

## Evaluation of selected papaya lines for the preservation of desirable traits

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### Abstract

There are currently few varieties of papaya in Mexico and the dominant one is 'Maradol', which over time has been vulnerable. Nonetheless, developing varieties for seed production is challenging. The objective was to evaluate outstanding and adapted papaya lines for the conservation of desirable traits. In the field, 23 lines of the 'Maradol' type were evaluated in Antúnez Michoacán, Mexico, in 2022. Initially, plant height, stem circumference, number of leaves and height to first fruit were recorded. During plant development, outstanding plants were identified and their pollination was controlled. In developed fruits, polar and equatorial circumference, weight, width and firmness of the pulp, and soluble solids were recorded. The development of plants presented differences, whose variability between lines allowed the identification of morphological characteristics of interest. Only 10 lines had this condition. In pollination control, the number of fruits formed over the flower buds decreased and the fruits collected over the fruits formed decreased. The characterization of fruits, except for soluble solids, showed differences. Multivariate analysis indicated variability associated with each principal component. It is concluded that of 23 papaya lines, only 43.48% presented outstanding plants. Within the lines, between 5 and 10% of the plants were selected. In the pollination control, they tended to decreased among the stages since only 28% of fruits were obtained. The selected lines showed fruit variability.

### Keywords:

*Carica papaya*, hermaphrodite, 'maradol' genotype, plant sexing.

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## Introduction

Papaya (*Carica papaya* L.), a species native to the American tropics, is the most economically important of 22 species that appear within the genus *Carica*. Traditionally, it has been commercially cultivated in regions of the Americas, Africa, Asia, Australia, the Philippines, and the United States (Hawaii and Florida) and recently in Europe (Honoré *et al.*, 2020). Papaya provides Ca, vitamins A and C in greater proportions and exceeds the minimum daily requirements in adults (Karunamoorthi *et al.*, 2014).

Mexico ranked fifth among countries with areas established with papayas, and fourth in terms of production volume (FAOSTAT, 2021). The area harvested in the last official record of 2022 in Mexico was 19 698 ha, of which Veracruz, Colima, Michoacán, Oaxaca, Chiapas, and Guerrero stand out. In particular, 3 135 ha were harvested in Michoacán, which produced 112 586 t (SIAP, 2023).

In Mexico, the dominant variety is 'Maradol' (SIAP, 2017). For its propagation, the type of seed used varies from the original F1 to Fn descendant selections. Under this diversity of seed quality, genotypic and phenotypic segregation is common. In addition, papaya has a complex flower biology since this species has female, male and hermaphrodite plants (Damasceno *et al.*, 2018). This influences the production and quality of fruits. Thus, the development of papaya varieties with better agronomic traits, fruit quality and high level of disease resistance is a challenge, since all these characteristics cannot be concentrated in a single genotype (Vivas *et al.*, 2017).

Papaya is generally an open-pollinated species (Urasaki *et al.*, 2012), which limits the uniform development of plantations in later periods because of their genetic makeup. In Mexico, the varieties used have originated by selection and breeding, where controlled pollination is key. Consequently, suitable hermaphrodite plants and pollination control must be chosen, so it can be expected that 66% of seeds will give rise to hermaphrodite plants (Ram, 2005).

Likewise, the genotypes improved for the regions must have the characteristics that influence the productive potential of the species 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 for seed production and the development of new materials (Álvarez and Tapia, 2019).

In addition, they must also be adapted to the regions of interest (SNITT-SAGARPA, 2016), for this, studies on genetic diversity are important (Silva *et al.*, 2017). The objective of this research work was to evaluate different lines of outstanding and adapted papaya plants derived from a preliminary selection in commercial environments.

## Materials and methods

Under field conditions in soil of the pelic vertisol (clayey) type, from the locality of Antúnez Michoacán, Mexico, as of May 30, 2022, 23 outstanding papaya lines of the 'Maradol' type were evaluated under an experimental design of randomized complete blocks, of these, 14 came from the exploration carried out in commercial orchards (H), selected because they are plants with outstanding traits; and the rest were previously collected materials (Table 1).

**Table 1. Outstanding papaya lines subjected to experimental field evaluation.**

Line (L)/Record (R)/Naming		
1 / 10 / H. Barocio 3, Antúnez, P1	9 / 5 / H. Adelo, Ceñidor P3	17 / IV / 42A
2 / 9 / H. Barocio, 2 Antúnez	10 / 12 / H. Barocio 4, Antúnez	18 / II / 21A
3 / 11 / H. Barocio 3, Antúnez, P2	11 / III / 42 A	19 / VIII / ARTM
4 / 14 / H. Andrade, Antúnez	12 / 3 / H. Adelo, Ceñidor P2	20 / X / P4
5 / 13 / H. Andrade, La Soledad	13 / 4 / H. Pista, Antúnez	21 / I / 9A
6 / 8 / H. Barocio 1, Antúnez P3	14 / IX / AR	22 / 2 / H. Adelo

Line (L)/Record (R)/Naming		
7 / 7 / H. Barocio 1, Antúnez, P2	15 / XI / P5	23 / 1 / H. Ramón Antúnez
8 / 6 / H. Barocio 1, Antúnez, P1	16 / III / 25A	

Twenty plants were established for each line; the planting frame used was 3 m between rows and 2 m between plants. The established plants were subjected to agronomic management, which consisted of supplying drip irrigation between 2 and 4 h daily, which is equivalent to a volume of 6.3 L per plant and a cumulative volume of 1 033 L per plant in the cycle; manual and chemical weed removal, monitoring and chemical management of pests and diseases, and fertilization management were adjusted to the progress of each phenological stage, the dosage was by supplying nutrients injected weekly to the irrigation water flow, varying the amounts in the solution without exceeding 4% concentration.

The total proportion was adjusted to the ratio of 1.5-1-2 of N-P-K, and foliar applications of secondary elements and microelements (Coria *et al.*, 2017); likewise, the plants interacted with the climatic conditions present during the months of May 2022 to March 2023: accumulated precipitation 558.8 mm, monthly average maximum temperature 36.6 °C, minimum temperature 15.5 °C, and average monthly evaporation 5.89 mm (Departamento de Hidrometría, Distrito de Riego 097, CONAGUA).

Initially, the matrix consisted of 23 treatments (lines) and five repetitions (plants), in an experimental design of randomized complete blocks. At 164 days after transplantation (dat), the following were recorded: plant height, measured from the base of the soil to the apex of the plant by using a tape measure; stem circumference, measured from 15 cm above the base of the soil with a tape measure; number of leaves, the number of leaves formed and height to the first fruit were recorded visually, which were only recorded at 94 dat, the length between the soil and the first fruit was measured with the use of a tape measure.

Likewise, during the development of the plants, all plants were observed weekly to identify outstanding plants based on the criterion that they were visually healthy plants with excellent vigor. Once the flower buds were formed, hermaphrodite plants were chosen under the criteria that height to the first flower was below 0.8 m and with fruit earliness, and we focused our attention on the selected plants, where some lines had at least one plant.

Plants that met these characteristics were marked with a plastic label wrapped around their stems. In anthesis, the flower buds were chosen from between four and seven fully developed buds, they were labeled with information necessary for their identification. The covering was made with 4.5 x 7.5 cm waxed glassine paper envelopes to ensure self-pollination. During this stage, the number of covered buds, the number of fruits formed, and the number of fruits perfect for seed collection were recorded.

The development of the fruits to physiological maturity was four months. With the 10 selected lines, an experiment was established with a randomized complete block design and five repetitions (fruits); data of the fruits was recorded, the variables were: polar and equatorial circumference, with the use of tape measure, which was obtained by dividing the circumference of the polar fruit by the equatorial circumference; fruit weight, they were weighed on a digital scale; pulp width, slices were cut and a graduated ruler was used to measure the middle part, the width of the mesocarp; fruit firmness, on the side of the fruit, the epicarp was removed and the mesocarp was pressed with a model GY3 manual penetrometer to record its hardness; and soluble solids, juice was extracted from the mesocarp on the base of a Hanna® Model HI 96801 handheld refractometer.

The variables of number of fruits and yield per plant were also recorded, the latter was obtained with the data obtained from the variables of fruit weight and the estimated number of fruits. Data analysis of the recorded variables was based on the type of study. The variables under experimental design were performed analyses of variance and comparison of means with Tukey's test ( $p= 0.05$ ).

The development progress of flower bud and transition to formed fruits and collected fruits, numerical values were compared percentage-wise. All variables were gathered and two multivariate analyses were performed. The first was through principal component analysis.

The second was by means of agglomerative and hierarchical classification using the Euclidean distance as a measure of dissimilarity and Simple Linkage (nearest neighbor) as a hierarchical agglomerative method, so for the proximity between groups, the distance between their closest objects or the similarity between their most similar objects was calculated, which was 20. Also, the basic statistical indicators of the variables under study were recovered in order to contrast them. The statistical packages used were (SAS version 9.3 2002) and PAST 3.2 (Hammer, 2018).

## Results and discussion

The analyses of variance applied to the plant development variables ordered the mean comparisons as shown in Table 2. The four variables evaluated expressed variation between lines. This makes it possible to identify morphological characteristics of the lines according to the purpose. The L7 R7 and L15 RXI lines were more vigorous, at least in the stem height and circumference variables.

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

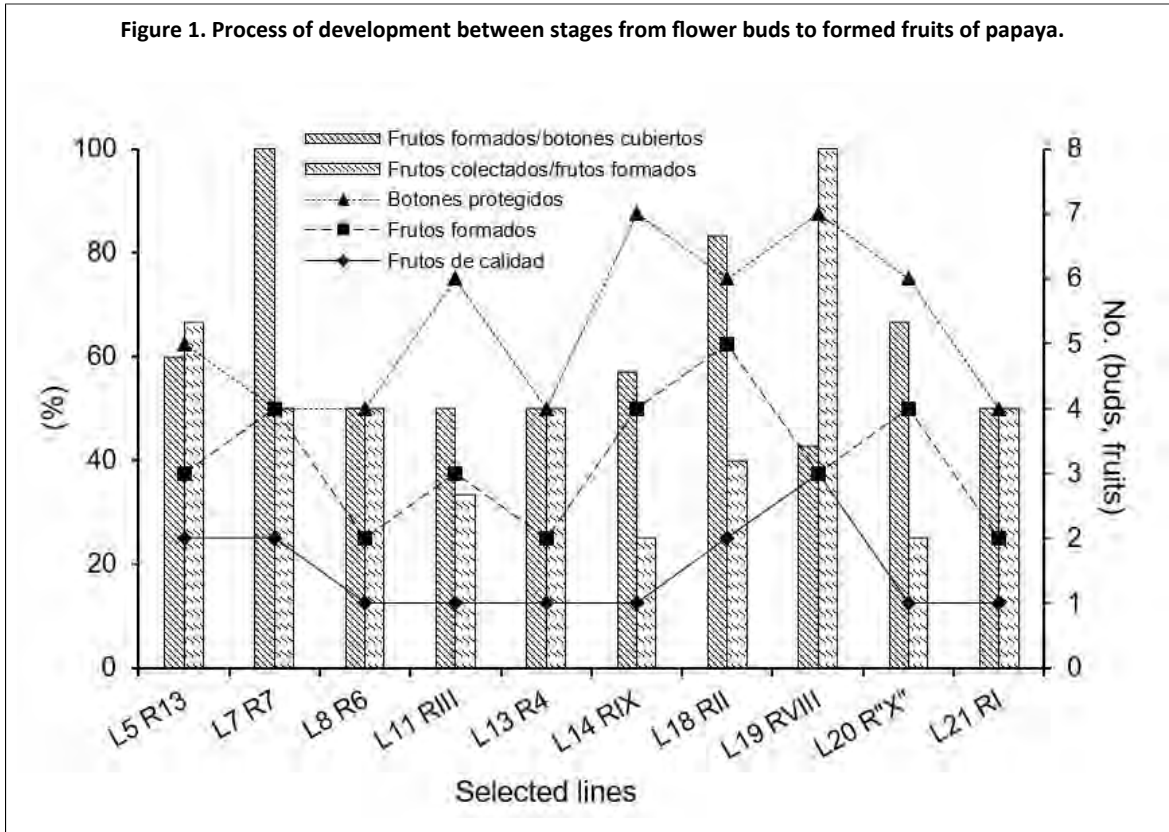
Id.	Plant height (dat)	Stem circumference (cm)	No. of leaves	Height to 1 <sup>st</sup> fruit (cm)
L1 R10	84.2 h	24.49 e	20 f	58 ef
L2 R9	110.8 efg	35.16 abcd	27.6 bcdef	55 f
L3 R 11	127.2 cdef	34.54 abcd	42 a	71.2 def
L4 R14	129.2 bcdef	33.91 abcd	31.6 bcde	68.8 def
L5 R13	128.6 cdef	34.54 abcd	31.4 bcde	71.2 def
L6 R8	131.4 bcde	35.79 abc	33.4 bcd	64.8 ef
L7 R7	140 bc	33.91 abcd	31.6 bcde	53 f
L8 R6	142.6 bc	36.42 ab	35.4 abc	69 def
L9 R5	133.2 bcd	32.02 abcd	32 bcde	93.6 bcd
L10 R12	143 bc	37.05 a	31 bcde	56.2 f
L11 RIII	113.6 defg	30.14 cde	32.8 bcde	74.8 cdef
L12 R3	130.2 bcdef	35.16 abcd	36.6 ab	85.6 bcde
L13 R4	134.2 bcd	33.28 abcd	30.4 bcde	100.6 bc
L14 RIX	114.6 defg	32.65 abcd	27 def	73.6 cdef
L15 RXI	194.8 a	37.05 a	26.6 def	134.4 a
L16 RIII	149.8 b	30.2 cde	25 ef	104.4 b
L17 RIV	129.6 bcdef	33.91 abcd	28.6 bcde	93.6 bcd
L18 RII	101.6 gh	29.51 de	26.8 def	64.6 ef
L19 RVIII	94.8 gh	30.77 bcd	26.2 def	60.6 ef
L20 RX	143.8 bc	32.65 abcd	27 def	100.6 bc
L21 RI	110.4 fg	32.65 abcd	28 bcdef	67.2 def
L22 R2	112 efg	32.65 abcd	27.2 cdef	51.4 f
L23 R1	111 efg	31.4 abcd	27 def	67 def
<i>P</i>	**	**	**	**
CV (%)	6.9	7.65	11.81	15.75

Means with equal letters are not statistically different (Tukey, 0.05), \*\* =  $p \leq 0.001$ ; CV= coefficient of variation.

Under this subsection, some lines evaluated showed marked advantages over the rest. The plants selected for propagation purposes must be vigorous, uniform, both in the size and shape of fruits,

these must be located at a low height, with tolerance to pests and diseases, among others (SNICS-SAGARPA, 2014). However, in order for the crop as a whole to achieve high yields and good fruit quality, several factors are involved, such as the genetic constitution of the cultivar, favorable soil and climatic conditions, efficient phytosanitary control, timely water supply, and correction of nutritional deficiencies (Santana *et al.*, 2019).

Only 10 lines presented plants with outstanding characteristics. And because these plants had their flower buds covered, the process of three stages was monitored: flower buds, formed fruits, and quality collected fruits. Thus, the number of fruits formed in relation to the covered buds decreased, and the number of fruits collected in relation to the fruits formed was also lower, due to the nature of the species, which influenced the process (Figure 1).



*Carica papaya* is generally propagated by seeds, so high plant heterogeneity is common (Bhattacharya and Khuspe, 2001). The variability of papaya genotypes is limited and there is a dependence on few alternatives of varieties and hybrids that do not meet the demands, this promotes that producers select generations F2 to F4 in continuous plantations, so the protection of flower buds is a common practice so as not to run the risk of loss of vigor and segregation (Marin *et al.*, 2006).

On the other hand, the comparison of means derived from the analyses of variance of the fruit characterization variables is shown in Table 3. As can be seen, except for the soluble solids variable, there were significant differences in all variables. The polar and equatorial circumference showed the largest fruit dimensions in the L14 RIX line, contrary values occurred in the L7 R7 line.



**Table 3. Characterization of the fruits collected in outstanding papaya plants.**

Line	Circumference (cm)		Weight (kg)	Pulp weight (cm)	Pulp firmness (kg cm <sup>-2</sup> )	Soluble solids (°Brix)
	Polar (P)	Equatorial (E)				
L5 R13	56.2 abc	31.8 ed	1.173 bc	2.74 bcd	2.14 ab	12.52
L7 R7	51 c	28.6 e	0.822 c	2.42 d	2.04 b	12.78
L8 R6	56.8 abc	34.6 bcd	1.239 bc	2.66 cd	2.2 ab	12.42
L11 RIII	62.4 a	27.2 abc	1.722 abc	3.1 abc	2.14 ab	12.34
L13 R4	61.2 ab	32.2 cde	1.297 bc	2.66 cd	2.2 ab	12.68
L14 RIX	62.2 a	40.2 a	1.73 abc	3.02 abc	2.08 b	12.38
L18 RII	61 ab	38.4 ab	1.806 ab	3.22 a	2.06 b	12.86
L19 RVIII	59.2 abc	38.8 ab	1.721 abc	3.16 ab	2.14 ab	12.6
L20 R"X"	53 bc	31 de	0.95 bc	2.74 bcd	2.08 b	12.16
L21 RI	61.8 a	38.8 ab	2.51 a	3.08 abc	2.32 a	12.1
P	**	**	**	**	**	ns
CV (%)	6.85	7.06	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; CV= coefficient of variation.

Fruit characteristics are important variables that allow the selection of genotypes (Oliveira *et al.*, 2012). The weight of the fruit was acceptable, ranging from 0.822 to 1 806 kg, and this in turn was reflected in the size of the fruit, width of the pulp, whose trend was similar. In addition, under the proposed scheme, the self-pollinated flowers of hermaphrodite plants, according to the flower proportion in papaya, are expected to produce offspring of 66.67% of hermaphrodite plants in a subsequent cycle (Santana *et al.*, 2019).

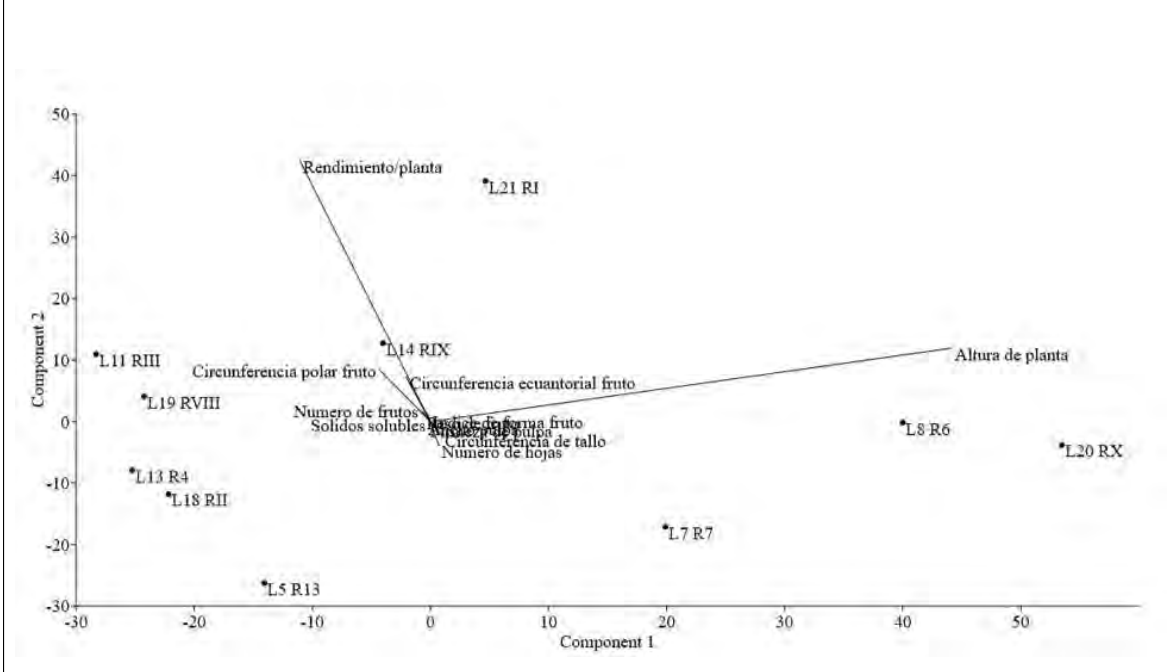
Regarding the multivariate analysis of the evaluated variables, it was interpreted from the eigenvalues, where the individual and cumulative variance in each component of the analysis are shown. The eigenvalue and variance of the correlation matrix are also shown. The values indicate the variability associated with each principal component, and it decreases as the number of components increases, showing cumulatively that the first component explains about 68% of the variability (Table 4).

**Table 4. Eigenvalues of plant and fruit characteristics and proportion of variability in outstanding papaya plants.**

Principal component	Variance-covariance matrix			Correlation matrix	
	Eigenvalue	Explained variance (%)	Cumulative variance (%)	Eigenvalue	Explained 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	0.995	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

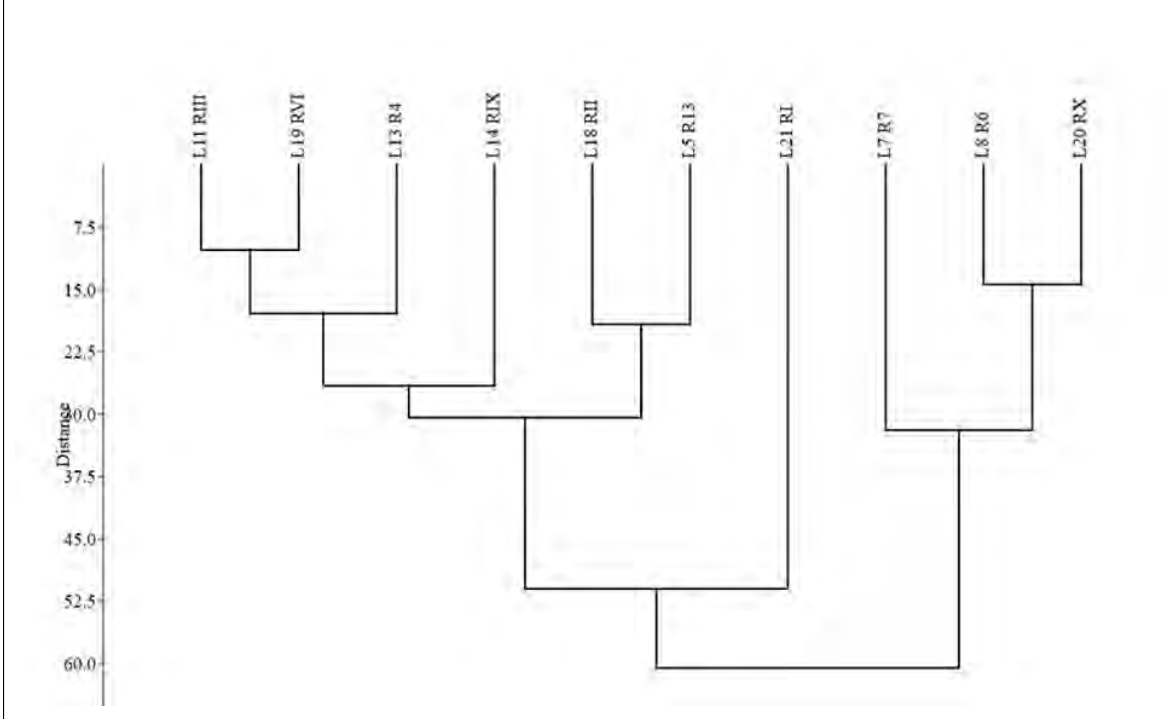
Likewise, the first two principal components explained the cumulative variability in the selected lines (Figure 2).

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



On the other hand, the cluster analysis was performed with the structures of the two principal components since they explained 96% of the variance and it facilitated the process of identifying groups of variants. The results showed that at a Euclidean distance of 20, three distinct 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 3).

Figure 3. Dendrogram of 12 variables of plants and fruits of selected lines, with three defined groups and three undefined lines at Euclidean distance of 20.



As already noted, with this information it was possible to identify three groups defined through the cluster analysis. Aikpokpodion (2012) evaluated 60 papaya materials using 21 variables defined based on descriptors, the multivariate analysis generated five groups, thus revealing a significant variation that can be exploited for papaya genetic improvement.

On the other hand, Saran *et al.* (2015) evaluated 24 papaya materials and 29 morphological characteristics. The multivariate analysis showed high morphological diversity in terms of fruit yield, weight, length, cavity, fruiting zone, pulp thickness, pulp color and soluble solids, whose response is similar to what was reported.

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

## Conclusions

The recorded values resemble the characteristics of the 'Maradol' type, whose variability allowed the identification of prospective materials. Of the 23 papaya lines evaluated, only 43.48% of the lines showed outstanding plants. Within the selected lines, only between 5 and 10% of the plants were chosen for presenting outstanding characteristics.

There was a gradual decrease in the progress of fruit development, of 60% in the stage of fruits formed over the stage of covered buds and of 47% in the stage of fruits collected over the stage of formed fruits, but in total only 28% of the fruits collected over the covered buds were recovered. The selected lines showed variability of fruit values, where fruit weight and pulp width were important indicators to define a mode preference. Multivariate analysis allowed us to classify three distinct groups.

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