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Genetic characterization by amplified fragment length polymorphism (AFLP) markers and morphochemical traits of *Carica papaya* L. genotypes

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***Carica papaya* L. is a native fruit from Central America and Mexico and it is an economically important fruit. As a pre-breeding genetic study, the variability of both parents (L7 and M22) and the F1 individuals derived from their crosses (L7 × M22), was evaluated in terms of 32 morphochemical traits, and contrasted with their genetic diversity indicated by amplified fragment length polymorphism (AFLP) markers. According to morphochemical traits, L7 and M22 were grouped in two different clades. The first group included L7 and 13 genotypes from the F1, while a second group included the parent M22 and 15 other genotypes from the F1 progeny. The analysis based on morphochemical traits showed an average correlation of 0.652 among genotypes. For AFLP analysis the combination of the primers E-ACA/M-CTA had the best polymorphic index (72.73%). When they were grouped based on AFLPs markers, it was confirmed that both parents are genetically distant, and they were again grouped in two different clades. Five genotypes from the F1 population were grouped in the same clade as L7 and shared 55% similarity. Twenty six genotypes were grouped in the same clade as M22, showing 63.3% similarity. Another 12 genotypes (mainly female genotypes) were grouped in a third independent clade. This relative general agreement between the grouping based on a large number of morphochemical traits (including both plant and fruit traits) and that based on its genetic diversity using AFLPs, suggests that morphochemical characterization, together with genetic analysis by AFLPs, can be complementary and useful techniques for the identification and assessment of genetic diversity within *C. papaya* L. genotypes, that should be useful for genetic breeding programs of this important species.**

Key words: Morphological markers, AFLP markers, genetic similarity, *Carica papaya* L.

INTRODUCTION

Carica papaya L. is grown in many tropical and sub-tropical countries and it has great economic value (Jobin-Décor et al., 1997). It originated in southern Mexico and Central America (Brown et al., 2012); and several wild populations have been detected in southern of Yucatan Peninsula in Mexico (Fuentes and Santamaría, 2014). *C. papaya* was about 70% dissimilar to other *Carica* species, which has had average dissimilarities around 50% (Jobin-

Décor et al., 1997). Wild papayas shows high contrast and variation in many morphochemical characters when compared to commercial genotypes, particularly in terms of leaf traits, type of flowers, size and shape of fruit, tolerance to pest and diseases (Ocampo et al., 2006). *C. papaya* L. plants develops fast; it has wide range of variability, and is extended all over America, with high seed production (Liu et al., 2004; Yu et al., 2008). Although, there are reports of collection, conservation and documentation of different accessions of papaya (Colunga and Zizumbo, 2004), studies related to genetic variability of this species in Mexico are very limited. The determination of genetic diversity using phenotypic and molecular tools in papaya, should be useful to understand the ability of these populations to adapt to their natural environment and to develop new cultivars (Moore, 2014). Molecular markers are a useful tool and have been used in the analysis of genetic diversity to facilitate genetic improvement of many crops, including *C. papaya* L. (Eustice et al., 2008). Different molecular techniques have been applied to the analysis of genetic diversity in papaya, including markers such as isoenzymes, RAPDs, AFLPs, ISSRs and SSRs (Kim et al., 2002; Esquivel et al., 2009; Oliveira et al., 2011; Madarbokus and Ranghoo-Sanmukhiya, 2012; Sudha et al., 2013; Vegas et al., 2013).

In particular, AFLP markers do not require prior genetic information; the technique process is faster, produces a large number of markers and is highly reproducible (Vos et al., 1995; Jones et al., 1997; Rojas et al., 2007). These markers are widely used in the assessment of genetic diversity, assessing genetic distance, DNA fingerprinting, analysis of germplasm collections, construction of genetic maps or saturation in certain areas of the genome (Mueller and Wolfenbarguer, 1999). In a previous study, a preliminary analysis of phenotypic variability was performed on genotypes of *C papaya,* using only a limited number (seven) of agronomical plant traits (Vázquez et al., 2014). In the present study, a genotype collected from a native population from undisturbed areas from Yucatan (L7) were crossed with a commercial genotype (M22), the genetic variability of both parents and their F1 progeny (L7 × M22), was characterized by AFLPs, and contrasted with their grouping when using 32 different morphochemical traits, that included both plant and fruit traits.

MATERIALS AND METHODS

Plant material

All plant material was grown in the germplasm bank in the Scientific Research Center of Yucatan (CICY), Mérida, Yucatan, Mexico. A wild genotype of *C. papaya* L. (L7) collected in undisturbed areas at southern of Yucatán Peninsula (Cancún, Quintana Roo), México, and the commercial M22 (maradol tpe) were selected as parents. Both genotypes are hermaphrodites but they have contrasting plant height, fruit size and pulp color characters. M22 are shorter plants bearing large red pulp fruits, while L7 are taller plants bearing small yellow pulp fruits. The F1 progeny derived from the crosses (L7 × M22), consisted in 43 individuals, 28 hermaphrodite plants, 14 female plants and one male plant.

Morphochemical characterization

Both parents and the F1 progeny were characterized and compared morphochemically. The morphological characterization was based upon UPOV (2010). 15 morphological characters and 17 physicochemical parameters were evaluated in L7, M22 and L7 × M22 individuals (Table 1). Fruit's pulp weight was measured with a granatary balance. Fruit's diameter and length parameters were measured with a graduated vernier (cm). Plant height and height of first fruit, were measured with a ruler (cm). pH was measured with a pH meter (Oakton, Singapur). Titratable acidity was measured with a Metrohom automatized system (Thermo Fisher Scientific Inc, USA). Total soluble solids or °Brix was measured using a digital refractometer (Gardco, Florida). Lycopene and β-carotene contents from fruit shells and pulps were measured following the protocol by Nagata and Yamashita (1992), using a DU6 spectrophotometer (Beckman Coulter, USA). CIELAB color components from shell and pulp were measured with a colorimeter by reflectance (Minolta, CR-200).

Molecular characterization

A fragment of leaves from the different individuals of *C. papaya* L. were sampled and placed in liquid nitrogen. DNA extraction was performed on freeze-dried leaf tissue using the CTAB method (Doyle and Doyle, 1990) with several modifications. DNA was quantified using a spectrophotometer NanoDrop (Thermo Fisher Scientific Inc. Wilmington USA) and visualized in agarose gel 1%. A dilution was performed at a final concentration of 100 ng DNA ng μL^{-1} for AFLP analysis. AFLP analysis was performed using the method reported by Vos et al. (1995) with some modifications. Digestion was performed from 100 ng μl^{-1} DNA with combination of EcoR1 (4U) and MseI (1U) enzymes. Ligation of complementary adapters was held by 1 unit of T4 ligase. After visualization with homogeneous intensity of digestion-ligation in agarose gel 1.5%, the samples were diluted in 1:5 ratios for uses in the pre-amplification. After that, the complementary primers EcoR1 and MseI with three selective nucleotides were applied. The simple product of PCR amplification was diluted 1:5, 1:10 and 1:50, according to the band intensity displayed. Selective amplification was carried out with the combination of EcoR1 and MseI primers with the presence of three selective bases each. The primer combinations were used as follows: E-AAC/M-CTT, E-AAC/M-CAC, E-AAC/M-CTA, E-ACG/M-CTT, E-ACG/M-CGA, E-ACT/M-CGA, E-ACT/M-CTT, E-ACC/M-CTA, E-AAG/M-CGC, E-ACA/M-CTA; from those, only the combinations E-ACT/M-CGA, E-ACC/M-CTA, E-ACT/M-CTT and E-ACA/M-CTA, offered better molecular information for the samples tested. The reaction mixture for pre-amplification was 20 µL per sample and it was amplified according to the following PCR conditions: 20 cycles of denaturation; 30 s at 94°C, 60 s annealing at 56°C, and 60 s of extension at 72°C. For selective amplification,

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Table 1. 32 morphochemical traits evaluated in *Carica papaya* L. (UPOV, 2010).

Plant		**Fruit**	
Plant height (cm)	AP	Fruit weight (g)	PF
Height of first fruit (cm)	APF	Fruit length (cm)	LF
Diameter of stem (cm)	DT	Diameter of fruit (cm)	DF
Length of petiole (cm)	LP	Pulp thickness (cm)	GP
Length of leaf (cm)	LH	Proportion of fruit length:diameter	LDF
Width of leaf (cm)	AH		
Proportion of leaf length:width	LAH		
Number of flowers per node	NFL		
Number of fruits per node	NFN		
Number of fruits per plant	NFP		
Physicochemical			
pH	pH	Color component *a* in shell	a-C
Acidity	AT	Color component *b* in shell	b-C
Total soluble solids	°B	Color component *C* in shell	C-C
Lycopene in shell	Li-C	Color component *h* in shell	h-C
β-carotene in shell	βC-C	Color component *L* in shell	L-C
Lycopene in fruit pulp	Li-P	Color component *a* in fruit pulp	a-P
β-carotene in fruit pulp	βC-P	Color component *b* in fruit pulp	b-P
		Color component *C* in fruit pulp	C-P
		Color component *h* in fruit pulp	h-P
		Color component *L* in fruit pulp	L-P

20µl of reaction mixture per sample was amplified with 13 cycles "touch down" of 94°C for 30 s, 65°C for 30 s with a decrease of 0.7°C per cycle, and 72°C for 2 min; followed by 30 cycles of annealing at 56°C for 30 s. For automated detection of AFLP, a dilution of product selective amplification (amplisel) was performed. 4 µL SLS and 2 µL of PCR product derived from the amplisel were placed in a plate with 45 wells with 25 µL of the mixture of SLS and STD 400 (molecular weight marker) subsequently, one drop of mineral oil was added to avoid the formation of bubbles. The plates were placed in the automated sequencer (Prism 310, Applied Biosystem) to detect AFLP markers by capillary electrophoresis. The detected fragments are shown as spikes, and the size of the detected peaks, were denoted. These markers were recorded as presence or absence for each genotype evaluated.

Statistical analysis

Morphochemical characterization

Data from 32 morphochemical traits were analyzed with the statistical package option NTSYS v2.1p running multivariate analysis (Crisci and Lopez, 1983). The variables were used for the construction of the correlation matrix. Cluster analysis was made on the program NTSYS pc v2.1p, based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA), in order to obtain the dendrogram based on the Ward method and Squared Euclidean distances.

Molecular characterization

The data generated from the detection of polymorphic fragments were analyzed. Specific amplification products were scored as present (1) or absent (0) for each DNA sample. Index of genetic similarity or distance was calculated (1-F); F values were initially calculated using Nei and Li (1979) matching coefficient method; $F = 2 \times N_{AB1}/(N_A + N_B)$, where N_A = the number of bands in accession A, N_B = the number of bands in accession B, N_{AB1} = the number of bands present in both accessions A and B (scored 1), N_{AB0} = the number of bands present upon amplification of some of the germplasm with this set of accessions, but not present in either accession A or B, and N_T = the total number of bands scored in the study. Later, F values were also calculated using the formulae: 1) $F' = N_{AB1}/(N_T - N_{AB0})$ (Jaccard's coefficient), 2) $F'' = (N_{AB1} + N_{AB0})/ N_T$ (similarity coefficient), 3) $F''' = N_{AB0}/(N_T - N_{AB1})$. An agglomerative method of clustering accessions was employed to analyze the data utilizing the UPGMA algorithm (SAS, 1985), that employs a contrasting method of classification based on a divisive clustering technique (Francisco-Ortega et al., 2000). Dendrogram was generated by UPGMA method using the similarity coefficient Dice from patterns generated by AFLPs.

RESULTS AND DISCUSSION

The F1 progeny from the cross (L7 × M22) and its parents, were evaluated both morphochemically and genetically (using AFLPs), to discriminate and identify genotypes for genetic improvement.

Morphochemical characterization

The 32 phenotypic (morphochemical traits) showed high

diversity among the evaluated papaya genotypes; these data can be used in the selection of different parents for improving this species. The Component 1, explained 33% of accumulated variance, the Component 2, explained 49% of accumulated variance and Component 3 explained 59% of the total variance from 32 morphochemical traits. The treats that show the high positive correlation for principal component (PC1) were b-P and c-P (0.89 and 0.83). For the PC2 they were Li-P and APF (both 0.56) and for the PC3 were LP (0.66), AH and LAH (both 0.61) (Table 2). The morphochemical markers formed two clear groups. A first group included L7 and 13 genotypes from the F1, and a second group included the parent M22 and 15 other genotypes from the F1 progeny (Figure 1). The F1 genotypes grouped with the parent L7, shared 55% similarity on average, being the genotype H66B, the one with the highest genetic similarity (0.688) with the parent L7. On the other hand, the genotypes grouped with the parent M22, shared on average of 63.3% similarity, and H90B genotype showed the highest genetic similarity (0.840) with M22 (Table 3). In relation to the lowest correlation of genetic similarity (0.281), the genotypes H15B, H70B and H90B had the greatest genetic distance from L7. With low genetic similarity correlation (0.130), the genotype H71B had relatively the highest genetic distance with M22.

Phenotypic similarity correlations analysis showed a 51.9% similarity, where H13B and H14B genotypes showed the highest degree of similarity (0.906), indicating that these genotypes share many of their phenotypic characters. The genotypes H66B and H12B showed the least similarity correlation (0.125) (Table 3). This indicates that among the genotypes of papaya evaluated, a contrasting morphological variability exists, which could serve as a source of genetic diversity for searching parents with desirable characteristics and it can be used in a breeding scheme to obtain new papaya varieties or genotypes adapted to the region.

Molecular characterization

The electropherograms from the four selected primer combinations of AFLP markers used to characterized papaya genotypes derived from the intraspecific crosses L7 × M22, showed a range of 22 to 74 fragments in E-ACA/M-CTA and E-ACT/M-CGA, respectively, and a total of 217 fragments with all combinations tested, with an average value of 54.48 DNA visualized fragments (Table 4 and Figure 3). The fragment sizes were in the range of 89 to 234 bp for E-ACA/M-CTA, 61 to 290 bp for E-ACT/M-CGA, 62 to 277 bp for E-ACC/M-CTA and 61 to 229 bp for E-ACT/MCTT. Monomorphic fragments showed a range from 6 to 36 fragments for E-ACA/M-CTA and E-ACT/M-CGA/E-ACT/M-CTT, respectively, with a total of 104 fragments, while polymorphic fragments showed a range from 16 to 42 fragments for E-ACA/M-CTA and E-ACC/M-CTA, respectively, with a total of 113 polymorphic fragments which represented a 54.48% polymorphism obtained with all combinations of evaluated individuals. Combinations with better percentage of polymorphic bands were E-ACA/M-CTA, E-ACC/M-CTA, E-ACT/M-CGA, with a value of 72.73, 61.74 and 51.35% of polymorphic fragments. The combination E-ACT/M-CTT, although generated one of the highest values of total fragments, had only 32.08% of polymorphic fragments (Table 4). This could be used in studies of genetic variability in other morphotypes of papaya, in order to support studies of morphological and genetic or accelerate breeding programs for the specie (Meerow, 2005; Esquivel et al., 2009).

Genetic variability among the 45 genotypes of *C. papaya* L. evaluated, was estimated by pairwise comparison of genetic similarity. The pairwise of genetic similarity showed a range of 0.35 to 0.84 (Figure 2), with an average genetic similarity of 0.639 within the population evaluated. The 81.6% of the pairwise comparison data showed a genetic similarity greater than 0.59. Cophenetic correlation values obtained from UPGMA cluster analysis and the genetic similarity matrix showed a correlation of 0.652 (Table 5). The molecular genetic similarity among all evaluated genotypes had correlation values that ranged from 0.35 to 0.84 suggesting that they are individuals with a narrow genetic similarity (Table 5). The generated dendrogram showed three groups at a distance of 0.715. The first group, was formed by six genotypes, including the parent L7. The second group was formed by 27 genotypes (20 hermaphrodite genotypes, six female and the parent M22) and the third group was formed by 12 genotypes, including 5 female genotypes and the male genotype (Figure 3). The analysis confirmed that the parents L7 and M22, that showed important phenotypical differences, also belong to a different genetic group. However, some genotypes from the F1 are genetically distant from both parents (L7 and M22). The *C. papaya* L. selection based on the progeny from complementary genetically distant parents maintains the genetic diversity, and it would allow the identification of superior progenies (“elite”) for commercial interest traits, such as pulp color and fruit size, towards pre-genetic improvement of the species. Oliveira et al. (2011), reported a value of average genetic distance of 0.735 in papaya genotypes of improved germplasm; similarly, Vegas et al. (2013) reported and classified as correlation of mean similarity of 0.899 in 28 accessions of *C. papaya* L. in Venezuelan germplasm; similar data were reported by Van-Droogenbroeck et al. (2002), who obtained a correlation of 0.873 of similarity in accessions of papaya from Ecuador. In turn, Janthasri et al. (2007) reported a correlation of 0.920 and similarity mention that these materials of papaya developed in Thailand have little genetic variability, perhaps because they were generated from the same materials of a germplasm bank. These reports support that the use of geographic provenance of plant material is important for

Table 2. Total variance in *Carica papaya* L. explained by principal component analyses. Correlations value for the different traits in the first three principal components (PC1, PC2, PC3).

Principal component	Eigen value	Explained proportion of variance (%)	
		Absolute	Accumulated
1	10.69	33	33
2	5.02	16	49
3	3.01	9	59
4	2.47	8	66
5	2.28	7	73
6	1.92	6	79
7	1.53	5	84
8	1.04	3	87
9	0.96	3	90
10	0.79	2	93
11	0.45	1	94
12	0.40	1	96
13	0.33	1	97
14	0.30	1	97
15	0.18	1	98
16	0.14	0	98
17	0.13	0	99
18	0.09	0	99
19	0.08	0	99
20	0.05	0	100

Trait	Principal components		
	PC1	PC2	PC3
AP	-0.37	0.45	0.27
APF	-0.54	0.56	0.28
DT	-0.15	-0.08	-0.20
LP	0.6	-0.17	0.66
LH	-0.21	-0.35	-0.65
AH	-0.44	-0.2	0.61
LAH	-0.66	0.3	0.61
NFL	0.40	-0.35	0.19
NFN	-0.66	0.11	-0.02
NFP	0.61	0.01	-0.25
PF	0.83	0.14	0.25
LF	-0.85	-0.13	0.12
DF	0.83	-0.04	0.23
GP	0.37	0.04	0.04
LDF	0.78	-0.05	0.29
pH	0.70	0.30	0.21
AT	-0.67	0.05	-0.02
°B	0.71	0.39	0.17
Li-C	0.92	0.12	0.08
_C-C	0.58	0.37	0.18
Li-P	-0.83	0.56	0.47
_C-P	-0.54	0.30	0.38
L-C	-0.15	-0.08	-0.20
a-C	0.79	-0.17	0.36
b-C	-0.21	-0.35	-0.15
C-C	-0.44	-0.20	0.14
h-C	-0.67	0.43	0.11

Table 2 contd.

L-P	0.40	-0.35	0.19
a-P	-0.89	0.11	-0.02
b-P	0.89	0.01	-0.25
c-P	0.83	0.14	0.25
h-P	-0.79	-0.13	0.12

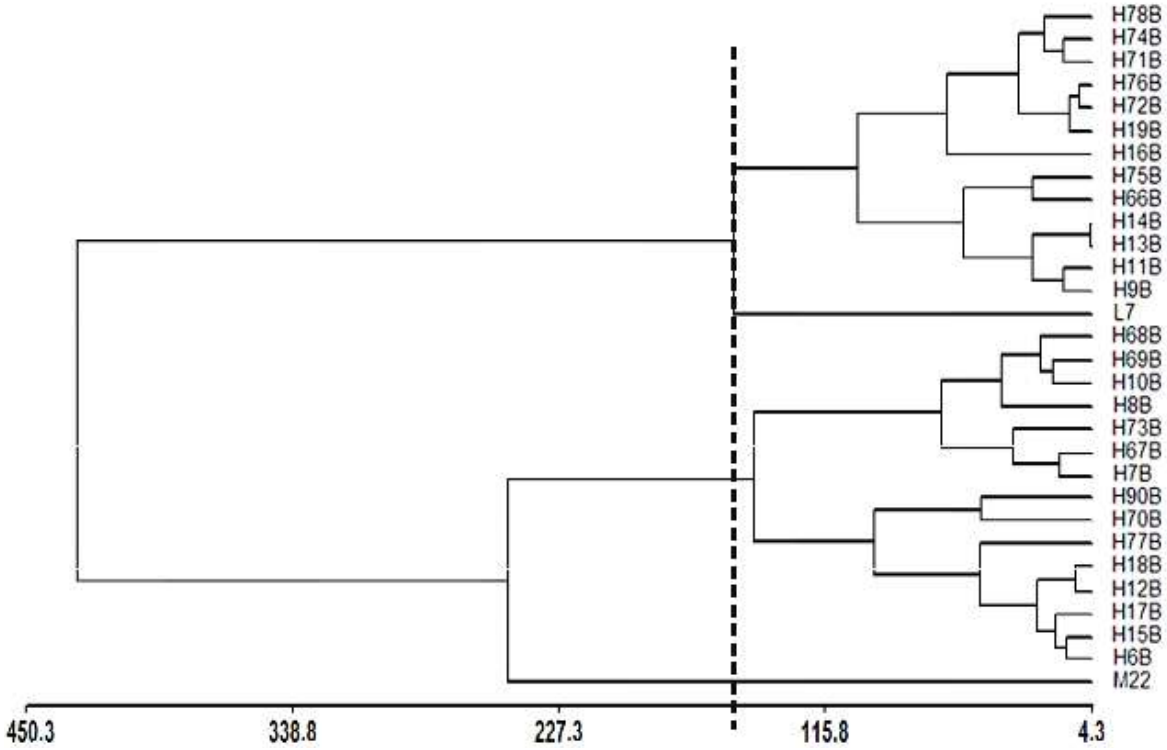

Figure 1. Dendogram of parents L7, M22 and their F1 progeny obtained from 32 morphochemical characters based on the Ward method and squared Euclidean distances.

the improvement of *C. papaya* L. Kim et al. (2002), obtained a similarity of 0.921 within Hawaiian accessions of papaya and 0.914 within Australian papayas; these exhibit little genetic diversity because they are materials that come from the same genetic pool. Brown et al. (2012), reported that the levels of genetic diversity in wild populations are higher than within cultivated (Commercial) papayas, which show heterozygote deficiency coupled with a high correlation of similarity between them. Regarding the usefulness of using AFLP, the combinations of primers of AFLP markers with better percentage of polymorphic bands were E-ACA/M-CTA, E-ACC/M-CTA and E-ACT/M-CGA, which may be employed in: 1) studies of genetic diversity in other morphotypes of papaya and 2) in studies searching for AFLP markers associated with other traits of economic interest which may in turn, accelerate breeding for the genetic improvement of this important species.

Relative agreement between the genetic and morphochemical characterization

Our data indicates that in general, the grouping of most genotypes coincides whether morphochemical or molecular markers are used. In the sense that both parents are clearly grouped in two different clades and some of the F1 genotypes from their progeny are grouped with either parent. Despite the fact that the grouping based on morphochemical traits formed two clear groups, while the grouping using AFLPs formed 3 groups, the lack of a third group when using morphochemical markers, was expected since no morphological data from the female or male individuals is available, because in the ongoing breeding

Table 3. Phenotypic similarity based on 32 morphological characters among both parents (L7 and M22) and the F1 progeny derived from intraspecific crosses (L7 × M22).

	L7	M22	H6B	H7B	H8B	H9B	H10B	H11B	H12B	H13B	H14B	H15B	H16B	H17B	H18B	H19B
L7	1.000															
M22	0.380	1.000														
H6B	0.469	0.660	1.000													
H7B	0.281	0.590	0.563	1.000												
H8B	0.563	0.480	0.594	0.531	1.000											
H9B	0.500	0.380	0.594	0.656	0.688	1.000										
H10B	0.563	0.480	0.656	0.656	0.688	0.625	1.000									
H11B	0.481	0.470	0.688	0.625	0.469	0.656	0.594	1.000								
H12B	0.375	0.750	0.656	0.594	0.500	0.375	0.438	0.406	1.000							
H13B	0.500	0.190	0.469	0.469	0.688	0.688	0.625	0.719	0.250	1.000						
H14B	0.488	0.160	0.438	0.438	0.719	0.719	0.594	0.625	0.281	0.906	1.000					
H15B	0.281	0.780	0.688	0.625	0.469	0.469	0.469	0.500	0.844	0.281	0.375	1.000				
H16B	0.688	0.310	0.406	0.469	0.563	0.563	0.500	0.406	0.438	0.563	0.656	0.406	1.000			
H17B	0.344	0.780	0.750	0.563	0.406	0.406	0.406	0.563	0.844	0.281	0.250	0.813	0.406	1.000		
H18B	0.406	0.660	0.688	0.500	0.469	0.344	0.531	0.500	0.844	0.281	0.375	0.813	0.531	0.750	1.000	
H19B	0.469	0.280	0.313	0.625	0.531	0.594	0.531	0.438	0.344	0.656	0.625	0.313	0.531	0.313	0.313	1.000
H66B	0.688	0.410	0.344	0.344	0.500	0.563	0.500	0.531	0.125	0.750	0.719	0.219	0.688	0.219	0.281	0.656
H67B	0.375	0.690	0.656	0.656	0.625	0.375	0.625	0.469	0.750	0.313	0.406	0.844	0.500	0.719	0.781	0.406
H68B	0.344	0.540	0.313	0.750	0.531	0.531	0.656	0.500	0.469	0.594	0.563	0.375	0.469	0.438	0.375	0.750
H69B	0.486	0.520	0.500	0.500	0.719	0.469	0.781	0.438	0.531	0.594	0.563	0.438	0.594	0.500	0.563	0.500
H70B	0.281	0.660	0.750	0.625	0.594	0.469	0.656	0.625	0.719	0.406	0.438	0.750	0.344	0.625	0.750	0.375
H71B	0.625	0.130	0.406	0.469	0.625	0.688	0.563	0.531	0.313	0.750	0.719	0.156	0.625	0.344	0.281	0.781
H72B	0.500	0.250	0.281	0.594	0.438	0.625	0.500	0.531	0.313	0.750	0.656	0.344	0.563	0.406	0.281	0.781
H73B	0.473	0.560	0.531	0.719	0.500	0.500	0.688	0.406	0.750	0.375	0.406	0.719	0.500	0.656	0.719	0.469
H74B	0.594	0.160	0.313	0.375	0.469	0.594	0.469	0.563	0.156	0.781	0.750	0.188	0.594	0.188	0.250	0.625
H75B	0.563	0.310	0.469	0.531	0.438	0.625	0.625	0.719	0.188	0.750	0.656	0.281	0.500	0.281	0.281	0.531
H76B	0.500	0.310	0.219	0.594	0.438	0.563	0.438	0.406	0.438	0.625	0.594	0.281	0.625	0.344	0.344	0.781
H77B	0.469	0.530	0.688	0.375	0.406	0.344	0.406	0.500	0.719	0.344	0.375	0.625	0.469	0.688	0.688	0.313
H78B	0.563	0.310	0.219	0.469	0.375	0.500	0.313	0.344	0.313	0.625	0.594	0.281	0.625	0.344	0.219	0.781
H90B	0.281	0.840	0.625	0.688	0.406	0.406	0.531	0.563	0.719	0.281	0.250	0.750	0.344	0.750	0.625	0.313
Máx.	0.688	0.840	0.750	0.750	0.719	0.719	0.781	0.719	0.844	0.906	0.750	0.844	0.688	0.750	0.781	0.781
Mín.	0.281	0.130	0.219	0.344	0.375	0.344	0.313	0.344	0.125	0.281	0.250	0.156	0.344	0.188	0.219	0.313
Prom.	0.466	0.471	0.512	0.555	0.530	0.529	0.541	0.513	0.493	0.545	0.525	0.477	0.524	0.473	0.450	0.576

Table 3. Contd.

	H66B	H67B	H68B	H69B	H70B	H71B	H72B	H73B	H74B	H75B	H76B	H77B	H78B	H90B
L7														
M22														
H6B														
H7B														
H8B														
H9B														
H10B														
H11B														
H12B														
H13B														
H14B														
H15B														
H16B														
H17B														
H18B														
H19B														
H66B	1.000													
H67B	0.250	1.000												
H68B	0.469	0.469	1.000											
H69B	0.469	0.594	0.688	1.000										
H70B	0.219	0.781	0.438	0.563	1.000									
H71B	0.688	0.250	0.719	0.594	0.281	1.000								
H72B	0.688	0.313	0.781	0.531	0.219	0.750	1.000							
H73B	0.313	0.688	0.656	0.656	0.531	0.438	0.563	1.000						
H74B	0.844	0.156	0.563	0.500	0.313	0.719	0.719	0.344	1.000					
H75B	0.813	0.250	0.594	0.469	0.344	0.625	0.688	0.438	0.844	1.000				
H76B	0.625	0.250	0.781	0.531	0.281	0.750	0.813	0.563	0.719	0.625	1.000			
H77B	0.344	0.531	0.375	0.438	0.688	0.469	0.281	0.469	0.438	0.406	0.406	1.000		
H78B	0.750	0.250	0.656	0.406	0.219	0.688	0.813	0.375	0.781	0.625	0.813	0.406	1.000	
H90B	0.156	0.719	0.500	0.500	0.750	0.219	0.344	0.594	0.188	0.344	0.406	0.625	0.281	1.000
Máx.	0.844	0.781	0.781	0.656	0.750	0.750	0.813	0.594	0.844	0.625	0.813	0.625	0.281	**Prom.**
Mín.	0.156	0.156	0.375	0.406	0.219	0.219	0.281	0.344	0.188	0.344	0.406	0.406	0.281	**gral.**
Prom.	0.510	0.438	0.614	0.519	0.403	0.582	0.603	0.464	0.594	0.500	0.542	0.516	0.281	0.512

Table 4. Combinations of primers used in obtaining DNA fingerprinting and distribution of total fragments, monomorphic and polymorphic parental and F1 progeny from *Carica papaya* L. L7 × M22.

Combination of AFLP	Number of total bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphic bands (%)
E-ACA/M-CTA	22	6	16	72.73
E-ACT/M-CGA	74	36	38	51.35
E-ACC/M-CTA	68	26	42	61.74
E-ACT/M-CTT	53	36	17	32.08
Total	217	104	113	54.48

program the male or female individuals were excluded from the field trials, as commercial papaya growers preferred hermaphrodites. It can be then concluded, that the molecular tool based on AFLP was efficient to detect high degree of polymorphism in a *C. papaya* L F1 population derived from the cross (L7 × M22), through the

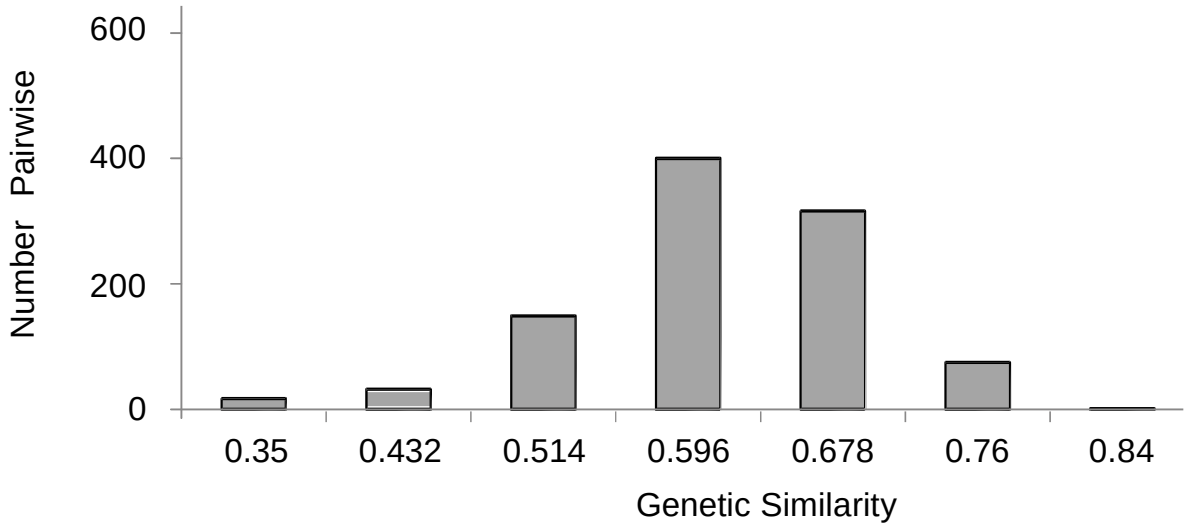

Figure 2. Distribution of data obtained by pairwise comparison of genetic similarity among 45 genotypes of *Carica papaya* L.

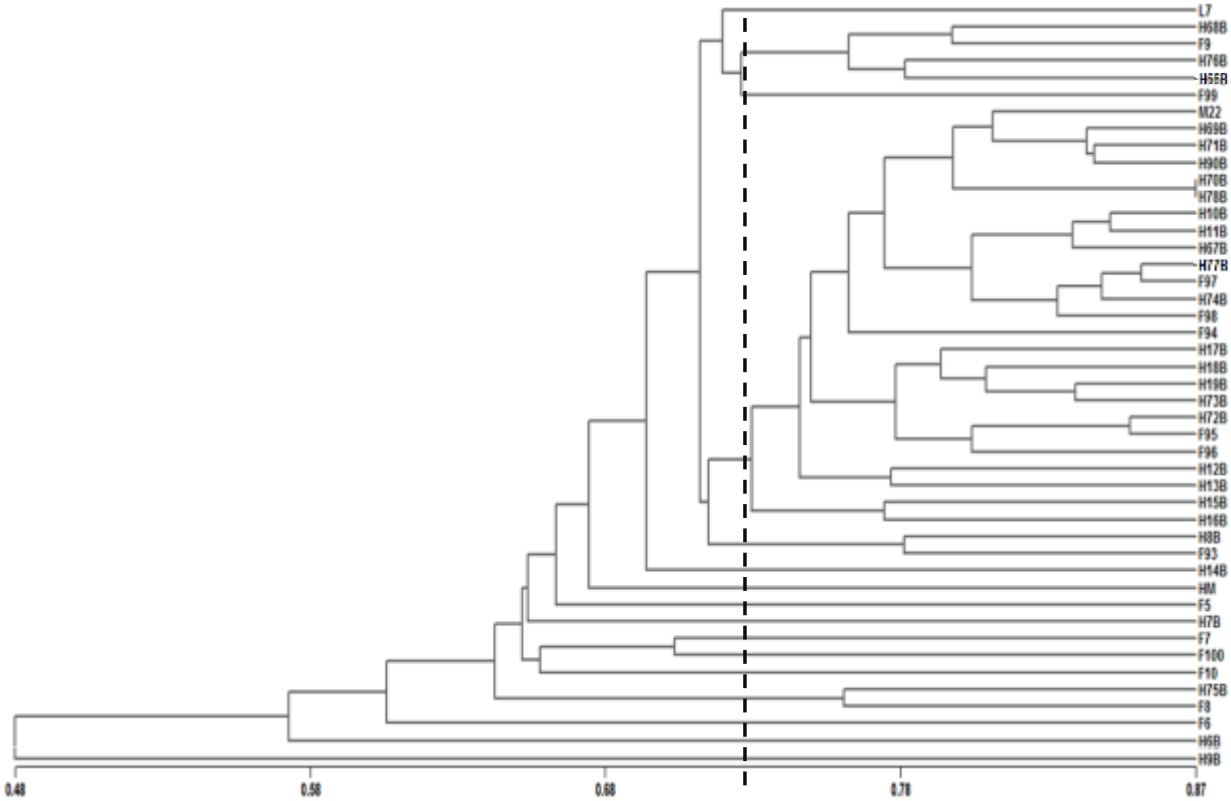

Figure 3. Dendrogram of parents L7, M22 and their F1 progeny, generated by UPGMA method using the similarity coefficient Dice from patterns generated by fragments for four combinations of AFLPs primers.

combinations E-ACA/M-CTA, E-ACC/M-CTA and E-ACT/M-CGA. The average molecular genetic similarity for L7 was 0.669 and for M22 it was 0.704.

The F1 segregated and some of them were grouped with M22, while some others were grouped with L7. A medium to high molecular genetic variation exists, associated with

Table 5. Genetic similarity of parental and F1 progeny from intraspecific crosses of *Carica papaya* L. L7 × M22 based on AFLP markers.

	L7	M22	H6B	H7B	H8B	H9B	H10B	H11B	H12B	H13B	H14B	H15B	H16B	H17B	H18B	H19B	H66B	H67B	H68B	H69B	H70B	H71B
L7	1.000																					
M22	0.620	1.000																				
H6B	0.510	0.540	1.000																			
H7B	0.600	0.620	0.620	1.000																		
H8B	0.630	0.750	0.640	0.730	1.000																	
H9B	0.540	0.510	0.460	0.480	0.580	1.000																
H10B	0.680	0.750	0.580	0.670	0.740	0.470	1.000															
H11B	0.640	0.651	0.600	0.630	0.700	0.420	0.820	1.000														
H12B	0.650	0.641	0.580	0.670	0.680	0.410	0.720	0.770	1.000													
H13B	0.670	0.680	0.590	0.580	0.660	0.460	0.740	0.730	0.770	1.000												
H14B	0.680	0.614	0.580	0.600	0.650	0.470	0.620	0.610	0.620	0.710	1.000											
H15B	0.640	0.720	0.590	0.650	0.680	0.440	0.680	0.690	0.700	0.690	0.740	1.000										
H16B	0.650	0.700	0.550	0.670	0.730	0.440	0.660	0.660	0.710	0.630	0.670	0.760	1.000									
H17B	0.660	0.670	0.550	0.680	0.630	0.360	0.740	0.720	0.760	0.690	0.660	0.750	0.730	1.000								
H18B	0.650	0.670	0.550	0.670	0.660	0.430	0.700	0.660	0.730	0.730	0.700	0.710	0.670	0.750	1.000							
H19B	0.580	0.621	0.590	0.620	0.660	0.410	0.670	0.690	0.740	0.650	0.670	0.720	0.700	0.720	0.780	1.000						
H66B	0.694	0.615	0.560	0.590	0.630	0.400	0.760	0.820	0.690	0.730	0.670	0.660	0.650	0.720	0.720	0.700	1.000					
H67B	0.660	0.670	0.610	0.640	0.720	0.460	0.830	0.800	0.730	0.740	0.660	0.660	0.670	0.720	0.690	0.660	0.760	1.000				
H68B	0.690	0.611	0.610	0.610	0.640	0.420	0.720	0.680	0.630	0.730	0.730	0.680	0.650	0.670	0.670	0.660	0.700	0.730	1.000			
H69B	0.630	0.740	0.540	0.610	0.720	0.410	0.740	0.770	0.660	0.660	0.610	0.700	0.640	0.690	0.620	0.620	0.730	0.710	0.720	1.000		
H70B	0.650	0.760	0.550	0.620	0.690	0.430	0.680	0.660	0.690	0.650	0.640	0.740	0.720	0.680	0.670	0.660	0.620	0.690	0.750	0.710	1.000	
H71B	0.660	0.740	0.560	0.600	0.680	0.420	0.750	0.780	0.700	0.700	0.650	0.750	0.700	0.760	0.680	0.660	0.770	0.740	0.690	0.790	0.710	1.000
H72B	0.640	0.700	0.520	0.620	0.660	0.350	0.740	0.750	0.770	0.740	0.650	0.710	0.690	0.800	0.730	0.750	0.770	0.730	0.670	0.700	0.670	0.770
H73B	0.620	0.660	0.530	0.640	0.660	0.390	0.700	0.710	0.770	0.700	0.660	0.740	0.720	0.780	0.800	0.820	0.740	0.700	0.690	0.650	0.680	0.750
H74B	0.660	0.720	0.540	0.620	0.690	0.380	0.780	0.780	0.700	0.680	0.630	0.680	0.670	0.730	0.700	0.740	0.820	0.750	0.730	0.750	0.660	0.750
H75B	0.670	0.610	0.590	0.600	0.640	0.520	0.640	0.590	0.630	0.640	0.580	0.580	0.570	0.560	0.590	0.610	0.550	0.640	0.620	0.560	0.590	0.570
H76B	0.710	0.616	0.580	0.630	0.670	0.480	0.710	0.640	0.630	0.740	0.690	0.660	0.640	0.660	0.640	0.590	0.680	0.690	0.760	0.730	0.660	0.640
H77B	0.610	0.710	0.580	0.690	0.750	0.550	0.690	0.660	0.660	0.680	0.650	0.670	0.670	0.650	0.650	0.600	0.660	0.680	0.720	0.710	0.680	0.670
H78B	0.650	0.770	0.560	0.650	0.740	0.440	0.750	0.740	0.670	0.630	0.610	0.730	0.750	0.740	0.630	0.600	0.700	0.750	0.720	0.800	0.830	0.780
H90B	0.640	0.750	0.550	0.570	0.640	0.370	0.740	0.750	0.700	0.700	0.650	0.770	0.670	0.790	0.680	0.690	0.750	0.720	0.720	0.780	0.700	0.810
F5	0.650	0.620	0.450	0.560	0.610	0.480	0.590	0.580	0.640	0.640	0.630	0.650	0.630	0.650	0.660	0.650	0.560	0.570	0.600	0.600	0.630	0.610
F6	0.660	0.530	0.450	0.500	0.500	0.390	0.540	0.520	0.620	0.620	0.630	0.530	0.600	0.660	0.650	0.610	0.590	0.560	0.590	0.530	0.480	0.580
F7	0.640	0.617	0.520	0.590	0.650	0.550	0.650	0.620	0.600	0.630	0.630	0.650	0.640	0.630	0.600	0.640	0.670	0.660	0.630	0.610	0.610	0.600
F8	0.630	0.610	0.590	0.590	0.680	0.530	0.630	0.610	0.610	0.680	0.680	0.620	0.550	0.570	0.630	0.650	0.610	0.680	0.660	0.580	0.590	0.570
F9	0.710	0.617	0.580	0.610	0.690	0.460	0.690	0.660	0.620	0.690	0.660	0.640	0.640	0.670	0.640	0.600	0.670	0.740	0.790	0.750	0.710	0.700
F10	0.600	0.618	0.450	0.640	0.680	0.570	0.560	0.560	0.570	0.560	0.600	0.620	0.650	0.570	0.570	0.590	0.580	0.590	0.630	0.630	0.650	0.590
F93	0.689	0.760	0.570	0.670	0.780	0.520	0.680	0.620	0.630	0.690	0.660	0.700	0.700	0.600	0.630	0.590	0.600	0.650	0.680	0.680	0.710	0.690
F94	0.670	0.690	0.540	0.560	0.670	0.390	0.720	0.750	0.680	0.730	0.620	0.670	0.630	0.730	0.640	0.660	0.710	0.710	0.690	0.720	0.670	0.740
F95	0.670	0.700	0.490	0.550	0.660	0.380	0.730	0.700	0.730	0.720	0.660	0.710	0.660	0.770	0.730	0.720	0.740	0.730	0.690	0.710	0.670	0.750
F96	0.660	0.640	0.490	0.570	0.620	0.410	0.650	0.660	0.690	0.680	0.650	0.690	0.690	0.690	0.730	0.750	0.700	0.660	0.650	0.600	0.640	0.670
F97	0.650	0.660	0.530	0.540	0.610	0.360	0.720	0.780	0.680	0.680	0.630	0.700	0.640	0.730	0.640	0.700	0.800	0.740	0.710	0.810	0.680	0.780
F98	0.680	0.720	0.590	0.620	0.700	0.440	0.770	0.750	0.700	0.740	0.660	0.650	0.650	0.720	0.680	0.660	0.790	0.790	0.740	0.710	0.700	0.740
F99	0.690	0.600	0.560	0.590	0.690	0.480	0.680	0.690	0.690	0.700	0.600	0.690	0.640	0.690	0.630	0.690	0.670	0.680	0.720	0.680	0.700	0.680
F100	0.610	0.610	0.540	0.580	0.650	0.570	0.580	0.540	0.570	0.610	0.590	0.590	0.570	0.580	0.550	0.590	0.580	0.630	0.640	0.580	0.620	0.590
HM	0.680	0.660	0.490	0.610	0.670	0.470	0.650	0.620	0.640	0.650	0.640	0.660	0.660	0.630	0.720	0.700	0.650	0.600	0.590	0.590	0.590	0.630
Max.	0.710	0.770	0.640	0.730	0.780	0.570	0.830	0.820	0.770	0.740	0.740	0.770	0.750	0.800	0.800	0.820	0.820	0.790	0.790	0.810	0.830	0.810
Min.	0.510	0.510	0.450	0.480	0.500	0.350	0.540	0.520	0.570	0.560	0.580	0.530	0.550	0.560	0.550	0.590	0.550	0.560	0.590	0.530	0.480	0.570
Prom.	0.647	0.662	0.552	0.613	0.669	0.444	0.695	0.684	0.676	0.681	0.649	0.680	0.658	0.687	0.665	0.661	0.685	0.686	0.685	0.678	0.660	0.681

Table 5. Contd.

	H72B	H73B	H74B	H75B	H76B	H77B	H78B	H90B	F5	F6	F7	F8	F9	F10	F93	F94	F95	F96	F97	F98	F99	F100	HM
L7																							
M22																							
H6B																							
H7B																							
H8B																							
H9B																							
H10B																							
H11B																							
H12B																							
H13B																							
H14B																							
H15B																							
H16B																							
H17B																							
H18B																							
H19B																							
H66B																							
H67B																							
H68B																							
H69B																							
H70B																							
H71B																							
H72B	1.000																						
H73B	0.790	1.000																					
H74B	0.760	0.730	1.000																				
H75B	0.580	0.540	0.600	1.000																			
H76B	0.640	0.600	0.670	0.650	1.000																		
H77B	0.650	0.600	0.680	0.710	0.770	1.000																	
H78B	0.680	0.660	0.770	0.560	0.700	0.740	1.000																
H90B	0.760	0.730	0.790	0.580	0.690	0.630	0.770	1.000															
F5	0.690	0.670	0.620	0.640	0.590	0.680	0.600	0.640	1.000														
F6	0.650	0.650	0.590	0.500	0.610	0.550	0.490	0.590	0.610	1.000													
F7	0.610	0.650	0.700	0.580	0.660	0.670	0.650	0.630	0.610	0.590	1.000												
F8	0.570	0.580	0.640	0.760	0.680	0.730	0.580	0.580	0.620	0.500	0.670	1.000											
F9	0.680	0.630	0.710	0.650	0.760	0.750	0.760	0.710	0.620	0.610	0.660	0.690	1.000										
F10	0.580	0.590	0.620	0.550	0.670	0.760	0.650	0.600	0.620	0.500	0.670	0.640	0.700	1.000									
F93	0.620	0.620	0.680	0.650	0.690	0.760	0.750	0.670	0.670	0.560	0.660	0.650	0.740	0.700	1.000								
F94	0.790	0.710	0.740	0.610	0.670	0.710	0.700	0.760	0.700	0.620	0.620	0.610	0.680	0.600	0.710	1.000							
F95	0.840	0.780	0.770	0.530	0.650	0.650	0.700	0.770	0.680	0.670	0.650	0.580	0.690	0.620	0.680	0.780	1.000						
F96	0.770	0.780	0.710	0.580	0.620	0.620	0.630	0.670	0.690	0.700	0.680	0.600	0.650	0.620	0.660	0.710	0.790	1.000					
F97	0.720	0.720	0.790	0.520	0.650	0.640	0.720	0.770	0.590	0.610	0.670	0.580	0.650	0.580	0.610	0.740	0.770	0.680	1.000				
F98	0.750	0.730	0.800	0.590	0.700	0.690	0.730	0.740	0.600	0.570	0.690	0.640	0.720	0.580	0.670	0.710	0.760	0.700	0.760	1.000			
F99	0.680	0.660	0.670	0.670	0.720	0.740	0.710	0.720	0.640	0.610	0.600	0.620	0.700	0.610	0.690	0.720	0.700	0.660	0.650	0.700	1.000		
F100	0.600	0.560	0.600	0.590	0.640	0.670	0.590	0.620	0.580	0.560	0.690	0.630	0.700	0.670	0.620	0.570	0.610	0.590	0.550	0.640	0.670	1.000	
HM	0.660	0.700	0.630	0.590	0.630	0.660	0.590	0.620	0.660	0.660	0.610	0.560	0.620	0.580	0.650	0.660	0.660	0.730	0.600	0.650	0.630	0.590	1.000
Max.	0.840	0.780	0.800	0.760	0.770	0.760	0.770	0.770	0.700	0.700	0.690	0.690	0.740	0.700	0.710	0.780	0.790	0.730	0.760	0.700	0.670	0.590	**Prom**
Min.	0.570	0.540	0.590	0.500	0.590	0.550	0.490	0.580	0.580	0.500	0.600	0.560	0.620	0.580	0.610	0.570	0.610	0.590	0.550	0.640	0.630	0.590	**gral.**
Prom.	0.685	0.661	0.689	0.606	0.672	0.685	0.664	0.673	0.635	0.597	0.656	0.618	0.685	0.618	0.661	0.699	0.715	0.672	0.640	0.663	0.650	0.590	0.652

an equivalent variability defined morphochemically. The existence of such correlation between the markers with the grouping based on 32 morphochemical traits, should favor the search of new QTL associated with morphological characters within F2 or backcross populations of *C. papaya* L. In addition, the detected genetic variability can be also useful in: a) the selection of distant or complementary elite genotypes that could in turn, generate F2 populations or backcrosses, to maintain variability of the species. b) as a basis to find new parents with features usable in breeding schemes, seeking new varieties with better adaptation to the regional environments.

Conflict of interests

The authors have not declared any conflict of interest.

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