

Article

Selection of Habanero Pepper F1 Hybrids (*Capsicum chinense* Jacq.) at the Yucatan Peninsula, Mexico with a High Potential for Different Markets

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Abstract: This study evaluated 29 F1 lines and the 11 genotypes of habanero peppers used in the crossbreeding program developed by the Scientific Research Center of Yucatan, México. A randomized complete block design with four repetitions was used. Eight plants of each of the genotypes were studied per block. A total of 22 qualitative and 18 quantitative descriptors established in the manuals of the International Plant Genetic Resources Institute (IPGRI) and the National Service for Seed Inspection and Certification (SNICS) was used. The multiple correspondence analysis of the qualitative traits explained 38.2% of the total variability. The trait that contributed the most to the qualitative variability identified was the presence of anthocyanins in the node. Principal component analysis showed that the first two axes explained 85.1% of the total variability and that capsaicin content and fruit pericarp thickness were the major contributors to the variation recorded. Based on these results, four F1 hybrids of habanero pepper were selected because of their promising traits for the different markets, i.e., high productive potential and/or high pungency. These traits are described in the section on Results.

Keywords: diversity; hybrid; pungency; habanero pepper

1. Introduction

All peppers belong to the *Capsicum* genus, which includes around 42 species with broad diversity in the shape, color, and size of the fruit, and in the sensory attributes such as taste, aroma and hotness [1], which make them stand out from the rest of plants used as spices. Only five of these species have been domesticated and cultivated: *C. annuum* L., *C. frutescens* L., *C. baccatum* L., *C. pubescens*, and *C. chinense* Jacq. [2]. Besides being used fresh and in a broad variety of dishes in international cuisine, chili peppers are a raw material of many industries; among them the food industry (powder, pastas, sauces), the military (self-defense sprays, projectiles), pharmaceutical (creams, sprays, patches, ointments), and chemical (protective coatings for electrical wires and as an additive in ship paints) [3–5]. Habanero

pepper is widely cultivated in the Yucatan peninsula, a region in Mexico acknowledged as the center of genetic diversity of the species (*Capsicum chinense* Jacq.). Its aroma, taste and hotness, set it apart from the habanero peppers grown in any other part of the world. In 2010, these attributes earned it the denomination of origin “Habanero pepper of the Yucatan peninsula” [6]. Habanero pepper pungency is classified between 100,000 and 300,000 Scoville Heat Units (SHU) [7]. However, the native varieties of southeastern Mexico are considered among the hottest in the world, with pungency ranging between 145,950 and 892,719 SHU [8], probably due to the edaphoclimatic conditions of the region.

Recently, Muñoz-Ramírez et al. [9] evaluated the pungency of ‘Bhut Jolokia’, ‘Trinidad Moruga Scorpion’ and ‘Carolina Reaper’ peppers cultivated at Yucatan, Mexico, which have been published in the Guinness Book of World Records at different periods of time as the hottest peppers in the world [10,11], and the three varieties significantly surpassed the pungency for which they were originally acknowledged. The ‘Carolina Reaper’, grown in Yucatán and regarded as the hottest variety in the world with 2,200,000 SHU, reached 3,006,330 SHU. This allows the inference that in the region of the Yucatan peninsula some of the attributes of habanero pepper, particularly hotness, may be exacerbated. Despite the economic, social and cultural importance of the crop, no improved varieties of habanero peppers had been developed until recently to increase fruit yield and quality while preserving the traits that distinguish Yucatan’s habanero pepper from those grown in other parts of the world.

Paradoxically, the yield of the native varieties of habanero pepper are low, fruits are small and heterogeneous, and fruits from the same plant may present different numbers of locules (2–4). For the last 15 years, special attention has been paid to the genetic improvement and technological development of the habanero pepper in southeastern México. Pungency is one of the most important traits considered in the improvement efforts that have been conducted. Canto-Flick et al. [8] worked with a collection of native habanero peppers and determined the capsaicinoids content of 18 accessions collected at the Yucatan peninsula. Whole fruit, placenta, and pericarp total capsaicinoids contents were determined by high-performance liquid chromatography (HPLC). The 18 accessions of habanero peppers assessed had a great variety of colors, shapes and sizes of the fruits. The results showed that 83.3% of the collection exceeded the pungency levels reported for habanero peppers [11] from other regions of the world. Interestingly, 33% of the accessions surpassed 500,000 SHU and 44.4% recorded above 600,000 SHU. A pungency level of 892,719 SHU was recorded in the whole fruit of accession NP1EG (yellow habanero), and the pericarp of accession NP3EC (orange habanero) reached 1,382,889 SHU. The pungency levels of both cultivars (NP1EG and NP3EC) were like those of ‘Bhut Jolokia’, but their shape and color were noticeably different, sharing the physical traits of the habanero peppers typical of the region.

In 2018, Santana-Buzzy et al. [12] presented ‘Mayan Kisin’, a high-yielding, red-fruited habanero hot pepper variety (*C. chinense* Jacq.). It is characterized by its high performance, and its bright red and very spicy fruits. ‘Mayan Kisin’ was the result of four selection cycles carried out on landraces collected in the region of Valladolid, in the state of Yucatan, Mexico. A study by Peña-Yam et al. [13] estimated the genetic parameters of seven agronomic characteristics for 11 habanero pepper genotypes. The aim of the study was to select potential progenitors to produce F1 hybrids. They found high values of the phenotypic coefficient of variation (PCV) of capsaicin content (CC). Heritability (h^2) was high (0.98) for yield per plant (YP) and CC (0.93). The principal component analysis (PCA) revealed that the first three components explained 94.02% of the total variation; hence genotypes with high yield and high fruit weight (FW) were obtained (AKN-08, ASBC-09). The genotypes with a greater content of capsaicin were MBI-11 and RES-05. The fruit of RNJ-04 had the greatest length. The greatest number of fruits per plant belonged to genotypes NBA-06, RKI-01, RHC-02, RHN-03, NKA-07, and MSB-12. Thus, the studied genotypes were found to be an excellent source of genetic material for habanero pepper improvement programs. Knowledge of the reproductive biology of a species is crucial to develop an efficient program of genetic improvement by hybridization. Peña-Yam et al. [14] conducted the first reported study on the floral biology of the habanero pepper. The aim of the study was to establish the best times to collect pollen, assess its viability and define the development state of the flower bud in

which the anthers are closed and stigma is receptive. The goal was to use the acquired knowledge in the implementation of the crossbreeding program. The objective of the present study was to select, from among the diversity of habanero peppers in the region, F1 hybrids of high productivity, hotness, and potential for both the fresh and the industrial markets.

2. Materials and Methods

A total of 29 F1 lines obtained by a Top Cross design were evaluated; 11 improved varieties of habanero pepper that were used as progenitors in the breeding program were also assessed (Figure 1). The improved varieties were obtained within the habanero pepper genetic improvement program developed in the Scientific Research Center of Yucatan (CICY) and are registered in the National Catalogue of Vegetables Varieties (CNVV).

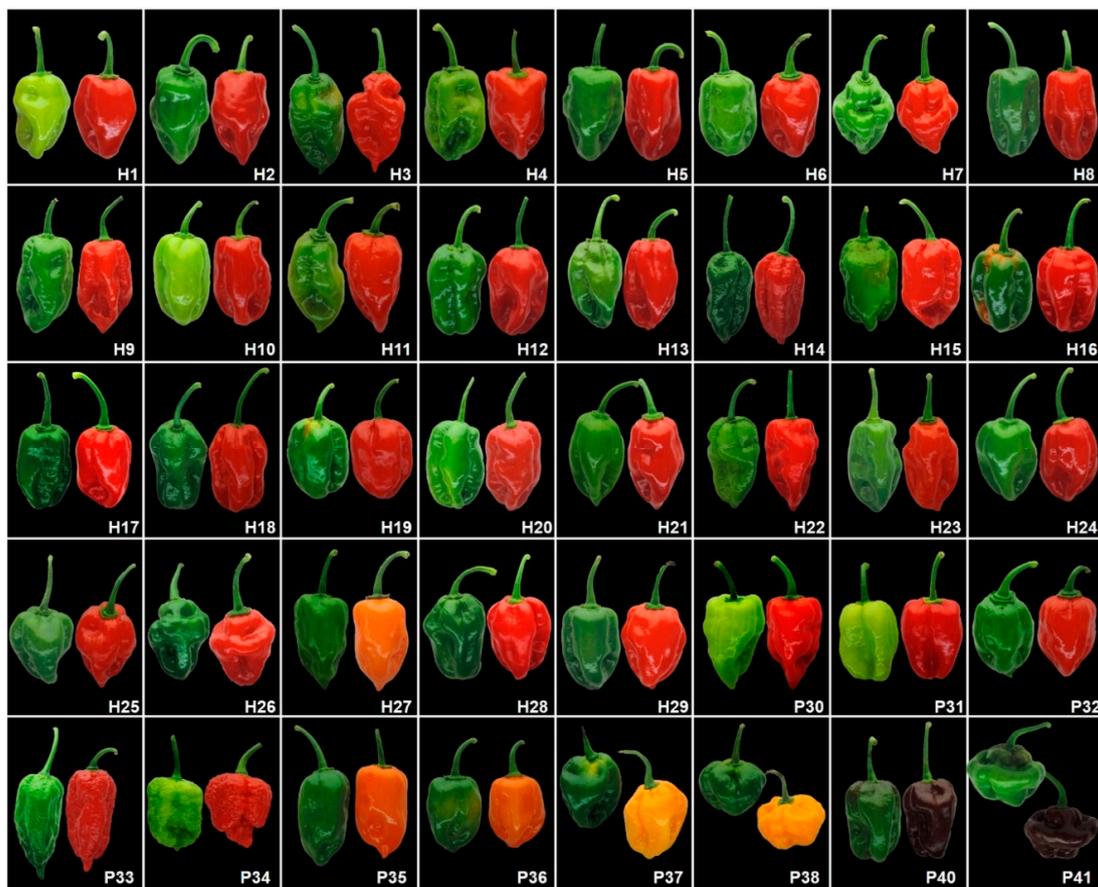


Figure 1. Evaluated F1 lines and the progenitors of habanero peppers. (Numbers H1–H29 correspond to F1 lines, and numbers P30–P41 correspond to progenitors).

A randomized complete blocks experimental design with four repetitions was used, assessing 10 fruits per replica. The plants grew under greenhouse conditions in the Scientific and Technological Park of Yucatan, located in Sierra Papacal, Merida, Yucatan at 21°07'20" N 89°43'41" O, at an altitude of 9 m above sea level, in the period between June 2018 and March 2019. Plants were transplanted into 1 m long bags (Pelemix, Guadalajara, México). The substrate was a thick and fine mix of coconut fiber in a 70:30 proportion; the distance between plants and rows was 20 cm and 160 cm, respectively. Total of eight plants of each genotype were randomly selected from each block and 22 qualitative and 18 quantitative traits were evaluated using the descriptors referenced in the IPGRI Manual [15] for *Capsicum*, and the SNICS Manual [16] for habanero pepper (*C. chinense* Jacq.) (Table 1).

Table 1. Qualitative and quantitative traits used to evaluate F1 lines and progenitors of habanero peppers, according to the IPGRI Manual (1995) and the SNICS Manual (2015).

Acronym	Descriptors	Unit/Scores
Qualitative descriptors		
NFA	Number of flowers per axil (IPGRI)	1: one, 2: two, 3: three or more, 4: many flowers in bunches
FP	Flower position (IPGRI)	3: pendant, 5: intermediate, 7: erect
CCO	Corolla color (IPGRI)	1: white, 2: Light yellow, 3: Yellow, 4: Yellow-green, 5: Purple with white base, 6: White with purple base, 7: White with purple margin, 8: Purple, 9: other
CA	Color of anthers (SNICS)	3: violet, 5: violet blue, 7: blue
FC	Filament color (SNICS)	1: light green, 2: yellow green, 3: light blue violet, 4: violet, 5: blue
STE	Stigma exertion (IPGRI)	3: inserted, 5: same level, 7: exerted
ACN	Anthocyanin coloration of nodes (SNICS)	1: absent, 3: weak, 5: medium, 7: strong
SS	Stem shape (SNICS)	1: cylindrical, 2: angled
IGC	Intensity of Green color (SNICS)	3: light, 5: medium, 7: dark
LS	Leaf shape (SNICS)	1: lanceolate, 5: ovate, 7: broad ovate
TL	Texture of leaf blade (rugosity) (SNICS)	3: weak, 5: moderate, 9: strong
LP	Leaf position (SNICS)	1: erect, 2: horizontal
LBM	Leaf blade margin (IPGRI)	1: entire, 2: undulate, 3: ciliate
IFC	Immature fruit color (SNICS)	3: light green, 5: green, 7: dark green
RFC	Ripe Fruit Color (SNICS)	2: yellow, 4: orange, 5: red, 6: greyed purple
FS	Fruit shape (SNICS)	1: triangular, 2: campanulate, 3: square, 4: rectangular
FST	Fruit surface texture (SNICS)	1: smooth, 2: slightly wrinkled, 3: strongly wrinkled
FSA	Fruit shape of apex (SNICS)	1: acute, 3: rounded, 5: depressed, 7: depressed and acute
MC	Margin of calyx (SNICS)	1: entire, 2: crenate, 3: dentate
FUS	Fruit undulation in cross section (SNICS)	3: weak, 5: medium, 7: strong
DPF	Density of placenta of fruit (SNICS)	3: laxa, 5: semi-distributed, 7: compacta
NL	Number of loculi (SNICS)	2: two, 3: three, 4: four, 5: five
Quantitative Descriptors		
G%	Germination IPGRI	Registered on 7, 10, 15, 18 and 21 days
PH	Plant height (cm) IPGRI	Recorded when in 50% of the plants the first fruit has begun to ripen
SL	Stem length (cm) SNICS	Height to first bifurcation. Measured immediately after first harvest
SD	Stem diameter (cm) SNICS	Measured in the middle part to first bifurcation, immediately after first harvest
LLB	Leaf: length of blade (cm) SNICS	Measured in the leaves that belong to the middle part of the plant, after the first harvest.
LWB	Leaf: width of blade (cm) IPGRI, SNICS	Measured on the widest part of the leaf.
LLP	Leaf: length of petiole (cm) SNICS	Measured in the leaves that belong to the middle part of the plant, after the first harvest.
DFL	Days to flowering (IPGRI)	Number of days from sowing/transplanting until 50% of plants have at least one open flower
DFR	Days to fruiting (IPGRI)	Number of days from transplanting until 50% of the plants bear mature fruits at the first and second bifurcation
FL	Fruit length (cm) IPGRI	Average fruit length of 10 ripe fruits of the second harvest
FWI	Fruit width (cm) IPGRI	Measured at the widest point. Average fruit width of 10 ripe fruits of the second harvest
FW	Fruit weight (g) IPGRI	Average fruit weight of 10 ripe fruits of the second harvest
FWT	Fruit wall thickness (cm) IPGRI	Average fruit weight of 10 ripe fruits of the second harvest
FPL	Fruit pedicel length (cm) IPGRI	Average fruit weight of 10 ripe fruits of the second harvest
FTP	Fruit: thickness of pedicel (cm) SNICS	Average fruit weight of 10 ripe fruits of the second harvest
NSF	Number of seeds per fruit (IPGRI)	Average of at least 10 fruits selected from random plants
FYP	Fruit yield/plant (g·plant ⁻¹)	Fruit yield average on 10 plants
CC	Capsaicin content in mg·g ⁻¹ of dry weight (DW)	20 fruits of each variety from random plants, extraction and quantification by the Collins et al. (1995) method.

2.1. Capsaicinoids Extraction and Quantification

The extraction and quantification of capsaicinoids followed the methodology reported by Collins et al. [17] with slight modifications. The separation and quantification of the capsaicinoids was conducted by HPLC (Agilent series 1200, Agilent Technologies, Santa Clara, CA, USA) equipped with an automatic injector and a fluorescence detector. The capsaicinoids were separated by a Zorbax Eclipse Plus C18 (Agilent Technologies, Santa Clara, CA, USA) column (4.6mm.i.d. × 250 mm) at a temperature of 25 °C and an injection volume of 20 µL. The wavelengths used for the detection were 280 nm (excitation) and 338 nm (emission). The mobile phase was isocratic with 70% of solvent B (100% methanol) and 30% of solvent A (10% methanol solution v/v). The HPLC operating conditions to determine total capsaicinoids were flow rate of 1 mL/min and 15 min runtime. Standards of capsaicin and dihydrocapsaicin were used to develop a calibration curve based on the relationship of the maximum areas for the known concentrations of external standards. The stock solution was prepared in 100% (v/v) methanol and five different concentrations (20, 60, 100, 200, and 300 ppm) were used to generate the calibration curve. The concentrations of the two major capsaicinoids (capsaicin and dihydrocapsaicin) were estimated using the calibration curve obtained. The concentration of capsaicinoids is reported in milligrams per gram of dry weight (DW).

2.2. Statistical Analyses

The data obtained from the qualitative traits were subjected to a multiple correspondence analysis (MCA), while data obtained from the quantitative traits were processed by variance analysis and the means were contrasted using the *t* Tests LSD (least significant difference) with $p < 0.05$ in order to determine the significance of the differences among variables. The associations between quantitative traits were determined using Pearson's correlation. The principal component analysis (PCA) used a traits correlation matrix. PCA results were plotted in a bidimensional plane. This was done with the IBM SPSS Statistics version 22 [18]. For the traits showing the greater contribution to variation in the PCA, a Euclidean distance matrix was calculated based on standardized data for cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) [19].

3. Results

3.1. Variation of Qualitative Traits

The results of the analysis of the 22 qualitative traits assessed in 29 F1 Lines and 11 progenitors of habanero pepper (Table 2) showed that 82.5% of the genotypes presented three or more flowers per axil (NFA); the position of the flower (PF) was in the middle in most of the genotypes (55%). The anthocyanin color of the node (ACN) was absent in only 7.5% of assessed progenitors and F1 lines. The predominant shape of the stem (SS) was cylindrical (80%), and 80% of genotypes had an oval leaf shape (LS) while the shape of the rest was lanceolate. The predominant leaf position (LP) was horizontal (55%), and 95% had a ciliated leaf blade margin (LBM).

Table 2. Percentage of distribution of the 22 qualitative traits in F1 lines and progenitors of habanero peppers.

Descriptor		Descriptor Occurrence Frequency (%)			
NFA	Three or more = 82.5	Many flowers in bunches = 17.5			
FP	Intermediate = 55	Erect = 45			
CCO	Light yellow = 15	Yellow = 82.5	Yellow-green = 2.5		
CA	Violet = 85	Violet blue = 2.5	blue = 12.5		
FC	Light green = 77.5	Yellow-green = 12.5	Violet = 10		
STE	Same level = 25	Exserted = 75			
ACN	Absent = 7.5	Weak = 37.5	Medium = 32.5	Strong = 22.5	
SS	Cylindrical = 80	Angled = 20			
IGC	Light = 7.5	Medium = 42.5	Dark = 50		
LS	Lanceolate = 20	Ovate = 80			
TL	Weak = 62.5	Moderated = 37.5			
LP	Erect = 45	Horizontal = 55			
LBM	Ciliate = 95	Undulate = 5			
IFC	Light green = 10	Green = 45	Dark green = 45		
RFC	Yellow = 5	Orange = 7.5	Red = 82.5	Purple = 5	
FS	Triangular = 45	Campanulate = 10	Square = 17.5	Rectangular = 27.5	
FST	Smooth = 67.5	Slightly wrinkled = 25	Strongly wrinkled = 7.5		
FSA	Acute = 42.5	Rounded = 22.5	Depressed = 35		
MC	Entire = 80	Dentate = 20			
FUS	Weak = 20	Medium = 60	Strong = 20		
DPF	Laxa = 17.5	Semi-distributed = 80	Compact = 2.5		
NL	Two = 2.5	Three = 82.5	Four = 15		

Abbreviations are as in Table 1.

As to the color of the fruit, three different color shades were apparent in the immature fruit (IFC), with a predominance of green and dark green colors (45%). The genotypes of plants with ripe fruits of different colors (RFC)—yellow, orange, red and purple—were identified. Red was the most frequent color (82.5%) in the assessed genotypes. The shape of the fruit (FS) also exhibited great diversity. The most common was the triangular shape (45%), followed by the rectangular (27.5%); bell and square shapes were found with less frequency (10% and 17.5%, respectively). A total of 42.5% of the fruits had pointed apex (FSA). The smooth texture of the pericarp (FST) was most common (67.5%). Most of the fruits had three loculi (NL) with the density of the placenta (DPF) semi-distributed (80%). Data of the qualitative descriptors itemized for the F1 lines and the progenitors of habanero peppers are shown in Table S1.

Multiple Correspondence Factorial Analyses

The results of the multiple correspondence analyses (MCA) performed for the 22 qualitative traits evaluated in the 40 genotypes of habanero pepper identified two dimensions that explain 38.2% of the total variability (Table 3). The traits that contributed the most to axis 1 were anthocyanin coloration of nodes (ACN), the margin of the calyx of the fruit (MC), leaf position (LP), fruit shape (FS) and fruit undulation in cross-section (FUS). The traits contributing most to the second axis were leaf blade margin (LBM), the shape of the fruit apex (FSA) and fruit surface texture (FST).

Figure 2 shows the distribution of the F1 lines and progenitors, as well as the modalities of the traits with a major contribution to axes 1 and 2. As can be appreciated, P33 and P34 move significantly away from the rest of the group. Both genotypes differ from the rest because their pericarps are markedly rugged (fruit surface texture), and their leaf blade margins undulated, in notable contrast with the rest of the genotypes.

Table 3. Results of the multiple correspondence analysis (MCA) carried out from the qualitative and quantitative traits of the assessed F1 lines and progenitors of habanero pepper.

Descriptors	Dimension	
	1	2
NFA	0.026	0.002
FP	0.059	0.229
CCO	0.103	0.112
CA	0.043	0.142
FC	0.199	0.202
STE	0.230	0.040
ACN	<u>0.758</u>	0.319
SS	0.090	0.124
IGC	0.303	0.000
LS	0.165	0.200
TL	0.089	0.001
LP	<u>0.406</u>	0.003
LBM	0.080	<u>0.426</u>
IFC	0.116	0.145
RFC	0.170	0.167
FS	<u>0.397</u>	0.314
FST	<u>0.215</u>	<u>0.360</u>
FSA	0.297	<u>0.420</u>
MC	<u>0.498</u>	0.084
FUS	<u>0.334</u>	0.057
DPF	0.184	0.194
NL	0.102	0.007
Total active	4.864	3.548
% variance	22.109	16.126
Total	38.235	

The underlined descriptors are those with the highest contribution in each dimension. Abbreviations are as in Table 1.

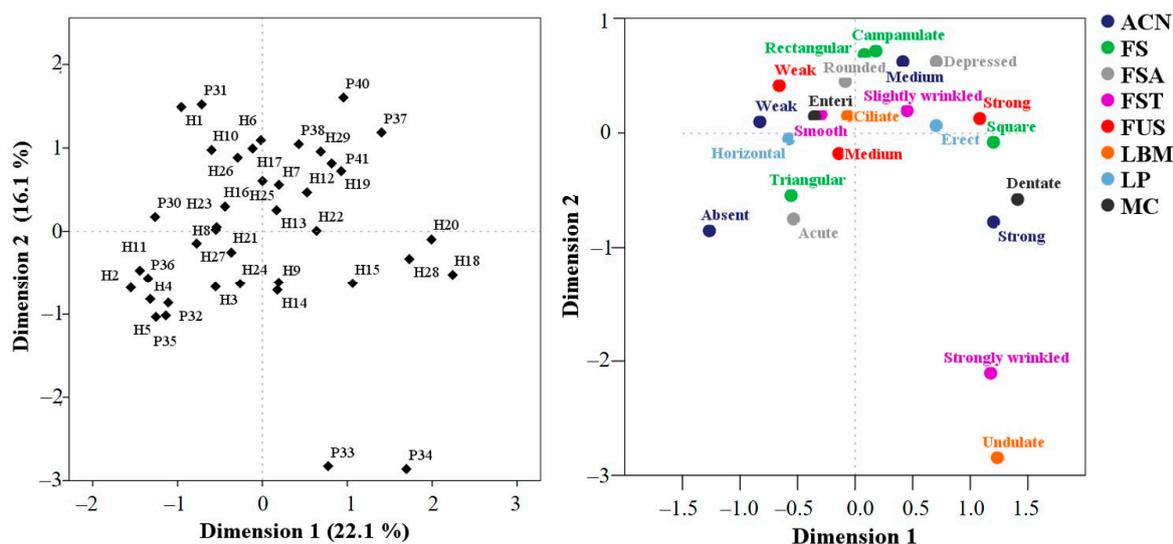


Figure 2. Distribution of the assessed F1 lines and progenitors, as well as the modalities of the main traits contributing to the axes 1 and 2, observed from the multiple correspondence analyses (MCA). ACN: Anthocyanin coloration of nodes, FS: Fruit shape, FSA: Fruit shape of apex, FST: Fruit surface texture, FUS: Fruit undulation in cross section, LBM: Leaf blade margin, LP: Leaf position, MC: Margin of calyx.

3.2. Variation of the Quantitative Traits

As can be seen in Table 4, most of the evaluated quantitative traits exhibit high variability, with the exception of the thickness of the pedicel of the fruit (FTP) that did not differ significantly ($p < 0.05$), so the trait was not considered in the principal component analysis (PCA).

Table 4. Analysis of variance (ANOVA) of the quantitative traits of the evaluated genotypes of habanero pepper.

Traits df	Mean Squares			p Value
	Replication 3	Genotype 39	Error 120	
G (%)	18.23	514.75 *	11.45	0
PH (cm)	47.19	900.77 *	55.5	0
SL (cm)	12.78	103.96 *	7.03	0
SD (cm)	0.014	0.115 *	0.008	0
LLB (cm)	2.05	4.45 *	0.512	0
LWB (cm)	0.874	1.55 *	0.120	0
LLP (cm)	0.951	0.621 *	0.09	0
DFL	60.91	132.65 *	1.97	0
DFR	8.75	122.5 *	0.67	0
FL (cm)	1.34	2.39 *	0.67	0
FWI (cm)	0.868	1.20 *	0.34	0
FW (g)	9.66	28.20 *	5.05	0
FWT (cm)	0.031	0.007 *	0.002	0
FPL (cm)	0.097	0.111 *	0.066	0.019
FTP (cm)	0.073	0.004	0.004	0.634
NSF	156.75	101.8 *	42.53	0
FYP (g·plant ⁻¹)	7519	199,983,866.76 *	13,185.38	0
CC (mg·g ⁻¹ DW)	0.0116	2608.631 *	0.161	0

* Significant difference ($p < 0.05$), df: degrees of freedom. Abbreviations are as in Table 1.

The results of the analysis of variance carried out for the evaluated traits in the plants and leaves are presented in Table 5. The germination values of F1 lines ranged between 60% and 100%, similar to those recorded for the progenitors (62–94%). Lines H8 and H22 showed 100% germination. The progenitor P34 had the greatest height (155.3 cm), while the lowest height (87.2 cm) belonged to Line H11. Variation was also found for the following traits: stem diameter (SD), with values ranging between 0.99 and 1.9 cm; length of blade (LLB) and width of blade (LWB) showed variations between 9.45 and 15 cm and between 5.2 and 8 cm, respectively; the length of the leaf petiole (LLP) variation values oscillated from 2.5 to 4.35 cm. Line H20 had the highest values for the traits of stem diameter (SD), length of blade (LLB) and width of blade (LWB) (Table 5).

Table 5. Variation in the morphoagronomic traits evaluated in F1 lines and progenitors of habanero pepper.

F1 Lines		Plant Traits					Leaf Traits		
No.	Cross	G (%)	PH (cm)	SL (cm)	SD (cm)	LLB (cm)	LWB (cm)	LLP (cm)	
H1	P31 × P30	75 jk	104.8 lmnopq	45.4 klmno	1.31 defgh	12.5 ghijklm	6.15 ijklm	3.15 lmn	
H2	P32 × P30	88 ef	142.7 bc	52.7 bcd	1.09 lmn	13.7 bcd	6.3 ghijklm	3.5 efghijk	
H3	P33 × P30	85 fg	111.9 hijklmn	55.5 b	1.16 ijklm	13.05 cdefg	6.15 jklm	3.45 fghijk	
H4	P35 × P30	60 o	97 pqrs	46.6 ijklmn	1.16 ijklm	13.1 cdefg	6.8 defgh	3.35 ghijkl	
H5	P36 × P30	68 mn	121.1 efgh	50.6 cdefgh	1.14 ijklm	12.35 hijklm	6.3 ghijklm	3.85 cde	
H6	P37 × P30	83 gh	113.5 ghijklmn	51.5 cde	1.15 ijklm	13.9 bc	6.55 defghij	3.2 jklm	
H7	P38 × P30	80 hi	123.2 defg	47.8 fghijkl	1.19 hijklm	12.2 hijklmn	6.25 hijklm	3.6 defghi	
H8	P40 × P30	100 a	104.2 mnoopq	49.2 defghij	1.25 efghi	12.35 hijklm	6.9 cdef	3.25 hijkl	
H9	P30 × P34	78 ij	122.4 efgh	62.4 a	1.16 ijklm	13.65 bcdef	7.1 bcd	3.55 defghij	
H10	P30 × P31	83 gh	93.5 rs	39.2 qrs	1.2 hijklm	14.25 ab	7.45 abc	3.6 defghi	
H11	P30 × P32	75 jk	87.2 s	45 lmnno	1.12 ijklm	12.25 hijklmn	6.15 ijklm	2.9 mn	
H12	P40 × P31	73 kl	126.7def	46.1 ijklmn	1.35 cdef	14.15 ab	6.7 defghij	3.65 defgh	
H13	P40 × P32	83 gh	106.9 jklmnop	42.4 opq	1.22 ghij	11.5 o	5.95 lmn	3.75 cdef	
H14	P40 × P33	73 kl	115.1 ghijkl	44.5 lmno	1.37 cde	13.15 cdefg	6.85 defg	3.55 defghij	
H15	P40 × P34	93 cd	148.5 ab	54.2 bc	1.37 cde	12.4 ghijklm	6.85 defg	3.35 ghijkl	
H16	P40 × P35	80 hi	123 defg	51.2 cdefg	1.41 cd	11.7 klmno	6.4 fghijkl	3.2 jklm	
H17	P40 × P36	90 de	120.3 efghi	49 efghijk	1.35 cdef	12 ijklmno	6.45 fghijkl	3.7 defg	
H18	P40 × P37	80 hi	133.4 cd	45.7 jklmno	1.47 c	12.05 ijklmno	6 klm	3.2 jklm	
H19	P40 × P38	95 bc	122.6 efg	45.3 klmno	1.39 cd	11.75 jklmno	6.05 klm	3.5 efghijk	
H20	P41 × P34	67 n	138 jklmno	44 mno	1.9 a	15 a	8 a	3.5 efghijk	
H21	P32 × P31	70 lmn	116.9 fghij	51.5cdef	1.21 hijklm	12.8 defghi	6.05 klm	3.15 klmn	
H22	P31 × P33	100 a	120.8 efgh	52.3 bcde	1.22 ghij	11.75 jklmno	6.45 fghijkl	3.75 cdef	
H23	P31 × P32	90 de	107.2 jklmnop	46.9 ijklm	1.11 jklmn	11.65 lmno	5.2 p	3.15 klmn	
H24	P37 × P35	98 ab	108.5 jklmno	47.8 ghijkl	1.17 ijklm	12.6 ghijkl	6.05 klm	3.45 fghijk	
H25	P38 × P35	70 lmn	104.4 mnoopq	42 opqr	1.19 hijklm	11.3 no	5.95 lmn	3.5 efghijk	
H26	P40 × P35	67 n	128.5 de	42 opqr	1.7 b	14 bc	7.5 ab	4.25 ab	
H27	P36 × P35	60 o	106.5 klmnop	45.5 klmno	1.16 ijklm	12.1 ijklmno	6.25 hijklm	3 lmn	
H28	P37 × P40	90 de	115.8 ghijk	40.1 pqrs	1.38 cd	14.25 ab	7.45 abc	4.1 abc	
H29	P37 × P36	83 gh	114.2 ghijklm	46.75 ijklmn	1.21 ghijkl	13.65 bcde	6.75 defghi	4.25 ab	
PROGENITORS									
P30	RK-01	88 ef	95.9 qrs	47 hijklm	1.23 fghi	11.95 ijklmno	6.1 klm	3.6 defghi	
P31	RC-02	72 klm	109.9 ijklmn	46.7 ijklmn	1.23 fhij	13.05 cdefg	6.45 fghijkl	2.9 mn	
P32	RN-03	74 jkl	115.3 ghijk	51.9 bcde	1.1 klmn	11.7 klmno	5.35 op	3.2 jklm	
P33	RJ-04	94 bcd	130.6 de	49.7 defghi	1.17 ijklm	12.25 hijklmn	6.2 ijklm	3.3 hijkl	
P34	RS-05	94 bcd	155.3 a	55.5 b	1.39 cd	12.6 ghijkl	6.85 defg	4.35 a	
P35	NB-06	88 ef	95.3 qrs	45.6 jklmno	0.99 n	13.35 bcdefg	6.5efghijkl	3.4 fghijk	
P36	NK-07	72 klm	97.3 pqrs	43.2 nop	1.09 nm	11.6 mno	5.4 nop	2.8 no	
P37	AK-08	100 a	98.2 opqr	38.6 rs	1.34 defg	12.65 efghij	7.1bcd	3.3 hijkl	
P38	AS-09	82 ghi	105.7 klmnopq	42.1 opqr	1.31 ijklm	9.45 p	5.3 op	2.5 o	
P40	MB-11	62 o	130.3 de	37.7 s	1.43 cd	11.8 jklmno	5.8 mno	3.9 bcd	
P41	MS-12	90 de	103.2 nopqr	49.3 defghi	1.17 ijklm	12.7 fghijk	7.05 bcde	3.65 defgh	
LSD (0.05)		4.70	10.45	3.67	0.12	0.96	0.59	0.36	

G: Germination, PH: Plant height (cm), SL: Stem length (cm), SD: Stem diameter (cm), LLB: Leaf length of blade (cm), LWB: Leaf width of blade (cm), LLP: Leaf length of petiole (cm). The averages within each column followed by the same letter are not significantly different from each other based on the probability level $p < 0.05$ of the LSD.

Germination percentage (G%), days to fruiting (DFR), average fruit length (FL), average fruit width (FWI), fruit weight (FW), number of seeds per fruit (NSF), fruit pericarp thickness (FWT), thickness the pedicel of the fruit (FTP) and the content of capsaicin (CC) are traits closely related with the yield and quality of the fruit [20]. The fruit traits variation for F1 lines and progenitors are shown in Table 6. According to the results, line H22 is characterized by greater fruit length (FL) (average value 5.52 cm), while P41 had the lowest length (3.41 cm). As for fruit width (FWI) and fruit weight (FW), the greater average values were recorded for P38 (4.32 cm and 16.82 g, respectively). P30 was the genotype with the greatest number of seeds per fruit (53.7). Genotype P41 stood out for having the thickest pedicel (FTP), while H7 had the thickest pericarp (FWT). Fruit yield per plant (FYP) showed a wide range of variation (846.78–99952.50 g·plant⁻¹), and genotype H20 (F1 line) had the highest yield while progenitor P34 had the highest capsaicin content (CC) (147.11 mg·g⁻¹ DW).

Table 6. Variation in the quantitative traits evaluated in F1 lines and progenitors of habanero pepper.

No.	CROSS	DFL	DFR	FRUIT TRAITS								
				FL (cm)	FWI (cm)	FW (g)	FWT (cm)	FPL (cm)	FTP (cm)	NSF	FYP (g·plant ⁻¹)	CC (mg·g ⁻¹ DW)
H1	P31 × P30	26 d	70 b	4.46 jkl	3.20 fghijk	13.61 ef	0.242 cde	3.08 abcde	0.47 abc	48.9 abcde	2803.38 h	53.38 h
H2	P32 × P30	33 c	77 a	4.76 gh	3.14 ghijkl	12.04 j	0.255 bc	2.75 degf	0.41 cdefg	41.9 defghijk	2802.46 h	30.30 v
H3	P33 × P30	33 c	63 c	5.37 ab	2.98 nopq	11.55 k	0.182 qr	3.02 abcdef	0.38 fg	50 abcd	3213.19 e	71.85 e
H4	P35 × P30	33 c	70 b	5.03 de	3.01 lmnop	13.40 efg	0.247 bcde	2.7 gf	0.4 defg	41.6 defghijk	2990.92 f	45.12 k
H5	P36 × P30	26 d	70 b	5.12 cd	3.13 ghijklm	12.71 i	0.237 efgh	3.18 abc	0.42 bcdefg	45.5 abcdefg	2964.19 fg	33.61 t
H6	P37 × P30	33 c	63 c	4.89 efg	3.30 ef	12.88 hi	0.240 defg	3.01 abcdefg	0.43 abcdef	50 abcd	2943.13 fgh	39.53 p
H7	P38 × P30	33 c	63 c	3.91 pq	3.87 b	15.12 c	0.247 bcde	2.72 egf	0.49 a	49.3 abcde	3375.54 cd	48.17 i
H8	P40 × P30	33 c	63 c	4.81 g	2.96 opq	10.90 l	0.237 efgh	2.69 gf	0.43 abcdef	40.3 fghijk	2813.07 gh	41.99 m
H9	P30 × P34	33 c	63 c	4.63 hi	3.08 jklmno	11.95 j	0.232 fghij	3.28 ab	0.39 efg	45.5 abcdefg	3242.48 de	55.85 g
H10	P30 × P31	33 c	77 a	4.89 efg	3.21 fghi	13.33 fg	0.225 hijk	3.16 bcdegf	0.46 abcd	39.1 ghijk	2619.78 i	30.16 v
H11	P30 × P32	26 d	70 b	4.84 fg	3.05 lmnop	13.34 fg	0.235 efghi	2.71 cdegf	0.44 abcdef	49.3 abcde	3416.95 c	31.73 u
H12	P40 × P31	42 b	70 b	4.83 fg	3.21 fghij	12.52 i	0.217 klm	3.33 abcde	0.42 bcdefg	47.9 abcdef	2905.59 fgh	44.02 l
H13	P40 × P32	33 c	63 c	4.19 mn	2.97 nopq	9.83 o	0.217 klm	2.79 abcd	0.42 bcdefg	39.6 fghijk	1865.47 pq	32.30 u
H14	P40 × P33	33 c	63 c	4.98 def	2.87 qr	9.47 op	0.192 pq	3.3 cdegf	0.4 defg	36.9 hijk	2090.69 o	85.05 c
H15	P40 × P34	33 c	63 c	4.06 nop	3.23 fg	12.02 j	0.222 ijkl	3.02 abcdefg	0.45 abcde	40.1 fghijk	1550.25 r	73.54 d
H16	P40 × P35	26 d	63 c	4.40 kl	2.96 opq	9.18 pq	0.202 nop	3.56 degf	0.44 abcdef	37.5 ghijk	1726.72 q	40.78 o
H17	P40 × P36	33 c	77 a	4.45 jkl	3.11 ghijklm	8.91 q	0.207 mno	2.83 cdegf	0.41 cdefg	40 fghijk	2139.69 no	38.81 q
H18	P40 × P37	42 b	70 b	4.75 gh	3.50 d	12.74 hi	0.207 mno	3.22 a	0.39 efg	40.6 efgghijk	2100.85 o	41.70 mn
H19	P40 × P38	42 b	63 a	4.13 no	3.28 ef	13.11 gh	0.277 a	3.3 abcd	0.44 abcdef	52.1 ab	1996.91 op	33.33 t
H20	P41 × P34	42 b	63 a	4.86 fg	3.28 ef	15.58 b	0.260 b	2.93 gf	0.46 abcd	38.8 ghijk	9952.50 a	48.22 i
H21	P32 × P31	26 d	70 b	4.54 ijk	3.22 fgh	12.58 i	0.217 klm	2.84 abcdegf	0.43 abcdefg	38.8 ghijk	2089.06 o	35.32 s
H22	P31 × P33	33 c	63 c	5.52 a	2.87 qr	10.25 n	0.170 rs	3.08 abcd	0.4 defg	35.7 ijk	2808.57 gh	58.36 f
H23	P31 × P32	26 d	77 a	4.62 hij	3.08 ijklmno	12.78 hi	0.230 fghijk	3.09 cdegf	0.41 cdefg	40.4 fghijk	2407.16 jklm	35.41 s
H24	P37 × P35	33 c	63 c	4.85 fg	3.20 fghijk	12.58 i	0.210 lmn	2.87 abcdefg	0.42 bcdefg	41.7 defghijk	2386.57 jklm	37.18 r
H25	P38 × P35	26 d	63 c	4.20 mn	3.97 b	13.74 de	0.257 b	2.98 bcdegf	0.43 abcdefg	48 abcdef	3385.87 cd	34.91 s
H26	P40 × P35	42 b	77 a	3.83 q	3.44 d	10.82 lm	0.260 b	2.81 degf	0.44 abcdef	42.9 cdefghij	6940.00 b	29.76 v
H27	P36 × P35	33 c	77 a	4.88 efg	3.08 klmno	11.48 k	0.257 b	2.88 abc	0.43 abcdefg	34.1 k	2821.75 gh	27.33 x
H28	P37 × P40	33 c	70 b	4.80 g	3.40 de	13.69 ef	0.197 nop	3.31 abcdegf	0.46 abcd	43.7 bcdefghi	2538.07 ij	41.40 n
H29	P37 × P36	26 d	70 b	4.52 ijk	3.09 hijklmn	11.51 k	0.210 lmn	3.09 abcde	0.47 abc	51.3 abc	2286.72 mn	46.17 j

Table 6. Cont.

PROGENITORS												
No.	CROSS	DFL	DFR	FL (cm)	FWI (cm)	FW (g)	FWT (cm)	FPL (cm)	FTP (cm)	NSF	FYP (g·plant ⁻¹)	CC (mg·g ⁻¹ DW)
P30	RK-01	26 d	70 b	4.90 efg	3.01 mnop	14.10 d	0.220 jklm	2.66 gf	0.44 abcdef	52.7 a	2393.75 jklm	23.68 z
P31	RC-02	33 c	63 c	4.31 lm	3.13 ghijklm	12.72 i	0.195 opq	2.96 abcdegf	0.39 efg	42.5 cdefghij	2342.32 klm	15.71 z
P32	RN-03	26 d	70 b	4.02 op	2.81 r	9.75 o	0.240 defg	3.08 abcde	0.4 defg	41.8 defghijk	2276.00 mn	20.34 z
P33	RJ-04	33 c	70 b	5.28 bc	2.42 s	6.75 s	0.147 t	2.86 cdegf	0.44 abcdef	35.1 jk	1482.29 r	120.38 b
P34	RS-05	33 c	77 a	3.63 r	2.97 nopq	7.77 r	0.165 s	3 abcdefg	0.45 abcde	42.9 cdefghij	846.78 s	147.11 a
P35	NB-06	26 d	77 a	4.81 g	2.94 pq	12.70 i	0.252 bcd	2.95 bcdegf	0.44 abcdef	44.2 bcdefghi	2315.25 lm	28.95 w
P36	NK-07	26 d	70 b	5.03 de	3.01 lmnop	10.46 mn	0.227 ghijk	2.76 degf	0.36 g	45.3 abcdefgh	2501.32 ij	29.09 w
P37	AK-08	33 c	70 b	4.48 ijkl	3.67 c	16.67 a	0.235 efghi	3.19 abc	0.46 abcd	49.3 abcde	2485.28 ijk	26.52 y
P38	AS-09	42 b	70 b	3.62 r	4.32 a	16.82 a	0.277 a	2.98 abcdegf	0.48 ab	50.5 abc	2610.28 i	23.82 z
P40	MB-11	33 c	77 a	4.23 mn	2.95 pq	13.52 ef	0.197 nop	3.08 abcde	0.45 abcde	43.5 bcdefghi	1418.63 r	44.54 l
P41	MS-12	48 a	77 a	3.41 s	3.65 c	9.55 op	0.282 a	2.97 abcdegf	0.49 a	49.1 abcde	2448.00 jkl	21.62 z
LSD (0.05)		0.94	0.94	0.17	0.13	0.38	0.012	0.35	0.06	8.81	161.69	0.568

DFL: Days to flowering, DFR: Days to fruiting, FL: Fruit length (cm), FWI: Fruit width (cm), FW: Fruit weight (g), FWT: Fruit wall thickness (cm). FPL: Fruit pedicel length (cm), FTP: Thickness the pedicel of the fruit (cm), NSF: Number of seeds per fruit, FYP: Fruit yield/plant (g·plant⁻¹), CC: Capsaicin content (mg·g⁻¹ DW). The averages within each column followed by the same letter are not significantly different from each other based on the probability level $p < 0.05$ of the LSD.

3.2.1. Correlation Analysis

The analysis of the quantitative traits' correlations (Figure 3) indicated that the trait width of blade (LWB) presented a positive and significant correlation with the length of blade (LLB), while fruit width (FWI) correlated negatively with fruit length (FL). On the other hand, fruit width (FWI) had a positive correlation with fruit weight (FW) 0.642. Plant fruit yield (FYP) shows an association with stem diameter (SD), length of blade (LLB) and width of blade (LWB), which allows us to infer that there is a direct relation between the bearing of the plant and the productivity. Capsaicin content (CC) showed a positive and significant correlation (0.557) with plant height (PH), and a negative correlation with fruit pericarp thickness (FWT), with a value of -0.620 , which indicates that fruits with a thin pericarp have a greater capsaicin concentration. The values of the correlations can be consulted in Table S2.

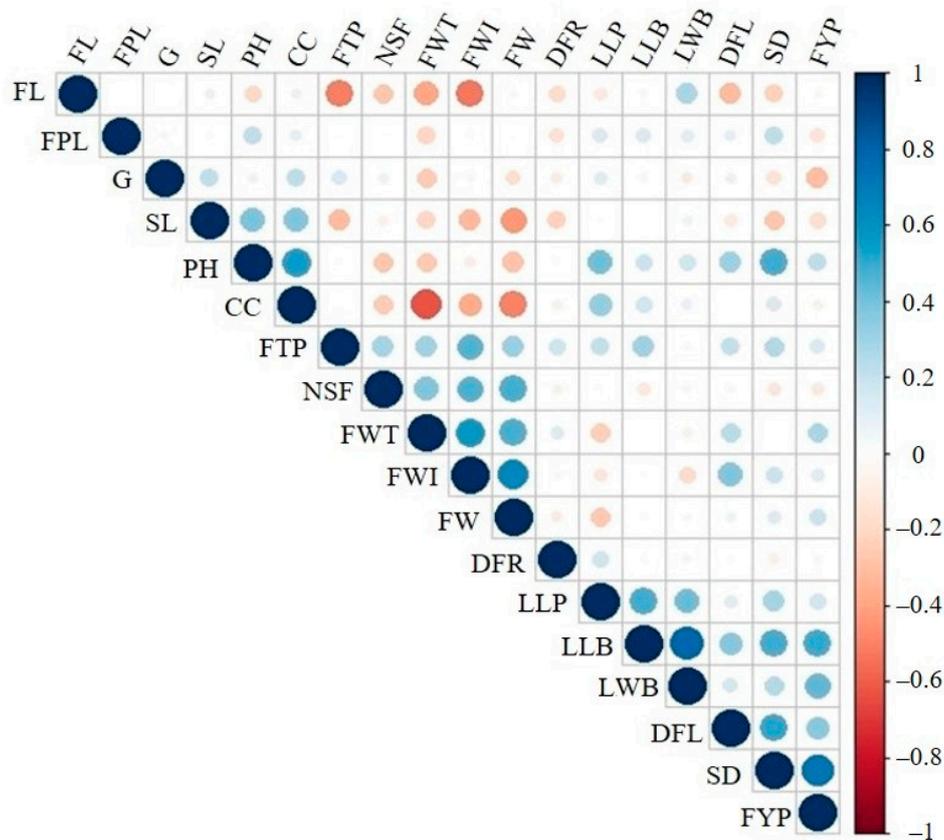


Figure 3. Pearson correlation matrix for the 18 quantitative traits of the 29 F1 lines and the 11 progenitors of habanero pepper. FL: Fruit length, FPL: Fruit pedicel length, G: Germination, SL: Stem length, PH: Plant height, CC: Capsaicin content, FTP: Thickness the pedicel of the fruit, NSF: Number of seeds per fruit, FWT: Fruit wall thickness, FWI: Fruit width, FW: Fruit weight, DFR: Days to fruiting, LLP: Leaf length of petiole, LLB: Leaf length of blade, LWB: Leaf width of blade, DFL: Days to flowering, SD: Stem diameter, FYP: Fruit yield/plant.

3.2.2. Principal Component Analysis

Results of the PCA (Table 7) showed that the first six axes represented 85.1% of the total variation, and that the first component explained most of the variation (%), ordering the genotypes by capsaicin contents (CC) and by fruit pericarp thickness (FWT). The second principal component was composed of the traits stem diameter (SD) and yield per plant (FYP), the third component was associated with fruit length (FL), the fourth with the number of seeds per fruits (NSF), the fifth component with the pedicel length (FPL), and the sixth component was related to stem length (SL). Figure 4 shows the

distribution of the evaluated traits for the first two components, according to their contribution to the explained total variation.

Table 7. Principal components, own values, self-values, and percentage of explained total variance.

Eigenvalues	Principal Components					
	1	2	3	4	5	6
Own values	1.942	1.762	1.328	1.163	1.057	0.938
% Explained variance	0.251	0.207	0.118	0.090	0.074	0.059
% Accumulated variance	0.251	0.458	0.576	0.666	0.799	<u>0.851</u>
Traits						
G (%)	0.118	-0.149	-0.285	0.417	-0.047	0.364
PH (cm)	0.315	0.182	-0.360	-0.236	-0.050	-0.254
SL (cm)	0.249	-0.178	-0.165	0.084	-0.445	<u>-0.602</u>
SD (cm)	0.100	<u>0.460</u>	-0.118	-0.271	0.230	0.106
LLB (cm)	0.190	0.415	0.136	0.298	-0.095	0.052
LWB (cm)	0.194	0.335	0.319	0.360	-0.148	-0.188
LLP (cm)	0.260	0.243	-0.164	0.273	-0.083	0.320
DFL	0.057	0.245	0.015	-0.020	-0.142	0.065
DFR	-0.039	0.261	0.066	-0.241	-0.313	0.051
FL (cm)	0.153	-0.139	<u>0.563</u>	0.180	0.164	-0.117
FWI (cm)	-0.352	0.216	-0.325	0.065	0.043	-0.030
FW (g)	-0.363	0.205	0.065	0.187	0.173	-0.135
FWT (cm)	<u>-0.383</u>	0.177	-0.031	-0.056	-0.330	-0.202
FPL (cm)	0.112	0.079	-0.186	0.198	<u>0.697</u>	-0.451
NSF	-0.279	0.042	-0.242	<u>0.463</u>	-0.128	-0.081
FYP (g·plant ⁻¹)	0.020	<u>0.458</u>	0.180	-0.261	-0.191	0.008
CC (mg·g ⁻¹ DW)	<u>0.395</u>	-0.039	-0.216	-0.031	-0.014	0.072

The underlined traits are the ones with the highest contribution in each component. The underlined traits are the ones with the highest contribution in each component. Abbreviations are as in Table 1.

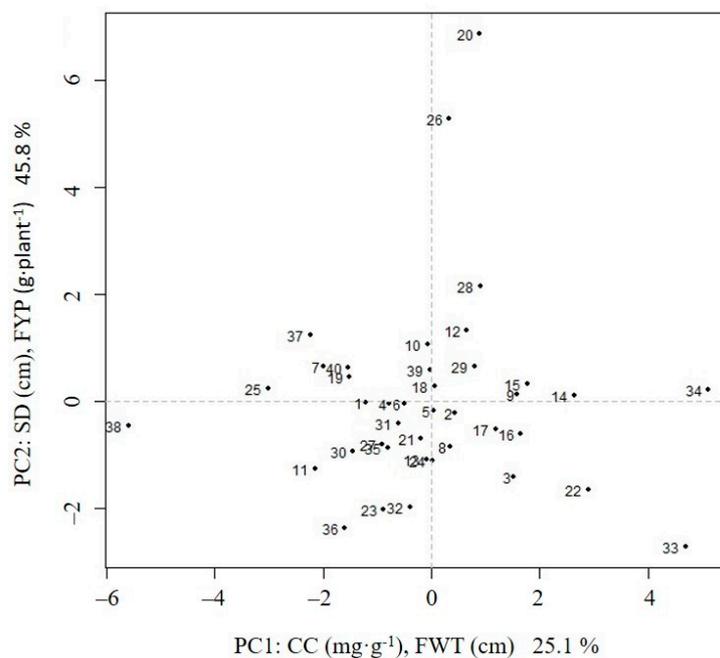


Figure 4. Dimensional representation of the distribution of F1 lines and progenitors of evaluated habanero peppers in the first two principal components. (Numbers 1–29 correspond to F1 lines H1–H29, and numbers 30–41 correspond to progenitors P30–P41).

3.2.3. Cluster Analysis

Five groups were identified in the dendrogram obtained from the cluster analysis (Figure 5) based on the plant and fruit traits, which are described in Table 8. As can be seen, the F1 lines H20 and H26, which presented the highest values of stem diameter (SD) and fruit yield per plant (FYP), comprised group 1. Five Lines F1 (H9, H14, H15, H16, and H22) and two progenitors (P33 and P34) were located in group 2, characterized by presenting the highest mean values for stem length (SL), fruit pedicel length (FPL) and capsaicin content (CC). F1 lines (H7, H19 and H25) and two progenitors (P38 and P41) were allocated in group 3, with the highest mean values of fruit pericarp thickness (FWT) and of number of seeds per fruit (NSF). Four F1 lines (H10, H12, H18, and H28) and two progenitors (P37 and P40) with the lowest mean values for stem length (SL) comprised group 4. Group 5 was composed of most of the F1 lines (15) and five progenitors that exhibited the highest values of fruit length (FL).

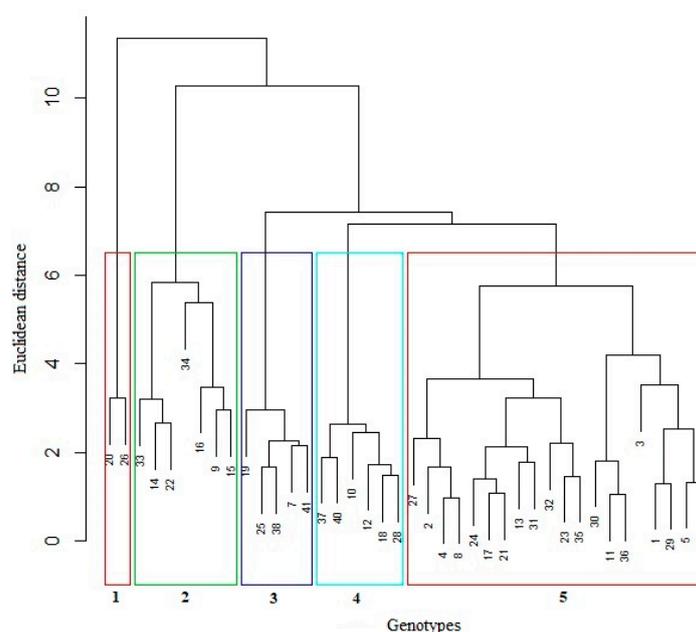


Figure 5. Dendrogram of the hierarchical cluster analysis showing the clusters of the 29 F1 lines and 11 progenitors habanero pepper based on the traits that made greater contributions to the PCA.

Table 8. Mean values of the evaluated qualitative and quantitative traits for each of the groups established by cluster analysis.

GROUPS	SL (cm)	SD (cm)	FL (cm)	FWT (cm)	FPL (cm)	NSF	FYP (g·plant ⁻¹)	CC (mg·g ⁻¹ DW)
Group 1	43.00	1.80 *	4.35	0.26	2.87	40.85	33,785.00 *	38.99
Group 2	52.83 *	1.30	4.65	0.19	3.16 *	39.10	1963.97	83.01 *
Group 3	45.30	1.25	3.85	0.27 *	2.99	49.80 *	2763.32	32.37
Group 4	41.23	1.36	4.67	0.21	3.22	44.02	2344.70	38.06
Group 5	47.79	1.18	4.77 *	0.23	2.88	44.54	2594.50	36.06

* Highest value between groups. SL: Stem length (cm), SD: Stem diameter (cm), FL: Fruit length (cm), FWT: Fruit wall thickness (cm), FPL: Fruit pedicel length (cm), NSF: Number of seeds per fruit, FYP: Fruit yield/plant (g·plant⁻¹) y CC: Capsaicin content in mg·g⁻¹ of dry weight (DW).

4. Discussion

The variance analysis of traits with agricultural interest is critical for the development of programs aiming to obtain the varieties and hybrids with high productivity and/or that carry other important traits and attributes of agricultural interest [21]. The results of the analysis of the evaluated qualitative and quantitative traits of the 40 genotypes of habanero pepper revealed a broad genetic diversity, which notably fosters the work on the genetic improvement of the crop. As for the variation in the

qualitative traits, the behavior of the ACN trait was interesting. Only 7.5% of the evaluated habanero pepper genotypes showed the absence of anthocyanins in the node of the plants, in contrast to what has been reported by Bozokalfa et al. [21] in another species of the genus (*C. annuum* L.). Their study found that node anthocyanins were absent in 47 of the 48 genotypes they assessed. This allows us to infer that, probably, this trait may be associated with the species.

Fonseca et al. [22] studied the genetic diversity of habanero peppers in the Amazon, and observed the predominance of some traits, such as the dark red color of the ripe fruit (42%), triangular-shaped fruits (42.1%) and rugged fruit surface (42.1%), in most of the evaluated genotypes. In our study, the MCA explained 38.2% of the total variability found, and identified some qualitative traits that contributed to clustering the genetic materials in a bidimensional plane. Castellón et al. [23], in a study on variations of native *C. annuum* L. accessions in Oaxaca, Mexico, found that MCA for the examined qualitative variables explained 88.5% of the variability, far higher than the findings in our study, which indicates greater variability in the habanero pepper. These same authors found statistically significant differences for all evaluated variables, except for the number of fruits per plant. In contrast, our study of 18 qualitative traits found that they all differed significantly ($p < 0.05$), except for the thickness of the pedicel of the fruit (FTP).

The width of the fruit (FWI) correlated negatively with fruit length (FL). On the other hand, the descriptor fruit width (FWI) had a positive correlation with fruit weight (FW), with a value of 0.642. Similar results were reported by Bharath et al. [24] and by Sharma et al. [25]. In our study, the trait pericarp thickness (FWT) had a positive association with fruit width (FWI) and fruit weight (FW). Moreira et al. [26] also worked with *C. chinense* and their results were similar to ours. Fruit yield per plant (FYP) shows an association with stem diameter (SD) and length of blade (LLB), indicating a direct influence between the bearing of the plant and the productivity. Likewise, capsaicin content (CC) had a significant positive correlation with plant height (PH), with a value of 0.557, and correlated negatively with fruit pericarp thickness (FWT), with a value of -0.620 . Butcher et al. [27] identified an association between capsaicin and fruit wall thickness of -0.083 , a lower value than the one we found in our study.

Both the PCA and the cluster analysis provide information showing the presence of an important genetic variability in the set of evaluated genotypes, which agrees with what has been reported by different authors [28,29]. Multivariate analyses have been used widely to evaluate the genetic variability of many crops [30,31]. Bozokalfa et al.'s results [21], including the PCA of the variation of 48 *C. annuum* accessions and lines, showed that the first six axes explained 54.29% of the variability, a value slightly lower than what we found in this study. Finally, the results of the cluster analysis obtained by Bianchi et al. [32] clustered *Capsicum* accessions in eight groups.

Our PCA showed that the traits capsaicin contents (CC), fruit pericarp thickness (FWT), stem diameter (SD) and fruit yield per plant (FYP) contributed the most to the accumulated variation. On the other hand, the cluster analysis generated five groups. Group 1 recorded the highest values for the following traits: Stem diameter (SD) and fruit yield per plant (FYP). Group 2 exhibited the highest values for stem length (SL), fruit pedicel length (FPL) and capsaicin content (CC), while the highest values for fruit pericarp thickness (FWT) and the number of seeds per fruit (NSF) were located in group 3. The lowest mean values for stem length (SL) were found in group 4 and group 5 had the highest value for fruit length (FL).

5. Conclusions

The Yucatan peninsula is a particularly privileged region of the world for habanero pepper genetic diversity. Based on the analysis of the data of the qualitative and quantitative traits, and taking into account the color, weight, shape and size of the fruit, as well as the yield and capsaicin content, we selected four F1 lines (H7, H14, H20, H27) (Figure 6) because of their high yield potential and/or high pungency. The data presented in this study, associated with productivity and other important traits, as well as the broad morphologic description of the evaluated genotypes, allowed us to identify four F1 lines with high potential for different markets—on one hand, for the fresh fruit market, because of

the high fruit quality, attractive colors, pretty shapes and high yields. On the other hand, the market is looking for a reliable and efficient source of high-quality capsaicin for different industries, particularly for the pharmaceutical industry. However, the most relevant result of our study is the fact of being able to obtain for the first time a habanero pepper hybrid (H14) with the greatest content of capsaicin to date (1,300,000–1,500,000 SHU). The hottest peppers in the world are not of the habanero type, and lack the attributes that give the habanero pepper from Southeast Mexico its high demand at international level. The germplasm of the habanero pepper that has supported this program of genetic improvement is stored in the Habanero Pepper Gene Bank of the Scientific Research Center of Yucatan (CICY), Yucatan, Mexico.

HYBRID	DESCRIPTION
<p>a)</p> 	<p>HYBRID H14: New hybrid of habanero pepper of dark green color as an unripe fruit and intense red when ripe. Vigorous plant of great height and productive. Short cycle plant (70–75 days). Its fruit is large (4–5 cm), with a triangular shape and low weight (9–10 g), thin and slightly rugged pericarp. Extremely hot (1,300,000–1,500,000 SHU). Recommended for capsaicin extraction.</p>
<p>b)</p> 	<p>HYBRID H7: New habanero pepper hybrid with high yields, bell shape fruits of intense green color when unripe and bright red when ripe. It is very productive, and its fruit is weighty (14–16 g). Short cycle plant (70–75 days). It is characterized by its high pungency (700,000–800,000 SHU). Recommended for the industry.</p>
<p>c)</p> 	<p>HYBRID H20: New hybrid of habanero pepper of dark green color that turns intense red when ripe. Vigorous, short cycle (70–75 days) plant with high yields. Its fruit is large (4–5.5 cm) and very heavy (15–16 g), squared shape as has an excellent shell life. It is very hot (700,000–800,000 SHU). Recommended for industrial purposes and for capsaicin extraction.</p>
<p>d)</p> 	<p>HYBRID H27: New habanero pepper hybrid with deep green color as an unripe fruit and bright orange when ripe, triangular-shaped (4–5 cm), with long shell life. Plant with a short cycle of 70 to 75 days. Moderately hot fruit (400,000–500,000 SHU). Recommended for fresh fruit markets.</p>

Figure 6. F1 hybrids (a) H14, (b) H7, (c) H20 and (d) H27 were selected because of their high productivity potential and high hotness.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0472/10/10/478/s1>, Table S1: Evaluated qualitative descriptors in F1 lines and progenitors of habanero pepper, Table S2: Pearson correlation matrix for the 18 quantitative descriptors of 29 F1 lines and 11 progenitors of habanero pepper.

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