Colegio de Postgraduados

Comparing outstanding papaya lines for selecting and preserving improved characters

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ABSTRACT

Objective: To evaluate outstanding and adapted papaya lines derived from selection to conserve desirable characteristics.

Design/methodology/approach: 23 lines of the 'Maradol' type were evaluated at Antunez Michoacan, Mexico. Initially, the plants' height, stem circumference, number of leaves, and first fruit height were recorded. During their development, outstanding plants were identified, and their self-pollinating was promoted. In the fruits, their polar and equatorial circumference, shape index, weight, width and pulp firmness, and soluble solids were assessed.

Results: The plants' development was different, their variability between lines allowed identifying morphological characteristics of interest. Only 10 lines had this condition. The number of fruits formed over covered flower buds and collected fruits on formed fruits was reduced. The fruits' characterization, except for their soluble solids, showed differences. Multivariate analysis indicated variability associated with each principal component.

Limitations on study/implications: Currently in Mexico, there are few papaya varieties, the 'Maradol' variety being dominant, and vulnerable to phytosanitary problems over time. However, developing varieties and seed production is challenging and the pollination control of the plants necessarily intervenes.

Findings/conclusions: Out of 23 assessed papaya lines, only 43.48% reported outstanding plants. Inside these lines, between 5 and 10% of the plants were chosen. In the developmental progress from the covered flower buds' stage to formed and collected fruits, only 28% of fruits were obtained. The selected lines showed fruit variability.

Keywords: *Carica papaya*, 'Maradol' genotype, hermaphrodite, plant sexing.

Citation: Álvarez-Hernández, J. C., & Castillo-Martínez, C. R. (2023). Comparing outstanding papaya lines for selecting and preserving improved characters. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i11.2725

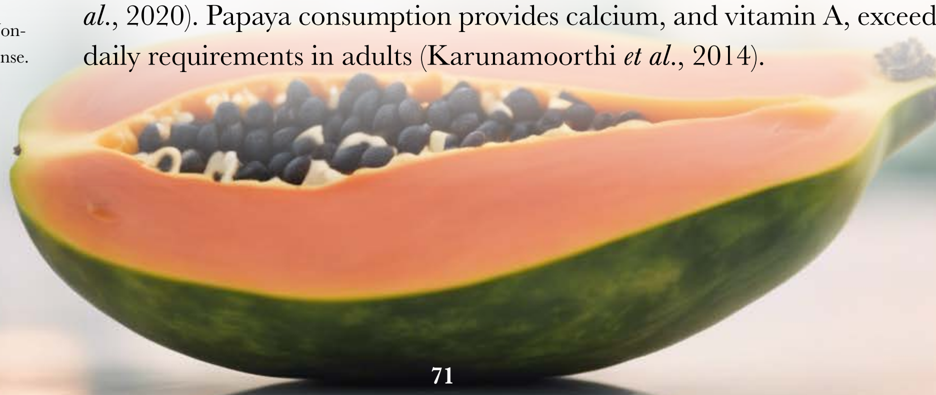
Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: May 13, 2023.
Accepted: September 28, 2023.
Published on-line: December 28, 2023.

Agro Productividad, 16(11). November. 2023. pp: 71-81.

INTRODUCTION

Of the 22 species in the *Carica* genus, the papaya (*Carica papaya* L.), native to the American tropics, is the one with the greatest economic importance. It has traditionally been cultivated in different regions of America, Africa, Asia, Australia, countries like the Philippines, and the United States (Hawaii and Florida); and recently in Europe (Honoré *et al.*, 2020). Papaya consumption provides calcium, and vitamin A, exceeding the minimum daily requirements in adults (Karunamoorthi *et al.*, 2014).



Mexico ranked fifth among the countries with the largest established area, and fourth by production volume (FAOSTAT, 2021). During 2022, the harvested area in Mexico was 19,698 ha, mainly in Veracruz, Colima, Michoacán, Oaxaca, Chiapas, and Guerrero states. Particularly, Michoacán registered 3,135 ha, and 112,586 tons of production (SIAP, 2023). The papaya production activity generates direct and indirect jobs and boosts regional economies. In Mexico, the 'Maradol' variety is dominant (SIAP, 2017). For its propagation, the seed type varies from original "F1" to "Fn" selections. Given this, the possibility of degeneration exists, leading to a low number of offered varieties, adapted to the different agroecological regions of the country, and a marked imbalance in the materials resistance capacity introduced in the papaya-producing areas, making them vulnerable to pests and diseases. Also, this species has a complex floral biology, since it has female, male, and hermaphrodite plants (Damasceno *et al.*, 2018), which influences the fruit's production and quality. Thus, developing papaya varieties with enhanced agronomic traits, fruit quality, and high disease resistance levels is a challenge (Vivas *et al.*, 2017). Generally, papaya is an open-pollinated species (Urasaki *et al.*, 2012), which limits the uniform development of plantations in later periods.

In Mexico, the utilized varieties were originated by selection and improvement, where controlled pollination is key. Consequently, outstanding plants with some characteristics of interest are chosen, and their pollination is subsequently controlled. If crosses are made between plants, these should preferably be between hermaphrodite plants, or self-pollination should be promoted so that, depending on the floral proportion, 66% of their seeds are expected to originate hermaphrodite plants (Ram, 2005). Thus, using improved genotypes must meet criteria that influence the species productive potential and the appropriate environment for its development (Nunes *et al.*, 2018). Therefore, it is necessary to rescue genetic material that can be used in the improvement of papaya seed production and new materials development (Álvarez and Tapia, 2019), adapted to regions of interest (SNITT-SAGARPA, 2016). For this, genetic diversity research is important (da Silva *et al.*, 2017). Based on the above, different lines of outstanding papaya plants derived from selection in commercial environments were evaluated to preserve improved productive characteristics.

MATERIALS AND METHODS

Starting in 2022 at the town of Antúnez, state of Michoacán, Mexico, 23 outstanding papaya lines of the 'Maradol' type were experimentally evaluated. From these, 14 lines of selected plants came from a commercial plot exploration; the rest were previously collected materials (Table 1).

For each line, 20 plants were established, their planting framework was 3 m between rows and 2 m between plants. The established plants had basic agronomic management for this crop, consisting of supplying drip irrigation between 2 to 4 h daily; manual and chemical weed elimination, monitoring and chemical managing pests and diseases, and fertilization management with N-P-K nutrient solutions (Coria *et al.*, 2017); likewise, the plants developed with the local environmental conditions (Figure 1).

Table 1. Outstanding papaya lines evaluated in the experimental field.

Line No.	Registry No.	Nomenclature	Line No.	Registry No.	Nomenclature
1	10	H. Barocio 3, Antúnez, P1	13	4	H. Pista, Antúnez
2	9	H. Barocio, 2 Antúnez	14	IX	AR
3	11	H. Barocio 3, Antúnez, P2	15	XI	P5
4	14	H. Andrade, Antúnez	16	III	25A
5	13	H. Andrade, La Soledad	17	IV	42A
6	8	H. Barocio 1, Antúnez P3	18	II	21A
7	7	H. Barocio 1, Antúnez, P2	19	VIII	ARTM
8	6	H. Barocio 1, Antúnez, P1	20	X	P4
9	5	H. Adelo, Ceñidor P3	21	I	9A
10	12	H. Barocio 4, Antúnez	22	2	H. Adelo, Ceñidor, P1
11	III	42 A	23	1	H. Ramón, Antúnez
12	3	H. Adelo, Ceñidor P2	-	-	-

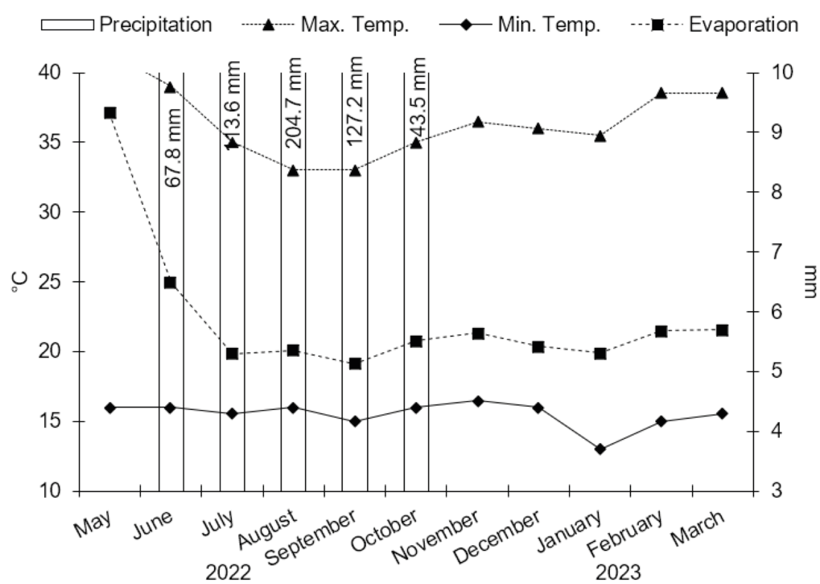


Figure 1. Climatic variation during the essay (Department of Hydrometry, Irrigation District 097, CONAGUA, Mexico).

From the beginning, 23 lines (treatments) and five plants (repetitions) were formed, in a randomized complete block experimental design. In two periods, 94 and 164 days after transplanting (dat), the following was recorded: plant height, using a flexometer, measured from the base of the soil to the plant apex; stem circumference, assessed approximately 15 cm above the soil base using a measuring tape. The number of leaves, visually recorded; and the height of the first fruit, recorded at 94 days, measuring with a flexometer the length between the ground and the first fruit.

During plant development, reviews were conducted to identify outstanding ones. This characteristic consisted of plants with a healthy visual appearance and excellent vigor.

Once they presented their flower buds, hermaphrodite plants were chosen, preferably with heights at the first flower below 0.8 m and fruit precocity. This allowed discarding plants from lines that did not meet the characteristics to be considered outstanding plants. Further attention was directed to the selected plants, where some lines presented one plant. Plants that met these characteristics were differentiated with a plastic signal coiled on the stem. At anthesis, flower buds were chosen from between four and seven fully developed buds and labelled with basic identification information. The flower buds were protected with 4.5×7.5 cm waxed glassine paper bags to ensure self-pollination. During this stage, the number of covered buds, formed fruits, and quality fruits were recorded, that is, morphologically normal fruits for subsequent seed collection. The development of the fruits until their physiological maturity lasted approximately four months. In a complete randomized blocks experimental design, 10 treatments were formed (only the outstanding lines) and five repetitions (fruits). The recorded fruit variables were: polar and equatorial circumference, with a measuring tape, where the fruits were surrounded by two crossing axes; fruit shape index, assessed by dividing the polar with the equatorial circumference of the fruits; fruit weight, weighed with a digital scale; pulp width, slices were cut, and with a graduated ruler, the middle part and mesocarp width were measured; pulp firmness, on a fruit side, the epicarp was removed and the mesocarp pressed with a penetrometer to record its hardness; and soluble solids, juice was extracted from the mesocarp onto a refractometer.

The data analysis of the recorded variables depended on the test. On variables under experimental design, analysis of variance, and comparison of means were performed with the Tukey statistical test ($P=0.05$). Progress of floral bud development and transition to formed fruits and collected fruits, the numerical values were percentagewise compared. All variables were concentrated to perform a multivariate principal components and clusters analysis. Also, the basic statistical indicators of the variables under study were compared. The SAS version 9.3 (2002) and PAST 3.2 (Hammer, 2018) statistical software were used.

RESULTS AND DISCUSSION

Table 2 presents the plant development of the evaluated lines. The analysis of variance showed significant differences in the plant development variables. The four evaluated variables, in their respective samples, expressed variation between lines. Which identifies the morphological characteristics of the lines according to their purpose. The L7 R7 and L15 RXI lines reported higher vigor in the height and stem circumference variables. Regarding the height to the first fruit variable, given that the lowest height materials are the ones required, the L1 R10, L2 R9, L7 R7, L10 R12, and L22 R2 lines meet this condition, as their height to the first fruit did not reach 60 cm. The L3 R11line had the greatest quantity for the number of leaves variable.

As observed in Table 2, the developments of the 23 lines of papaya plants were different during their development stage, which distinguished plant responses to the conditions of the study area. In fact, the national agenda for research, innovation, and agricultural technology transfer suggests these research initiatives and emphasizes that the used materials must be developed for each region of interest (SNITT-SAGARPA, 2016).

Table 2. Development of papaya plants of 23 lines in two sampling periods.

Id.	Plant height (cm)		Stem circumference (cm)		Leaf number		Height of 1st fruit (cm)
	94	164	94	164	94	164	
L1 R10	44.4 hij	84.2 h	10.68 ef	24.49 e	17.2 i	20 f	58 ef
L2 R9	56.4 efgh	110.8 efg	13.82 bcde	35.16 abcd	22.2 abcdef	27.6 bcdef	55 f
L3 R 11	63.8 bcdefg	127.2 cdef	14.01 bcde	34.54 abcd	25.2 a	42 a	71.2 def
L4 R14	49.6 fghi	129.2 bcdef	12.56 efd	33.91 abcd	21.4 cdefgh	31.6 bcde	68.8 def
L5 R13	59.8 bcdefg	128.6 cdef	14.13 bcde	34.54 abcd	22.8 abcde	31.4 bcde	71.2 def
L6 R8	49.2 fghi	131.4 bcde	12.56 def	35.79 abc	20.2 efghi	33.4 bcd	64.8 ef
L7 R7	64.6 bcdefg	140 bc	16.02 abcd	33.91 abcd	24.4 abc	31.6 bcde	53 f
L8 R6	68.2 bcde	142.6 bc	16.96 abc	36.42 ab	23.8 abcd	35.4 abc	69 def
L9 R5	68.2 bcde	133.2 bcd	16.96 abc	32.02 abcd	23.8 abcd	32 bcde	93.6 bcd
L10 R12	64.4 bcdefg	143 bc	18.53 a	37.05 a	23.6 abcd	31 bcde	56.2 f
L11 RIII	48 ghi	113.6 defg	14.13 bcde	30.14 cde	20.8 defgh	32.8 bcde	74.8 cdef
L12 R3	59 defgh	130.2 bcdef	13.50 cde	35.16 abcd	19.2 fghi	36.6 ab	85.6 bcde
L13 R4	74.2 ab	134.2 bcd	17.59 ab	33.28 abcd	24.4 abc	30.4 bcde	100.6 bc
L14 RIX	41.8 ij	114.6 defg	9.67 f	32.65 abcd	25 ab	27 def	73.6 cdef
L15 RXI	82.8 a	194.8 a	17.59 ab	37.05 a	19 fghi	26.6 def	134.4 a
L16 RIII	62.8 bcdefg	149.8 b	15.39 abcd	30.2 cde	20.8 defgh	25 ef	104.4 b
L17 RIV	68.6 bcd	129.6 bcdef	15.70 abcd	33.91 abcd	23.2 abcde	28.6 bcde	93.6 bcd
L18 RII	55.6 fghi	101.6 gh	14.13 bcde	29.51 de	23.2 abcde	26.8 def	64.6 ef
L19 RVIII	36.2 j	94.8 gh	10.68 ef	30.77 bcd	18.6 ghi	26.2 def	60.6 ef
L20 RX	71.6 abc	143.8 bc	14.45 bcde	32.65 abcd	20.6 defgh	27 def	100.6 bc
L21 RI	40.4 ij	110.4 fg	10.68 ef	32.65 abcd	18.2 hi	28 bcdef	67.2 def
L22 R2	54.2 efgh	112 efg	14.13 bcde	32.65 abcd	21.8 bcdefg	27.2 cdef	51.4 f
L23 R1	67 bcdef	111 efg	16.33 abcd	31.4 abcd	20.6 defgh	27 def	67 def
P	**	**	**	**	**	**	**
DMS	11.80	20.62	3.38	5.96	3.32	8.29	28.11
CV (%)	8.51	6.90	11.29	7.65	6.49	11.81	15.75

Means with equal letters are not statistically different (Tukey, 0.05), **: P≤0.001, MSD: Minimum significant difference, CV: coefficient of variation.

Under this assessment, some evaluated lines showed marked advantages over the rest. According to SNICS-SAGARPA (2014), plants for multiplication purposes must have some characteristics to guarantee quality, such as being vigorous, from varieties with uniform plants, both in size and fruit shape, in addition to low height, pests and disease tolerant, among others. However, for the crop as a whole to achieve high yields and fruit quality, several factors are involved, such as the genetic constitution of the cultivar, favourable soil-climatic conditions, efficient phytosanitary control, timely water supply, and nutritional deficiency corrections (Santana *et al.*, 2018).

Derived from the plants periodic evaluation, only 10 lines reported outstanding characteristics plants. Because the flower buds in these plants were covered, the three-stage flower buds, formed fruits and collected fruits quality process was followed. As observed in Figure 2, the number of formed fruits was reduced in percentage terms compared to the covered buds, as well as the number of fruits collected was reduced in relation to the formed fruits, due to natural factors influencing the process. Thus, the L7 R7 line reached 100% formed fruits in the covered buds, that is, from four covered buds, four fruits were formed. However, in collected fruits quality terms the formed fruits reached only 50%, That is, from four formed fruits, only two quality fruits were collected for seed extraction. In the L19 RVIII line case, the quality collected fruits from the formed fruits was 100%, that is, from three formed fruits, three quality fruits were collected. The same did not happen in the fruits formed from the covered buds at the beginning, since, out of seven covered buds, only three fruits were formed.

Carica papaya is generally propagated by seeds, therefore, high plant heterogeneity is common (Bhattacharya and Khuspe, 2001). The genetic base is limited and depends on a few alternative varieties and hybrids that do not satisfy their demand. This encourages producers to select F2 to F4 generations in continuous plantations, which is why flower bud protection is common practice (Stice *et al.*, 2016), so as not to run the risk of loss of vigor and segregation (Marin *et al.*, 2006). For this reason, the fundamental principle of having a broad genetic base is pursued to choose promising materials, where plant selection is a good beginning for crop improvement. Meanwhile, of 23 promising lines, only 10 were selected, the rest were purged.

The variance analyses on the fruit characterization variables are shown in Table 3. Except for the soluble solids variable, all the variables had significant differences. The polar and equatorial circumference in the L14 RIX line reported the largest fruit dimensions, opposite values were recorded in line L7 R7. For the shape index variable, line L13 R4 had the highest proportion. At the same time, the L19 RVIII line had the lowest shape index. Regarding fruit weight, the L21 RI line had the heaviest fruits, the opposite situation occurred in the fruits of the L7 R7 line, just as they had the smallest pulp width, while the L18 RII line had the largest pulp width. In pulp firmness, the L21 RI line presented the highest pulp hardness.

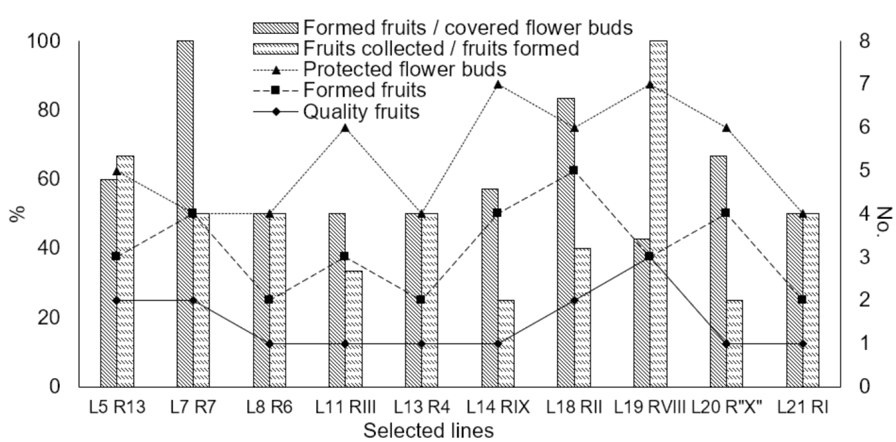


Figure 2. Development process between stages from flower buds to formed fruits of papaya.

Table 3. Characterization of fruits collected from outstanding papaya plants.

Line	Circumference (cm)		Form index	Weight (kg)	Pulp width (cm)	Pulp firmness (kg cm^{-2})	Soluble solids (°Brix)
	Polar	Equatorial					
L5 R13	56.2 abc	31.8 ed	1.76 abc	1.173 bc	2.74 bcd	2.14 ab	12.52
L7 R7	51 c	28.6 e	1.79 ab	0.822 c	2.42 d	2.04 b	12.78
L8 R6	56.8 abc	34.6 bcd	1.64 bcd	1.239 bc	2.66 cd	2.2 ab	12.42
L11 RIII	62.4 a	27.2 abc	1.67 abcd	1.722 abc	3.1 abc	2.14 ab	12.34
L13 R4	61.2 ab	32.2 cde	1.89 a	1.297 bc	2.66 cd	2.2 ab	12.68
L14 RIX	62.2 a	40.2 a	1.54 cd	1.73 abc	3.02 abc	2.08 b	12.38
L18 RII	61 ab	38.4 ab	1.58 bcd	1.806 ab	3.22 a	2.06 b	12.86
L19 RVIII	59.2 abc	38.8 ab	1.52 d	1.721 abc	3.16 ab	2.14 ab	12.6
L20 R"X"	53 bc	31 de	1.71 abcd	0.95 bc	2.74 bcd	2.08 b	12.16
L21 RI	61.8 a	38.8 ab	1.58 bcd	2.51 a	3.08 abc	2.32 a	12.1
P	**	**	**	**	**	**	ns
DMS	8.54	5.29	0.22	0.92	0.47	0.21	1.07
CV (%)	6.85	7.06	6.26	28.95	7.78	4.77	4.03

Means with equal letters are not statistically different (Tukey, 0.05), **: $P \leq 0.001$, ns: not significant, MSD: minimum significant difference, CV: coefficient of variation.

Fruit characteristics are commonly important variables that allow genotype choosing (Oliveira de *et al.*, 2012). The fruit weight was acceptable, ranging between 0.822 to 1.806 kg, this in turn was reflected in the fruit size, shape index, and pulp width, whose trend was similar. Both soluble solids and pulp hardness express fruit quality. These characteristics correspond to the 'Maradol' variety. Furthermore, under the proposed scheme, the self-pollinated flowers of hermaphrodite plants, according to the floral proportion in papaya, expected 66.67% hermaphrodite offspring plants for their next cycle (Santana *et al.*, 2019).

The multivariate analysis of the 12 considered variables was interpreted based on the eigenvalues where the individual and accumulated variance in each component of the analysis is shown. Likewise, the eigenvalue and variance of the correlation matrix. The shown values indicate the associated variability with each principal component, and it reduces as it increases the components number, cumulatively showing that the first component explains 68% of the variability (Table 4).

Table 4. Eigenvectors of plant and fruit characteristics, and variability proportion of variance in outstanding papaya plants.

Principal component	Variance-covariance matrix			Correlation matrix	
	Proper value	Explained variance (%)	Cumulative variance (%)	Proper value	Variance (%)
1	845.614192	0.6886	0.6886	845.614	68.858
2	336.012629	0.2736	0.9622	336.013	27.362
3	31.456645	0.0256	0.9878	31.4566	2.5615
4	8.823233	0.0072	0.9950	8.82323	0.71848
5	4.413165	0.0036	0.9986	4.411316	0.35936
6	1.343405	0.0011	0.9997	1.34341	0.10939

Likewise, the first two principal components explained the accumulated variability in the selected lines (Figure 3). Since the first component exceeds 68% of all variance it is positively correlated with plant height (Table 5). The second component represents 24% of all the variance and is positively correlated with the yield per plant (Table 5).

For its part, with the structures of the two principal components, a cluster analysis was performed. This analysis considered the structure of the first two principal components since they explained 96% of the variance and could facilitate identifying variant groups. The results showed that at a Euclidean distance of 20, three defined groups were formed: L11 RIII, L19 RVI, and L13 R4; L18 RII and L5 R13; L8 R6 and L20 RX. On the other hand, L14 RIX, L21 RI, and L7 R7 were not part of any group (Figure 4).

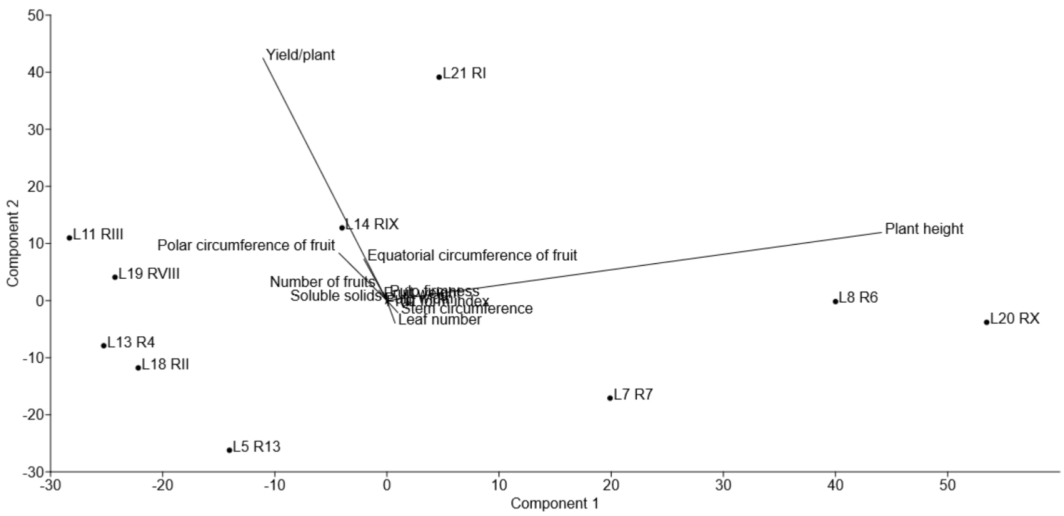


Figure 3. Diagrammatic dispersion of selected lines and variables of plants and fruits in principal components 1 and 2.

Table 5. Eigenvalues of principal components 1 and 2 in the recorded variables.

Variable	Principal component 1	Principal component 2
Polar circumference of fruit	−0.0934426	0.18166
Equatorial circumference of fruit	−0.044811	0.15857
Fruit form index	−0.0014479	−0.0021231
Fruit weight	−0.0064373	0.030484
Pulp width	−0.0040511	0.009336
Pulp firmness	−0.00063862	0.0038579
Soluble solids	−0.0029458	−0.012002
Plant height	0.96428	0.26089
Stem circumference	0.021186	−0.045933
Leaf number	0.01583	−0.085808
Number of fruits	−0.015518	0.038721
Yield / plant	−0.24171	0.92824

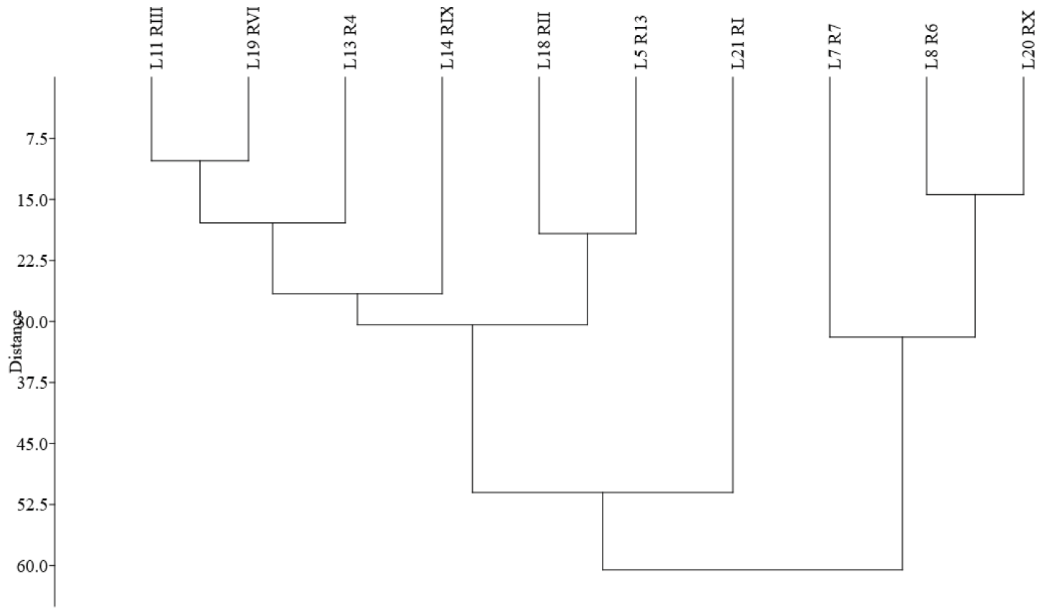


Figure 4. Dendrogram of 12 variables of plants and fruits of selected lines, with three defined groups and three undefined groups at a Euclidean distance of 20.

The principal components analysis required two components to explain 96% of all the variance, the first component contributed 68% of the total variance. With this information, three groups were defined through the cluster analysis. Aikpokpodions (2012) evaluated 60 papaya materials using 21 variables defined from descriptors. Their multivariate analysis generated five groups, thus revealing a significant variation that can be used for the papaya genetic improvement. For their part, Saran *et al.* (2015) evaluated 24 papaya materials and 29 morphological characteristics. Their multivariate analysis showed high morphological diversity in fruit yield, weight, length, cavity, fruiting zone, pulp thickness, pulp color, and soluble solids, whose response is similar to that reported here. Therefore, exploratory studies are important to identify promising materials, for the implementation of a strategy for multiplication, distribution, and improvement of the crop. In fact, the study of genetic diversity is essential in the preliminary selection of accessions with superior characteristics and for the successful use of these genotypes in breeding programs (Barbosa *et al.*, 2011).

Regarding the sample statistics derived from values calculated in variables recorded from papaya plants, these are shown in Table 6. The recorded values did not deviate from normality.

Given that the strategy is to increase productivity in a sustainable and balanced way, searching for new genotypes is crucial for performance improvement (Nascimento *et al.*, 2019). In germplasm collections, genetic diversity allows the assessment of qualitative or quantitative morphology, whose focus is on the evaluation of population segregation by genetic parameters estimation, using selection indices and estimates of correlations between traits related to yield and quality of fruit are necessary (Barbosa *et al.*, 2011).

Table 6. Sample statistics of the variables recorded in outstanding papaya plants.

Variable †	Mean	Standard error	variance	Standard deviation	Minimum value	Maximum value	Coefficient of variation
PCF (cm)	58.20	1.46	21.44	4.63	50.50	63.67	7.96
ECF (cm)	34.80	1.20	14.43	3.80	29.75	40.00	10.91
FFI	1.71	0.04	0.02	0.13	1.58	1.93	7.78
PW (kg)	1.52	0.21	0.44	0.66	0.93	3.15	43.36
PF (cm)	2.87	0.09	0.08	0.28	2.40	3.20	9.79
FP (kg cm^{-2})	2.14	0.03	0.01	0.10	2.00	2.33	4.47
SS (°Brix)	12.49	0.10	0.09	0.31	11.93	13.00	2.46
PH (cm)	222.40	9.00	809.16	28.45	196.00	273.00	12.79
SC (cm)	47.77	0.93	8.61	2.93	41.41	52.08	6.14
LN	42.63	0.74	5.50	2.34	38.88	47.00	5.50
NF	32.80	1.70	29.07	5.39	24.00	40.00	16.44
Y/P (kg)	49.04	5.82	339.22	18.42	28.49	85.02	37.56

† PCF=polar circumference of fruit; ECF=equatorial circumference of fruit; FFI=fruit form index; FW=fruit weight; PW=pulp width; PF=pulp firmness; SS=soluble solids; PH=plant height; SC=stem circumference; LN=leaf number; NF=number of fruits; Y/P=Yield/plant.

CONCLUSIONS

The plant morphological variables recorded values resemble the characteristics of the 'Maradol' type, whose variability allowed the identification of prospective materials. Of the 23 evaluated papaya lines, only 43.48% presented outstanding plants, so 13 lines were discarded. Within the selected lines, only between 5 and 10% of the plants were chosen for their outstanding characteristics. In the development progress from the covered buds stage to formed and harvested fruits, the average value of the 10 lines gradually reduced, in 60% of the formed fruits stage over the covered buds stage and 47% of the fruits stage collected during the stage of formed fruits. Overall, only 28% of the collected fruits on the covered buds were rescued. The selected lines showed variability in fruit values, where the shape index, fruit weight, and pulp width were important indicators to define a mode preference. The multivariate analysis classified three defined groups.

AKNOWELEMENTS

The authors thank the Instituto de Ciencia, Tecnología e Innovación del Estado de Michoacán for the financing granted within the framework of the Convocatoria Apoyo a proyectos de investigación científica de impacto regional 2022, PICIR-076; and to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias for the facilities granted and the SIGI: 22552436128 project registration.

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