percentage of graft survival and shortened the time spent taking care of grafted seedlings. Seedling vigor, which improved as time passed, was evaluated 12 d after cutting unwanted tissue (Fig. 2). However, cutting unwanted tissue 6 or 7 d after grafting was also adequate, because seedling vigor improved easily thereafter. Studies of grafting in different species have shown that the time of recovery increases the uniformity and vigor of seedlings, and makes them more likely to resist biotic and abiotic stresses (Leonardi and Romano, 2004), which is in agreement with the results of our study.

During the first evaluation, which involved two grafting methods and two fixation devices, ANOVA detected statistical differences in graft survival ($P \leq 0.001$). Treatment 1 (T-T) yielded 80% of graft survival, surpassing the other treatments. Treatment 4 [cleft grafting fastened with pin (C-P)] yielded only 50% graft survival, which is considered a low percentage (Table 4). Regarding the vigor of the grafted seedling only had significant differences on day 9 of observation ($P \leq 0.01$). Treatment 1 (T-T) statistically was better, because it kept below with value lower to 2, according to the scale of vigor (Table 1), compared with treatments 2 (T-P), 3 (CT), and 4 (C-P), for which the resulting value was higher. The 12-d vigor evaluation, even without differences, all treatments improved due to the time that elapsed favored the vigor (Table 4).

To date, there have been few studies of grafting, although they are not consistent for papaya. However, our study started by evaluating the tongue approach and cleft grafting methods (Khankahdani et al., 2012; Lee et al., 2010) in terms of graft survival and seedling vigor. The results obtained in the first evaluation served as the basis to propose modifications to the grafting methods under study. Of the two grafting methods and the two fastening devices, treatment 1 (T-T) was the most effective because it yielded a percentage of graft survival of 80% in the first evaluation. It should be noted that this method is commonly used in other species (Haghighi et al., 2016). It yielded good results in papaya, but it is worth noting that neither temperature nor humidity were controlled during the growth of the grafted seedlings, as is done for other species and as has been established in the literature (Hassell et al., 2008). With respect to seedling vigor, on days 9 and 12 after grafting, the values were 1.71 and 1.42, respectively. Thus, this method, which was used for treatment 1 (T-T), did not cause any problems in terms of seedling vigor (Table 4). The other treatments—2 [tongue approach grafting fastened with pin (T-P)], 3 (C-T), and 4 (C-P)—which used the cleft grafting method, yielded a low percentage of graft survival and lower seedling vigor after grafting (Table 4), and so they have room for improvement using other strategies (Van-Hong and Chung-Ruey, 2018).

During the second evaluation using modified grafting methods, there were statistically significant differences in graft survival ($P \leq 0.01$). Regarding the percentage of graft survival, treatment 2 (M-T-Bc-T) surpassed the rest of the treatments, with 90% graft survival. Treatments 1 and 4 [modified tongue approach grafting method, tongues formed above the stem-site cut, fastened with tape (M-T-Ac-T) and modified tongue approach grafting method, tongues formed below the stem-site cut, fastened with pin (M-T-Bc-P), respectively] both yielded a graft survival of 65%. Finally, treatments 3 [modified tongue approach grafting method, tongues formed above the stem-site cut, fastened with pin (M-T-Ac-P)] and 5 [modified cleft grafting method, fastened with lace (M-C-L)] yielded a lower percentage of graft survival than the other treatments (Table 5). Regarding the vigor of grafted seedlings, statistical differences were also detected between both observations (9 and 12 d) after grafting ($P \leq 0.01$ and $P \leq 0.001$, respectively). According to the quality scale, treatment 5 (M-C-L) yielded the lowest quality seedlings for both observation days. The observation to 9 d after grafting, all treatments (M-T-Ac-T, M-T-Bc-T, M-T-Ac-P, and M-T-Bc-P) yielded vigor values ranging from 1.4 to 2.6, but these values improved as time progressed until 12 d after grafting (Table 5).

Although M-C-L works well in tomato grafts (Alvarez, 2012), the results obtained in our study suggest that use should be discarded for papaya as a result of the low percentage of graft survival (only 12.5%) and the low vigor values on the two observation dates (Table 5). Of the modified grafting methods, treatment 2 (M-T-Bc-T) yielded a greater rate of graft survival (90%) and acceptable vigor (Table 5), surpassing our expectations for use in papaya under the conditions evaluated. With this modified method, the meristem at the upper part of the rootstock is removed at the beginning of the grafting procedure. This step allowed us to manipulate more easily both plants during the cutting and joining process, and it had a positive effect on the percentage of graft survival. Thus, grafting methods should be modified based on different influencing factors, and it is necessary to evaluate the effectiveness of each modification (Yassin and Hussen, 2015).

With respect to the evaluation of seedlings grown in different containers, ANOVA results showed statistically significant differences ($P \leq 0.05$) for the graft survival variable; the seedling vigor variable (according to the Kruskal-Wallis test), was statistically significant ($P \leq 0.05$) for the first observation date. Treatment 3 (M-T-Bc-T-B) and treatment 1 [modified tongue approach grafting method, tongues formed below the stem-site cut, fastened with tape, 128-cavity tray (M-T-Bc-T-128)] yielded higher percentages of graft survival. However, the percentage of graft survival obtained with the three treatments were considered acceptable because of to the spacing between plants. With respect to seedling vigor, the three treatments yielded good results, based on the observations made 9 d after grafting; at day 12, seedling vigor was still improving, although without differences (Table 6).

The grafting method used in this evaluation was the modified tongue approach grafting, which yielded the greatest percentage of graft survival and highest values of seedling vigor. The plants in the container involving the use of plastic bags (M-T-Bc-T-B) were more stable and had a greater percentage of graft survival compared with the tray containers. An advantage of the use of plastic bags was that the root balls were never exposed to the environment during the grafting procedure, which did happen in the treatments involving trays. *C. papaya* is very sensitive to the exposure of its rootlets during transplantation, which probably explains its

Table 4. Graft survival and vigor of papaya seedlings grafted using two grafting methods and two fixation devices.

			Vigor[x] (d after grafting)	
No.	Treatments	Graft survival (%)	9	12
1	T-T	80 a[zy]	1.71 b[w]	1.42
2	T-P	25 c	2.85 a	2.14
3	C-T	17.5 c	3.00 a	2.14
4	C-P	055 b	3.42 a	2.42
SMD		0.20		
cv		12.75		
H			10.35	5.90
HC			11.31	6.84
Significance		***	**	NS

[z]Different letters in the same column indicate significant differences (Tukey).
[y]Data subjected to arcsine square root transformation.
[x]Kruskal-Wallis (non parametric test).
[w]Mann-Whitney test.
[NS, **, ***]Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.
SMD = significant minimum difference; cv = coefficient of variation; H = statistical H calculated; HC = critical value.

Table 5. Graft survival and vigor of papaya seedlings grafted using two modified grafting methods and three fixation devices.

			Vigor[x] (d after grafting)	
No.	Treatments	Graft survival (%)	9	12
1	M-T-Ac-T	65 ab[zy]	2.00 bc[w]	1.42 bc
2	M-T-Bc-T	90 a	1.40 c	1.40 c
3	M-T-Ac-P	40 bc	2.60 b	2.20 b
4	M-T-Bc-P	65 ab	2.40 b	1.80 b
5	M-C-L	0.35 c	4.00 a	3.40 a
SMD		0.42		
cv		22.27		
H			13.84	15.54
HC			15.83	17.34
Significance		**	**	**

[z]Different letters in the same column indicate significant differences (Tukey).
[y]Data subjected to arcsine square root transformation.
[x]Kruskal-Wallis (non parametric test).
[w]Mann-Whitney test.
**Significant at $P \leq 0.01$.
SMD = significant minimum difference; cv = coefficient of variation; H = statistical H calculated; HC = critical value.

Table 6. Graft survival and vigor of papaya seedlings grafted using the same grafting method but grown in different containers.

			Vigor[x] (d after grafting)	
No.	Treatments	Graft survival (%)	9	12
1	M-T-Bc-T-128	82.5 ab[zy]	2.14 bc[w]	1.71
2	M-T-Bc-T-200	70 b	2.57 ab	2.00
3	M-T-Bc-T-B	92.5 a	1.57 c	1.42
SMD		0.26		
cv		10.52		
H			5.95	1.92
HC			7.19	2.39
Significance		*	*	NS

[z]Different letters in the same column indicate statistically significant differences (Tukey).
[y]Data subjected to arcsine square root transformation.
[x]Kruskal-Wallis (non parametric test).
[w]Mann-Whitney test.
[NS, *]Nonsignificant or significant at $P \leq 0.05$, respectively.
SMD = significant minimum difference; cv = coefficient of variation; H = statistical H value calculated; HC = critical value.

response to the treatments involving polystyrene trays, which was independent of the number of cavities in each tray. The favorable effect of plastic bags was also reflected in seedling vigor shown by the seedlings on the two observations dates after grafting (Table 6).

In the last evaluation of graft quality based on the physical and visual conditions of the grafting area, the grafting methods used yielded results between "regular" and "good," with the exception of grafting method M-C-L, which yielded "bad" results in terms of adherence and compatibility. M-T-Bc-T yielded good results in terms of the four parameters tested and was thus considered the best grafting method for *C. papaya* (Table 7).

The methods involving the tongue approach, in which the two root systems are preserved during the grafting procedure, yielded the best results. Cleft grafting can be considered an alternative method, but it has the disadvantage that temperature and humidity must be controlled rigorously controlled, which is not necessary for the tongue approach grafting method. In addition, in cleft grafting, the quality of the seedlings is highly influenced by the growing medium used in the nursery (Agbo and Omaliko, 2006) (Table 7).

Plant grafting onto specific rootstocks is used to induce vigor and precocity, improve yield and quality, increase survival rates, reduce the risk of infection by soil pathogens, and increase tolerance against abiotic stress (Kumar and Sanket, 2017). In our study, normal and modified grafting methods were evaluated several times, as the latter was derived from the former. The same was true

Table 7. Main parameters of graft quality in the different methods evaluated.

Treatments	Superficial adherence	Compatibility	Formation of graft callus	Absence of necrotic tissue
T-T	R[z]	G	G	G
T-P	R	G	R	G
C-T	R	G	R	G
C-P	R	G	G	G
M-T-Ac-T	R	G	G	R
M-T-Bc-T	G	G	G	G
M-T-Ac-P	R	G	G	R
M-T-Bc-P	R	G	G	G
M-C-L	B	B	R	R

[z]Quality: B = bad; G = good; R = regular.

for the different containers used for growing grafted papaya seedlings.

Conclusions

Graft survival and seedling vigor were the most important parameters used to determine the quality of grafted seedlings. Under normal environmental conditions in the Valley of Apatzingan, the seedlings were able to function as grafted seedlings 6 d after the grafting procedure. T-T and M-T-Bc-T were the most effective grafting methods, with 80% and 90% graft survival, respectively. With respect to the use of containers, the grafted plants grown in plastic bags had the highest percentage (92.5%) of graft survival. We therefore propose M-T-Bc-T and M-T-Bc-T-B, as reliable grafting methods for papaya that do not require special handling conditions.

Literature Cited

Abad, M., P. Noguera, R. Puchades, A. Maquieira, and V. Noguera. 2002. Physico-chemical and chemical properties of some coconut dusts for use as a peat substitute for containerised ornamental plants. Bioresour. Technol. 82(3):241–245.

Agbo, C.U. and C.M. Omaliko. 2006. Initiation and growth of shoots of *Gongronema latifolia* Benth stem cuttings in different rooting media. Afr. J. Biotechnol. 5(5):425–428.

Allan, P. 2007. Phenology and production of *Carica papaya* 'Honey Gold' under cool subtropical conditions. Acta Hort. 740:217–223.

Allan, P., C. Clark, and M. Laing. 2010. Grafting papayas (*Carica papaya* L). Acta Hort. 851:253–258.

Alvarez, H.J.C. 2012. Agronomic performance and incidence of diseases in tomato grafted plants (*Solanum lycopersicum* L.). Acta Agron. 61(2):117–125.

Alvarez-Hernández, J.C., H. Cortez-Madrigal, I. Garcia-Ruiz, L.F. Ceja-Torres, and J.F. Perez-Dominguez. 2009. Incidence of pests in grafts of tomato (*Solanum lycopersicum*) on wild relatives. Rev. Colomb. Entomol. 35(2):150–155.

Barchuk, A.H. and M.P. Diaz. 2000. Growth vigour and survival of *Aspidosperma quebracho-blanco* and *Prosopis chilensis* plantations in the arid Chaco. Quebracho 8:17–29.

Davis, A.R., P. Perkins-Veazie, Y. Sakata, S. Lopez-Galarza, J.V. Maroto, S.G. Lee, Y.C. Huh, Z. Sun, A. Miguel, S.R. King, R. Cohen, and J.M. Lee. 2008. Cucurbit grafting. Crit. Rev. Plant Sci. 27:50–74.

de Lima, L.A., R.V. Naves, O.K. Yamanishi, and H.L. Pancoti. 2010. Behavior of three papaya genotypes propagated by grafting in Brazil. Acta Hort. 851:343–348.

Desaid, A., B. Panchal, A. Trivedi, and D. Prajapati. 2017. Studies on seed germination and seedling growth of papaya (*Carica papaya* L.) cv. 'Madhubindu' as influenced by media, GA3 and cow urine under net house condition. J. Pharmacognosy Phytochem. 6(4):1448–1451.

Feitó, M.D. and B.M. Portal. 2013. La competitividad en las exportaciones de papaya de México: Un análisis cuantitativo. Perspectivas: Revista de Análisis de Economía, Comercio Negocios Internacionales 7(2):27–54.

Garcia, E. 1988. Modificaciones al sistema de clasificacion climatica de Köppen, para adaptarlo a las condiciones de la Republica Mexicana. 4th ed. UNAM, Mexico.

García, R.I. and A.L. Linares. 2012. Arboles y arbustos de la cuenca del rio Tepalcatepec (Michoacan y Jalisco, Mexico) para uso urbano. 1st ed. El Colegio de Michoacán A.C. e Instituto Politecnico Nacional, Mexico.

Haghighi, M., A. Sheibanirad, and M. Pessarakli. 2016. Cucurbit grafting: Methods, physiology, and responses to stress, p. 255–272. In: M. Pessarakli (ed.). Handbook of cucurbits: Growth, cultural practices, and physiology. CRC Press, Taylor and Francis Group, Boca Raton, FL.

Hammer, Ø. 2018. PAST V. 3.2 reference manual. Natural History Museum, University of Oslo, Oslo, Norway.

Hassell, R.L., F. Memmott, and D.G. Liere. 2008. Grafting methods for watermelon production. HortScience 43:1677–1679.

Honma, S. 1977. Grafting eggplants. Scientia Hort. 7(3):207–211.

INEGI. 2016. Anuario estadistico y geografico de Michoacan de Ocampo. Instituto Nacional de Estadistica y Geografia, Mexico.

Kawaguchi, M., A. Taji, D. Backhouse, and M. Oda. 2008. Anatomy and physiology of graft incompatibility in solanaceous plants. J. Hort. Sci. Biotechnol. 83(5):581–588.

Khankahdani, H.H., E. Zakeri, G. Saeedi, and G. Shakerdargah. 2012. Evaluation of different rootstocks and grafting techniques on graft union percent, yield and yield components of watermelon cv. 'Crimson Sweet'. World Appl. Sci. J. 18(5):645–651.

Kumar, B.A. and K. Sanket. 2017. Grafting of vegetable crops as a tool to improve yield and tolerance against diseases: A review. Intl. J. Agr. Sci. 9(13):4050–4056.

Kumar, A.S. and S. Kumari. 2013. Efficient procedure for clonal propagation of *Carica papaya* L. via shoot tip explant. Indian J. Plant Sci. 2(1):24–33.

Lange, A.H. 1969. Reciprocal grafting of normal and dwarf Solo papaya on growth and yield. HortScience 4:304–306.

Lee, J.M. 1994. Cultivation of grafted vegetables I: Current status, grafting methods, and benefits. HortScience 29:235–239.

Lee, J.M., C. Kubota, S.J. Tsao, Z. Bie, P.H. Echevarria, L. Morra, and M. Oda. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. Scientia Hort. 127(2):93–105.

Lee, J.M. and M. Oda. 2003. Grafting of herbaceous vegetable and ornamental crops. Hort. Rev. 28:61–124.

Leonardi, C. and D. Romano. 2004. Recent issues on vegetable grafting. Acta Hort. 631:163–174.

Maiti, R.K., P.S. Raju, and F.R. Bidinger. 1981. Evaluation of visual scoring for seedling vigour in sorghum. Seed Sci. Technol. 9:613–622.

Meena, A.K., O.P. Garhwal, A.M. Kumar, and S.P. Sing. 2017. Effect of different growing media on seedling growth parameters and economics of papaya (*Carica papaya* L) cv. 'Pusa Delicious'. Intl. J. Curr. Microbiol. Appl. Sci. 6(6):2964–2972.

Mirafuentes, H.F. and A.M. Azpeitia. 2008. 'AZTEC', the first papaya hybrid for the tropical conditions of Mexico. Rev. Fitotec. Mex. 31(3):291–293.

Mirafuentes, H.F. and F.B. Santamaria. 2014. 'MSXJ', hybrid of papaya without carpellody for southeastern Mexico. Rev. Mex. Cienc. Agr. 7:1297–1301.

Oda, M. 1995. New grafting methods for fruits bearing vegetables in Japan. Jpn. Agr. Res. Qrtly. 29:187–194.

Schmildt, O., E. Campostrini, E.R. Schmildt, N.A. Torres, P.A. Lopes, F.T. Massi, G.A. Ferreguetti, A.R. Sobreira, and G.J. Cuevas. 2016. Effects on indol butyric acid concentration on propagation from cuttings of papaya cultivars 'Golden' and 'Uenf/Caliman 01'. Fruits 71(1):27–33.

Senthilkumar, S., N. Kumar, K. Soorianathasundaram, and P. Jeya-Kumar. 2014. Aspects on asexual propagation in papaya (*Carica papaya* L.): A review. Agr. Rev. 35(4):307–313.

SIAP-SAGARPA. 2017. Estadisticas de la producción nacional de papaya. 2 Apr. 2018. http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ResumenProducto.do.

Singh, R., R.C. Yadav, N.R. Yadav, A. Yadav, and P. Sharma. 2010. Effect of plant growth regulators and silver nitrate of female papaya using shoot tip as explant. Indian J. Hort. 67:51–54.

Song, G., A.E. Walworth, and W.H. Loescher. 2015. Grafting of genetically engineered plants. J. Amer. Soc. Hort. Sci. 140:203–213.

Teixeira, S.J.A., Z. Rashid, D.T. Nhut, D. Sivakumar, A. Gera, M. Teixeira S., and P.F. Tennant. 2007. Papaya (*Carica papaya* L.) biology and biotechnology. Tree For. Sci. Biotechnol. 1(1): 47–73.

Van-Hong, N. and Y. Chung-Ruey. 2018. Rootstock age and grafting season affect graft success and plant growth of papaya (*Carica papaya* L.) in greenhouse. Chil. J. Agr. Res. 78(1):59–67.

Yassin, H. and S. Hussen. 2015. Review on role of grafting on yield and quality of selected fruit vegetables. Global J. Sci. Frontier Res. Agr. Vet. 15(1):1–16.