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Morphological, Sensorial and Chemical Characterization of Chilli Peppers (*Capsicum* spp.) from the CATIE Genebank

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Abstract: In order to assess the potential of 192 accessions of *Capsicum* L., from 21 countries, a morphological and agronomic characterization was carried out by applying 57 qualitative and quantitative descriptors. Multivariate analyses identified two large groups: the first including *C. annuum* (G3, G5, G7 and G8) and the second *C. frutescens*, *C. baccatum*, *C. chinense* and *C. pubescens* (G1, G2, G4, G6 and G9). The discriminant qualitative descriptors were the colour of the corolla, the colour of the anthers and position of the flower. The quantitative discriminant characteristics were length, weight and width of the fruit. The participatory selection identified 15 materials by colour, aroma, texture, flavour, size and thickness of fruits. Chemical analyses determined the highest concentration of flavonoids in the accessions 10,757 (16.64 mg/g) and 15,661 (15.77 mg/g). Accessions 17,750 (11.68 mg/g) and 10,757 (11.41 mg/g) presented the highest polyphenol contents. The highest capsaicin concentration was recorded in accessions 16,209 (55.90 mg/g) and 10,757 (48.80 mg/g). The highest antioxidant value was recorded in accessions 17,750 (90.85 mg/g) and 15,661 (87.03 mg/g). All these characteristics are important with a view to increasing industrial use and genetic improvement processes. These results show the existence of significant genetic variability within the genus *Capsicum*.

Keywords: germplasm; genetic resources; accessions; descriptors

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1. Introduction

Chilli pepper (*Capsicum* L.) is one of the most important spice and vegetable crops in the world in agricultural, cultural and economic terms [1–3]. The genus *Capsicum*, native to Tropical America, comprises 27 species [4,5]. The five most widely used species worldwide are: *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens* Ruíz & Pav. [6]. *Capsicum annuum* and *C. frutescens*—which according to The Plant List [7] are considered conspecific, but which are here maintained as separate taxa as they are in The Tropical Agricultural Research and Higher Education Center (CATIE) genebank—are known to have been domesticated in Mesoamerica [5], however the original description of *C. frutescens* by Linneaus was based on a cultivated specimen from India. In the Andean region, chilli peppers have been consumed for more than 5000 years; *C. baccatum*, *C.*

pubescens and *C. chinense* are believed to be native to South America. *Capsicum chinense* was domesticated in the tropical lowlands; while *C. baccatum* possibly was domesticated in the valleys of Bolivia, while the domestication site for *C. pubescens*, which is a tall chilli, remains unknown [8,9]. Although Ecuador has not been considered a centre of origin of the species, archaeological *Capsicum* starches have been found that date back to 6250 years before our era [10].

The five cultivated species are diploid and self-compatible and the number of chromosomes of the species is 2n = 24 [11]. Cross-pollination rate among *Capsicum* spp. is highly variable, ranging from 2 to 90% [12]. The species *C. annuum*, *C. chinense* and *C. frutescens*, form a morphological complex of overlapping characters from a common base of genes; qualitative characters, such as floral characteristics, differentiate the three species morphologically. These are solitary flowers, creamy white in *C. annuum*; the *C. chinense* species presents two or three flowers per leaf node of a whitish-green colour and with a constriction at the base of the calyx at the junction with the pedicel; while the *C. frutescens* species presents an erect white-green flower without calyx constriction. The other species are relatively easy to distinguish: *Capsicum baccatum* has yellow or white-yellow flowers with greenish spots towards the basal part of the petals and *C. pubescens* has either uniformly purple or purple flowers with a white base and black-rough seeds [13,14].

To promote the use of germplasm collections, morphological characterizations are performed to describe the existing genetic diversity, as an example, intraspecific diversity studies for *C. annuum* in Mexico [15] and in India [16,17] are cited. Morphological characterization has allowed identifying intra and inter-specific variability in the case of *Capsicum* [18–22], identifying resistance to pathogens such as *Phytophthora capsici* [23] or insects such as the *Bemisia tabaci* Begomo-virus complex [24]. A combination of morphological and molecular descriptors was used in India by Yumnam et al. [25].

The objectives of this study were: (a) to determine the level of morphological diversity present in 192 accessions of *Capsicum*; (b) to identify the qualitative and quantitative characters with high discriminating capacity; (c) to classify *Capsicum* species into groups based on their quantitative and qualitative characteristics; (d) to identify germplasm through participatory sensory evaluation; and (e) to determine the chemical characteristics of promising materials.

2. Methodology

2.1. Morphological Characterization

The morphological characterization was set up in a field experiment at CATIE location, in Turrialba, Costa Rica (9° 54′ North Latitude, 83° 40′ West Longitude, altitude 625 m a.s.l.). The average annual precipitation is 2700 mm, and the annual average temperature is 22 °C according to CATIE [Centro Agronómico de Investigación y Enseñanza Tropical, CR] [26]. For morphological characterization, we worked with 192 accessions of chilli (*Capsicum* spp.). These accessions come from various collections carried out in Central America and Mexico between 1976 and 2006 and germplasm exchanges carried out between institutions from five continents corresponding to 21 countries (Table 1).

Table 1. Origin and number of accessions of each of the species of *Capsicum* spp.

	***Capsicum* Species**					
Origin	***C. annuum***	***C. frutescens***	***C. chinense***	***C. baccatum***	***C. pubescens***	**Not Identified**
Peru	1	--	--	5	--	--
Panama	3	5	2	--	--	2
Costa Rica	8	30	2	1	--	4
Mexico	21	4	--	1	--	--
Honduras	--	7	2	--	--	1
Guatemala	30	6	--	--	1	6
El Salvador	4	8	--	--	--	--
Ethiopia	5	2	--	--	--	1
Zimbabwe	1	--	--	--	--	--
Spain	2	--	--	--		

Malaysia	10	--	--	--	--	2
U.S.A.	1	--	--	--	--	--
Syria	1	--	--	--	--	--
Russia	2	--	--	--	--	--
Ecuador	--	--	--	2	--	--
Brazil	1	--	--	2	--	1
Philippines	--	--	--	--	--	1
Cuba	--	--	--	--	--	1
Colombia	--	1	--	--	--	--
Nicaragua	--	1	--	--	--	--
Maldives	1	--	--	--	--	--

Twenty plants per accession were installed in the field and the middle 10 evaluated (to eliminate border effect). The descriptors jointly defined by IPGRI [International for Plant Genetic Resources Institute, now BIOVERSITY, AVRDC and CATIE [27] were used, which comprise 57 qualitative and quantitative descriptors (Tables 2 and 3). The data obtained from the morphological characterization were analysed using the InfoStat/Professional program version 2011 [28].

Table 2. *Capsicum* morphological qualitative descriptors.

Descriptor	State	Descriptor	State
Hypocotyl colour	White, Green, Purple	Corolla spot colour	White, Yellow, Yellowish Green, Green, Purple, Other
Hypocotyl pubescence	Sparse, Medium, Dense	Anther colour	White, Yellow, Pale Blue, Blue, Purple, Other
Cotyledon leaf colour	Light Green, Green, Dark Green, Light Purple, Purple, Dark Purple, Variegated, Yellow, Other	Anthocyanin spots or stripes	Absent, present
Cotyledon leaf shape	Deltoid, Oval, Lanceolate, Elongated-deltoid	Intermediate state fruit colour	White, Yellow, Green, Orange, Purple, Dark Purple, Other
Stem colour	Green, Green with purple stripes, Purple, Other	Mature state fruit colour	White, Lemon-Yellow, Pale Yellow-Orange, Yellow-Orange, Pale Orange, Orange, Light Red, Red, Dark Red, Purple, Brown, Black, Other
Node anthocyanin	Green, Light purple, Purple, Dark purple	Fruit shape	Elongated, Almost round, Triangular, Campanulate, Blocky, Other
Stem shape	Cylindrical, Angled, Flattened	Fruit shape at junction with pedicel	Acute, obtuse, truncated, chordate, lobate
Stem pubescence	Sparse, Medium, Dense	Neck at fruit base	Abscent, present
Plant growth habit	Prostrate, Intermediate, Erect	Shape fruit apex	Pointed, Blunt, Sunken, Sunken and pointed, Other
Branching density	Sparse, Medium, Dense	Appendix in the fruit	Absent, present
Tillering	Sparse, Medium, Dense	Fruit cross-sectional corrugation	Slightly corrugated, Inter-mediate, Very corrugated
Leaf density	Sparse, Medium, Dense	Fruit epidermis type (surface)	Smooth, Semi-wrinkled, wrinkled
Leaf colour	Yellow, Light Green, Green, Dark Green, Light Purple, Purple, Heather, Other	Persistence of pedicel with fruit	Slight, Intermediate, Persistent
Leaf shape	Deltoid, Oval, Lanceolate	Persistence of pedicel with stem	Slight, Intermediate, Persistent

Leaf blade margin	Whole, Wavy, Ciliated	Seed colour	Light Yellow, Dark Yellow, Black
Leaf pubescence	Escasa, Intermedia, Densa	Seed surface	Smooth, Rough, Wrinkled
Flower position	Pendant, Intermediate, Erect	Susceptibility to biological stress	Very low or no visible signs of incidence, Low, Intermediate, High, Very high
Corolla colour	White, Light yellow, Yellow, Greenish yellow, Purple with white base, White with purple base, White with purple margin, Purple, Other	Chilli flavour	Sweet, spicy

Table 3. *Capsicum* morphological quantitative descriptors.

Descriptor	Unit of Measure	Descriptor	Unit of Measure
Plant height	cm	Fruit length	cm
Plant width	cm	Fruit width	cm
Plant height/width ratio	cm	Fruit weight	g
Stem length	cm	Fruit pedicel length	cm
Stem width	cm	Fruit wall thickness	mm
Length mature leaf	cm	Days to fruiting	number
Width mature leaf	cm	Weight of 1000 seeds	g
Leaf l/w ratio	number	Placenta length	cm
Days to flowering	number	Seed size	mm
No. flowers leaf axil^{-1}	number	Classification seed no. fruit^{-1}	number
Fruit set	number		

Qualitative and quantitative variables were used in a hierarchical cluster by using Ward's method and Gower distance, Also, contingency tables were used, using Chi-square, and multiple correspondence analyses were made.

2.2. Participatory Sensory Evaluation

For this stage the 192 morphologically characterized accessions were evaluated by 50 invited untrained participants representing producers, industrialists, chefs, scientists and ordinary consumers from Costa Rica, Mexico, Colombia, Ecuador and Peru. The evaluation was carried out in four phases. In the first phase, farmers/producers were invited to the field trial in CATIE to select accessions based on criteria such as colour, shape, size and production. In the second phase, with previously selected accessions, participants made a second evaluation related to shape, colour and size of the fruit. In the third phase, a sensory evaluation was carried out using the 9-point hedonic test proposed by Lawless [29]. However, this scale caused confusion for the attendees, so we decided to change to a friendlier scale, in which six criteria were recorded colour, smell, texture, flavour, size and thickness of the pulp. Each criterion was evaluated based on a 5-point scale: Excellent 5; Very Good 4; Good 3; Regular 2; Bad 1, proposed by Hernández [30]. In the fourth phase, the samples were tasted to determine the degree of fruit spiciness as reported by Hernández [30], the following scale was used: Very spicy 3, Medium spicy 2 and Regular 1. In each scale the assistants marked with an X the criterion they considered adequate based on their preferences. For the analysis of the results, frequency tables were prepared.

2.3. Chemical Characterization

The chemical analysis of the samples was carried out on the best 15 accessions selected by the morphological characterization and the participatory process. One kilogram of fruits, at the same maturity state, was harvested per accession from different parts of the plants to avoid differences in biochemical properties due to plant position according to Zewdie et al. [31]; Kirschbaum-Titze et al.

[32]; Mueller-Seitz [33]. These samples were dried out in oven at 60 °C for 30 h, after which 100 g per accession were sent for analysis. The determination of flavonoid content was conducted using the method proposed by Miean and Mohamed [34]. The determination of antioxidant activity was completed using the method of measurement of the absorption capacity of oxygen radicals proposed by Álvarez-Parrilla et al. [35]. The determination of the content of total phenolic compounds was done according to the method proposed by Blainski et al. [36]. The determination of the concentration of capsaicin and dihydrocapsaicin was completed according to the method proposed by Juangsamoot et al. [37]. Finally, the analysis of the results was carried out through multivariate descriptive statistics.

3. Results

3.1. Descriptive Analysis of Morphological Characters

Evaluation of the characteristics of stem, leaves, flowers and fruits from 192 accessions are summarized follow.

3.2. Stem

The purple hypocotyl character was present in 70 accessions, purple stem in 5 accessions, purple knot anthocyanin in 78 accessions, light purple anthocyanin in 46 accessions and dark purple in one accession. Having accessions with purple characteristics determines the presence of anthocyanins, which are classified as nutraceuticals and appetizing agents. Bhattacharya et al. [38] indicate that anthocyanins minimize the proliferation of cancer cells, prevent lipid damage in food and protect against diseases of the heart. Likewise, Rodríguez and Kimura [39] mention that antioxidants can neutralize or reduce the activity of free radicals, associated with cardiovascular diseases.

3.3. Leaves

The variable leaf shape of the individual cotyledon was recorded according to the lanceolate, elongated-deltoid and oval categories, where the oval category was the most dominant since it was found in 134 accessions, corresponding to 69.79% of the materials evaluated. Likewise, the colour of the mature leaf was mostly green in 159 accessions corresponding to 82.81%. The majority of our accessions (170) had reduced leaf pubescence corresponding to 88.54%. This is in agreement with the results reported by Smith and Heiser [40] who mention that for *C. frutescens* leaf pubescence tends to be scarce.

3.4. Flowers

The colour of the corolla, the colour of the anther, position of the flower, the length of the placenta, and the pubescence of the stem had a marked influence on the discrimination of species. To the extent that the *C. annuum* were characterized by presenting white flowers and a single flower per leaf axil and *C. frutescens* presented a greenish-yellow flower without calyx constriction. It is worth noting that in our study the length of the ovary influenced the grouping of the samples, which differs from IPGRI [27], which do not consider it as highly discriminating for species differentiation. According to Sreelathakumary and Rajamony [41], the length of the ovary is correlated with the size and weight of the fruits and the most extended shelf-life at the market. This descriptor is more significant in *C. frutescens* than in *C. annuum*, and capsaicin is stored in it, also, both weight and size influence good filling of fruit cavities and seed production.

In the same way, this study agrees with the results reported by Hernández et al. [42] who mention that the characteristics of the flowers per leaf axil are an important variable to establish differences between the *C. annuum* and *C. frutescens* species. Note that the two taxa here are treated as separate taxa, though taxonomists in general consider them as being conspecific, as already mentioned; however, for practical reasons they are here considered as representing separate cultivar groups. *Capsicum annuum* accessions were characterized by having solitary flowers and *C. frutescens* for presenting more than one flower per leaf axil. The white colour of the corolla appeared in 47.40% of the accessions evaluated mainly in the G3 and G7, represented by the *C. annuum* species. The corolla's greenish-yellow colour appeared in 36.46% of the accessions, specifically in the G4, G9 and G6 constituted by the species *C. frutescens*. In comparison, the colour of the corolla was light yellow at 7.29%, and in G1 mainly made up of the *C. baccatum* species (Figure 1). The above agrees with Pickersgill [43], who mentions that in *Capsicum*, two groups of flowers are defined: white and purple. In the group of white flowers, there are two subgroups, the one made up of *C. baccatum* and a second that groups *C. annuum*, *C. chinense*, *C. frutescens*. The group of purple flowers are the species *C. eximium* Hunz., *C. cardenasii* Heiser & P.G.Sm., and *C. pubescens*. On the other hand, Smith and Heiser [35] reported that in *C. frutescens*, the flowers are greenish-yellow, and for *C. annuum*, they are white.

3.5. Fruits

According to Andrews [43], the accessions belonging to the *C. annuum* species are characterized by having small, ovoid fruits with two locules, the fruit—a bloated berry—with different colours, e.g., light green, green, purple, yellow, orange and deep red. The *C. frutescens* species is characterized by presenting elongated fruits ending in a blunt point, with two locules per fruit, which agrees with the results found in this work where most of the accessions belong to *C. frutescens* and *C. annuum*.

In the characterized accessions, the following high variation coefficients were registered for the fruit characteristics:

- anthocyanin spots or streaks on the fruit,
- colour of the fruit in the intermediate state,
- colour of the fruit in the mature state,
- the shape of the fruit,
- the shape of the fruit at the attachment of the pedicel,
- fruit apex shape,
- fruit appendix,
- traces of petals and anthers,
- fruit transverse ridges,
- type of fruit epidermis,
- pedicel persistence with fruit,
- pedicel persistence with stem,
- seed colour
- seed surface.

These characters indicate the importance of the descriptor to discriminate variability within a collection. The results corroborate those reported by Smith and Heiser [40,44] who maintain that in each species of chilli pepper there are various fruit shapes and colour of immature fruits. In the accessions evaluated, the shape of the fruit was mostly triangular and elongated; however, small-fruited species tended to be round and conical, especially sweet pepper species. The variability of the genus is mainly due to the characteristics of the fruit, followed by the architecture of the plant, flower structure and the number of flowers per leaf axil [16].

Pickersgill [45] mentions that in *Capsicum*, the annular constriction of the calyx is characteristic of *C. chinense* and is absent in the other four species. For the colour of the immature fruits, it is typical for the fruits among the *Capsicum* spp. to start with a green colour before reaching the final colour at full maturity; however, the fruits in a mature state have mostly red tones and an elongated shape, while *C. chinense* matures with fruit shades of yellow and orange. Yellow colours of fruits in the intermediate maturity stage, i.e., apparent from the results obtained, were recorded in some accessions of both *C. chinense* (7300, 12,154, 5489), and *C. frutescens* (10,946, 10,793, 165,654). Furthermore, the shape of the fruit apex was mostly pointed in the accessions studied and the epidermis of the fruit was smooth, i.e., characteristics that correspond to the *C. frutescens* and *C. annuum* species.

3.6. Grouping of Accessions Based on Morphological Variables

The hierarchical cluster, using the qualitative and quantitative variables identified significant differences among nine accession groups of *Capsicum* spp. Significance ($p < 0.0001$) was obtained employing a multivariate analysis and the differences between mean vectors were obtained using the Hotelling comparison test corrected by Bonferroni [46,47].

3.7. Combined Analysis of Qualitative and Quantitative Variables

The result of the grouping of the accessions—obtained with the Ward method and the Gower distance—allowed us to identify the taxonomic structure of the collection, where the relationship between the groupings could be seen, i.e., *C. annuum* (groups 7, 8, 5 and 3), *C. frutescens* (groups 9, 6, 4 and 2). However, within these groups there are also accessions of *C. chinense* and *C. pubescens* and group 1 formed by the *C. baccatum* species. Group 3 contains the highest number of accessions (36); while Group 1 is made up of nine accessions. The groups with the highest similarity for the qualitative and quantitative variables are Group 1 and Group 2 (Table 4, Figure 1).

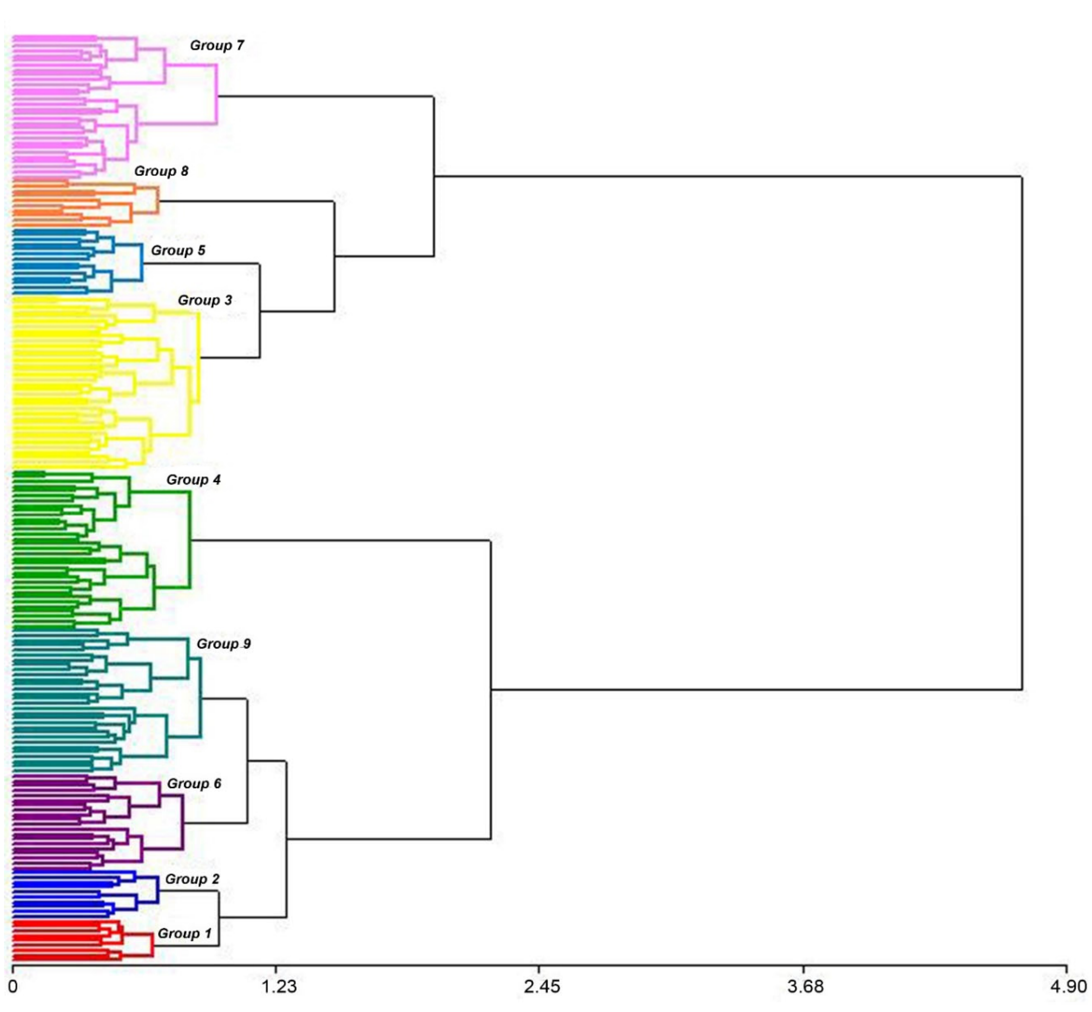

Figure 1. Dendrogram obtained by hierarchical cluster analysis (Ward's method, Gower's distance) with qualitative and quantitative variables from the morphological characterization of 192 accessions of chilli (*Capsicum* spp.).

Table 4. Description of the 9 groups of accessions from Figure 1, including species identification.

Group 1	5440 [b], 7203 [b], 7209 [b], 7417 [b], 16,463 [b], 16,209 [b], 17,268 [s], 18,060 [a], 18,645 [b]
Group 2	7300 [c], 8386 [f], 9892 [f], 10,757 [c], 10,792 [f], 10,793 [f], 14,757 [a], 16,308 [p], 22,115 [s], 22,119 [s]
Group 3	5445 [b], 6123 [a], 7816 [a], 8058 [s], 9135 [a], 9139 [a], 9186 [a], 10,886 [s], 11,757 [f], 12,911 [f], 14,376 [a], 15,239 [a], 15,407 [a], 15,412 [a], 15,422 [a], 15,449 [a], 15,587 [a], 15,632 [a], 15,646 [a], 15,653 [a], 15,661 [a], 15,983 [a], 16,270 [a], 16,297 [a], 16,304 [a], 16,458 [a], 16,462 [a], 16,467 [a], 17,151 [a], 17,294 [a], 18,314 [a], 18,631 [a], 18,651 [a], 18,660 [a], 18,757 [a], 19,259 [a]
Group 4	8248 [f], 8394 [a], 8998 [s], 8999 [f], 9015 [f], 9140 [a], 9159 [f], 9201 [a], 9777 [f], 9781 [f], 9801 [f], 9811 [f], 9832 [f], 9837 [f], 9902 [f], 9923 [f], 10,003 [c], 10,730 [f], 10,762 [f], 10,862 [f], 10,909 [f], 10,951 [f], 11,073 [f], 11,198 [f], 11,744 [f], 11,745 [f], 12,017 [f], 12,097 [f], 12,154 [c], 12,156 [c], 12,910 [b], 13,328 [s]
Group 5	9038 [a], 9096 [f], 9115 [a], 9183 [a], 9226 [a], 11,303 [a], 15,395 [f], 16,450 [a], 16,452 [a], 16,453 [s], 16,454 [a], 16,456 [a], 16,457 [a], 16,460 [a]
Group 6	6126 [f], 7216 [s], 8534 [s], 8567 [a], 9095 [f], 9200 [f], 9204 [f], 10,760 [f], 10,871 [f], 10,903 [f], 10,946 [f], 11,050 [f], 11,717 [s], 15,237 [a], 15,654 [f], 15,932 [a], 16,273 [a], 16,308 [a], 16,513 [f], 18,229 [f]
Group 7	6143 [a], 7818 [a], 7819 [a], 8047 [a], 8055 [s], 8064 [a], 9053 [f], 9110 [b], 9131 [a], 10,630 [a], 10,691 [a], 11,204 [s], 11,232 [a], 11,305 [a], 11,795 [a], 13,963 [a], 14,751 [a], 14,756 [a], 15,389 [s], 15,434 [f], 15,440 [a], 15,640 [a], 15,641 [a], 15,651 [a], 15,658 [a], 15,976 [s], 16,276 [a], 16,451 [s], 16,521 [a], 20,029 [a]
Group 8	9269 [a], 16,459 [a], 16,461 [a], 17,867 [a], 18,156 [a], 18,776 [a], 18,787 [a], 18,788 [a], 18,804 [a], 18,815 [a]
Group 9	6586 [f], 7218 [c], 7257 [a], 8395 [c], 8994 [s], 9016 [f], 9037 [f], 9040 [a], 9043 [f], 9079 [f], 9097 [f], 9103 [f], 9803 [f], 9835 [f], 9839 [f], 9841 [f], 9916 [f], 9917 [f], 9921 [f], 10,005 [f], 10,015 [f], 11,755 [f], 14,776 [f], 15,914 [f], 16,275 [a], 16,280 [s], 17,247 [a], 17,750 [c], 18,778 [a], 20,016 [s]

[a] *C. annuum*; [b] *C. baccatum*; [c] *C. chinense*; [f] *C. frutescens*; [p] *C. pubescens*; [s] *Capsicum* spp.

3.8. Discriminant Values

Regarding the qualitative descriptors, from the 39 characters analysed using the X^2 test, 30 of them were identified with high significance ($p < 0.0001$) (**), and nine were not significant (ns). These results indicated the presence of a large number of descriptors making an essential contribution to separate the nine genetic groups (Table 5), moreover they presented high association coefficients. Likewise, 15 characters with the highest discriminant value were recorded, which can be used to establish genetic differences between groups.

Table 5. Qualitative descriptors with the highest discriminant value influencing the genetic groups' separation of *Capsicum* species.

Character	X^2	Coeficient (P)	Cramer (V)
Colour of the corolla	325.34 **	0.79	0.58
Colour of the anthers	323.70 **	0.79	0.65
Position of the flower	166.04 **	0.68	0.54
Leaf distance	144.82 **	0.66	0.50
Branching distance	141.30 **	0.65	0.50
Susceptibility to biological stress	129.04 **	0.63	0.37
Corollar spot colour	126.36 **	0.63	0.47
Tillering	123.08 **	0.63	0.46
Anthocyanin of the node	106.07 **	0.60	0.37
Cross wrinkling of the fruit	102.59 **	0.59	0.42
Fruit shape at the junction with the pedicel	97.38 **	0.58	0.32
Stem pubescence	95.95 **	0.58	0.41
Leaf pubescence	86.83 **	0.56	0.39
Persistence of the pedicel with the stem	82.99 **	0.55	0.38
Fruit colour in the intermediate state	75.09 **	0.53	0.28
Type of epidermis of the fruit	73.85 **	0.53	0.36
Persistence of the pedicel with the fruit	70.41 **	0.52	0.35
Fruit shape	69.66 **	0.52	0.27
Shape of cotyledonal leaf	66.37 **	0.51	0.34
Colour of the hypocotyl	64.24 **	0.50	0.33
Shape of fruit tip	63.75 **	0.50	0.29
Leaf margin	63.14 **	0.50	0.41
Growth habit	62.76 **	0.50	0.33

Pubescence of hypocotyl	62.48 **	0.50	0.33
Leaf colour	59.32 **	0.49	0.32
Ovary length	51.49 **	0.46	0.30
Seed size	38.59 **	0.41	0.32
Neck at the base of the fruit	33.99 **	0.39	0.30
Cotyledonal leaf colour	31.91 **	0.38	0.24
Anthocyanin stains or streaks of the fruits	27.14 **	0.35	0.27

** = high significance with ($p < 0.0001$).

The colour of the corolla and the colour of the anthers were the characters with the highest discriminant value (325.34 and 323.7 respectively) and presented the highest association coefficients. The position of the flower presented an X^2 value of 166.04 and the highest value according to the Cramer test (0.54); therefore, it has a high contribution to discriminate between genetic groups, as has the colour of the corolla that also provides a discriminating value. These results indicate that the G4 and G9 groups are associated with the white character of the corolla.

Regarding the quantitative descriptors, six were identified with the highest discriminant value: leaf length/width ratio, width mature leaf, fruit length, fruit width, fruit wall thickness, plant height; these descriptors allowed to differentiate the nine groups (Table 6). In addition, we determined that accessions within the groups maintain a close relationship, once there is not much variation since they present small values of standard deviation.

Table 6. Eigenvalues determined by the canonical discriminant function discriminating grouping of *Capsicum* spp. accessions.

Variables	Axis 1	Axis 2
Leaf length/width ratio	2.35	−0.81
Width mature leaf	0.81	0.09
Fruit length	0.36	−0.08
Fruit width	0.23	0.82
Fruit wall thickness	0.09	−0.21
Plant height	0.03	−0.04
Days to flowering	0.01	−0.01
Fruit weight	1.80×10^{-3}	-2.20×10^{-3}
Plant width	−0.02	0.06
Stem length	−0.02	0.02
Days to fruiting	−0.02	−0.02
No. flowers leaf axil-1	−0.51	−0.38
Fruit pedicel length	−0.55	0.45
Weight of 1000 seeds	−0.6	0.19
Length mature leaf	−0.73	−0.1
Plant height/width radio	−1.07	0.47
Stem width	−1.22	0.6

The discriminant analysis found less distance between the species of *C. annuum*, *C. frutescens* and *C. chinense*. These three taxa are separated from *C. baccatum* and *C. pubescens*, because these two species grow in highlands (2800 m a.s.l.), where the climatic conditions differ from the low altitudes (200 m a.s.l.) where *C. pubescens* is distributed through the middle region of the Andes mountain range (1300 m a.s.l.). *Capsicum baccatum* is widely distributed throughout the lowlands of South America, as mentioned by Pickersgill [43]. The studies by García [48] corroborate the previous results; García [48] points out that the morphological characterization did not allow the species of *C. annuum*, *C. chinense* and *C. frutescens* to be differentiated. These observations are in agreement with Pardey et al. [18] who concluded that the species *C. annuum*, *C. chinense* and *C. frutescens* make up the same morphological group; like Vallejo et al. [49] who managed to discriminate the *C. pubescens* and *C. baccatum* species, but were unable to discriminate *C. annuum*, *C. frutescens*, *C. chinense*.

The *Capsicum* population presented morphological variation in the qualitative characteristics of the *C. annuum*, *C. chinense* and *C. frutescens* species because of the shared morphological features among the three species, making taxonomic classification difficult. This agrees with the results of García [48] and Palacios [50] who confirm this intraspecific variability; they also mention that as a result of the morphological description, it could be assumed that the three taxa constitute the same group. Similarly, the studies by Vallejo et al. [49], and Palacios Castro and García [51] managed to discriminate the species of *C. pubescens* and *C. baccatum*, but not between the species *C. annuum*, *C. frutescens* and *C. chinense*. The results of this study continue to corroborate the hypothesis that these three species are a large group in the process of differentiation, which is consistent with the studies conducted by Pickersgill [52].

The results of the present study are in agreement with those by Chávez-Servia [53] and Chávez-Servia and Castillo [54], who reported that variables such as length, width and shape of the *Capsicum* fruit showed considerable genetic variation. The purple colour of the anther was observed in the *C. chinense* species; while *C. baccatum* anthers presented a yellow colouration. On the other hand, *C. annuum* had pale blue anthers and *C. frutescens* blue. The species tended to have no stain on the corolla, except for *C. baccatum*, which is the typical characteristic of this species. The flowers in *C. frutescens* are erect; while in *C. annuum* and *C. chinense* the position of the flowers varied between intermediate and/or hanging.

According to Martín and González [55] and Fernández [56], chilli peppers with large-sized fruits and a thick epidermis tend to be less pungent. In contrast, in the accessions with smaller fruits where also the epidermis is thinner, the concentration of capsaicinoids increases, which is consistent with the results of the investigation, here accession 16,209 presented higher capsaicin content (5590 ug/g), while accession 16,450 registered low capsaicin content (200 ug/g).

3.9. Participatory Sensory Evaluation

The 50 people attending the workshop represented the following categories: producers (30), industry (5), chefs (4), scientists (5) and people who like to consume chilli (6). In the first phase, 134 accessions were selected as 'Very Good', presenting characteristics such as fruits with characteristics such as colour (pale orange, red, dark red and orange), shape (bell-shaped, triangular, elongated, bell-shaped and thick), size (medium and large) and fruit production. In the second phase, participants chose 64 accessions using the shape, colour and size of the fruit as selection criteria. In the third phase, 34 samples were selected with the criteria: taste, odour, texture, pulp thickness and size (Appendix B, Table A1); and, in the fourth phase, the most relevant fruits with orange, pale orange and red epidermis colours were selected. The shapes of the fruit are bell-shaped, and triangular, or bell-shaped and thick; the epidermis are of the fruit is semi-wrinkled and rough and fruits of medium to large size.

At the end of the process, the samples of the accessions were rated as follows:

- As excellent—accession 15,661 (dark red fruit colour, bell-shaped and thick shape, large size)
- As very good—accession 7818 (dark red fruit colour, triangular shape, medium size)
- As good—accessions 16,304 (red fruit colour, flared shape, large size), 10,757 and 22,119 (red fruit colour, flared shape and compact, small size), 9892 (red fruit colour, flared shape, medium size), 9916 and 17,750 (orange fruit colour, bell-shaped, large size), 8994 and 16,209 (red fruit colour, triangular shape, medium size), 17,268 and 9902 (red fruit colour, bell-shaped, medium size).

3.10. Chemical Characterization

The nutritional value was determined in 15 accessions, selected as promising in morphological characterization, corresponding to the taxa *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *Capsicum* spp. Furthermore, these accessions were rated as excellent in the participatory selection. The accessions that had the highest concentration of capsaicin (5.59 mg/g), polyphenol (18.68 mg/g) and flavonoid (16.64 mg/g) were 16,209, 17,750 and 10,757, respectively (Table 7). The content of total polyphenols, flavonoids and capsaicinoids varied in the accessions evaluated, with a tendency to present an association with the morphological classification described by Morán et al. [57]. Appedino [58] studied 13 cultivars of *C. annuum*, finding concentration levels of flavonoids (0.028 and 0.551 mg/g) lower than those found in the accessions in the present study.

Table 7. Flavonoid, polyphenol and capsaicin concentrations in seven accessions representing four species of *Capsicum* in the CATIE genebank.

Species	Accession No.	Flavonoid Concentration (mg/g)	Polyphenol Concentration (mg/g)	Capsaicin Concentration (mg/g)
C. chinense	10,757	16.64 [fh]	11.41	4.88
	15,661	15.77	10.69	0.32
C. annuum	7818	11.41	7.15	2.64
	14,757	11.90	6.91	1.37
	16,304	10.74	5.97	0.99
	16,450	8.94 [fl]	4.52 [pl]	0.20 [cl]
	16,454	11.68	7.83	1.34
	16,457	12.41	7.98	1.65
	16,462	11.48	6.79	1.10
	17,750	13.59	11.68 [ph]	4.60
C. frutescens	7816	12.05	7.46	4.61
	9892	13.30	8.56	3.14
	9902	11.09	5.12	2.80
C. baccatum	16,209	10.30	6.73	5.59 [ch]
Capsicum spp.	11,204	10.04	5.45	2.02

[fh]—highest flavonoid concentration; [fl]—lowest flavonoid concentration; [ph]—highest polyphenol concentration; [pl]—lowest polyphenol concentration; [ch]—highest capsaicin concentration; [cl]—lowest capsaicin concentration.

The results in Table 7 coincide with those found by Cázares et al. [59], who report that the populations of Ma'x ik and Sukurre belonging to the *C. chinense* species presented the highest capsaicin values (2.93 and 4.35 mg/g); while the lowest values reported for *C. annuum* sweet pepper populations (0.20 mg/g).

Additionally, Antonious and Jarret [60] studied different species of *Capsicum*, finding low concentration levels of capsaicin (0.0009 to 0.002 mg/g). Estrada et al. [61] reported increasing levels of capsaicinoids as maturation progresses, finding total capsaicinoid concentrations of between 0.15 to 0.70 mg/g (ps). However, these values are lower than those reported in the accessions studied here, where values between 5.59 to 0.20 mg/g were found (Table 8).

Table 8. Average, standard deviation, coefficient of variation, minimum and maximum values for the characteristics of the nutritional value of 15 accessions of chilli (*Capsicum* spp.).

Variable	Average	Standard Deviation	Coefficient of Variation	Mínimum Values	Maximum Values
Humidity %	6.48	2.87	44.32	2.77	13.75
Polyphenols mg/g	7.62	2.18	28.69	4.52	11.68
Flavonoids mg/g	12.09	2.06	17.02	8.94	16.64
Capsaicinoids mg/g	2.48	1.74	70.12	0.20	5.59
Antioxidants mg/g	68.66	13.69	19.93	47.84	90.85

The Biplot from the principal component analysis of the 15 selected accessions shows the first two principal components explaining variance greater than 92% (Figure 2). The contents of polyphenols, antioxidants and flavonoids are highly correlated with each other and these in turn are correlated with humidity. In the case of capsaicin, its content is independent of the amounts of polyphenols, antioxidants and flavonoids.

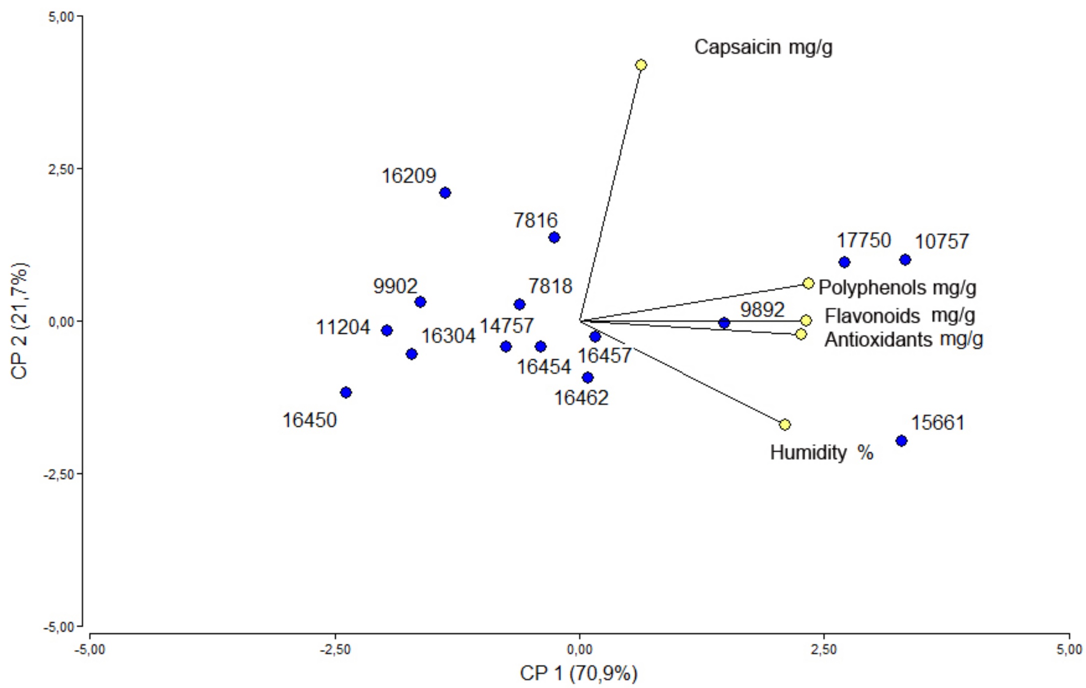

Figure 2. Biplot graph obtained from the principal component analysis of the 15 *Capsicum* accessions.

Accessions 17,750, 10,757, 9892 and 15,661 are those with the highest amount of polyphenols, antioxidants and flavonoids, in turn these four accessions together with accessions 16,209 and 17,262 were the ones that obtained the highest score in the selection made by the producers. Accessions 17,750, 10,757 contain the most capsaicin content, being only surpassed by accession 16,209. On the opposite side, accession 16,450 is the one with the least content of nutritional values and in turn has a low capsaicin content

Of the four selected accessions with the best nutritional content, two of them belong to the genus *C. chinense* (17,750 and 10,757) and the accession (15,661) belongs to the genus *C. annuum*, accession 9892 belongs to *C. frutescens*. Regarding the classification by conglomerates, these four accessions belong to groups G2, G3 and G9. The highest capsaicin content was recorded in accession 16,209 belonging to the species *C. baccatum*.

4. Discussion and Conclusions

The most discriminant qualitative characteristics were colour of the corolla, the colour of the anthers and position of the flower; while the most discriminant quantitative characteristics were leaf length/width ratio, width mature leaf, fruit length, fruit width, fruit wall thickness and plant height. This is similar to previous studies by Medina et al. [62], Pardey et al. [18] Ortiz et al. [20] and Castañón et al. [63] who have also identified colour of the corolla, corollar spot colour, fruit width and fruit length as discriminant variables within other *Capsicum* spp. collections. A practical morphological characteristic found in our *C. annuum* and *C. frutescens* accessions is the persistence of the pedicel with the fruit, According to Sreelathakumary et al. [41] this characteristic, along with the length of the placenta, is correlated with the mass of the fruits and, therefore, longer shelf life.

The agronomic characterization allowed classifying the genetic variability of the *Capsicum* germplasm collection into two large groups and nine subgroups. The two large groups are formed by the subgroups G7, G8, G5 and G3 represented by *C. annuum*, and the second group formed by subgroups G4, G9, G6, G2 having mostly *C. frutescens* accessions plus G1, represented mainly by *C. baccatum.* Subgroup 1 (G1) differentiates from the other subgroups because of the presence of spots on the corolla (Corollar spot colour) similarly observed by García [48], Palacios & García [51]. and Walsh & Hoot [64]. It is important to notice that within all subgroups (except G8) there are few intercalated accessions of *C. baccatum*, *C. pubescens*, *C. chinense* and *C.* spp. (Table 4). *Capsicum* phylogeny determined closer relation among *C. annuum*, *C. frutescens* and *C. chinense* which is known as *C. annuum* complex Pickersgill, [43], Vallejo et al. [49], Pardey et al. [18] and Palacios and García [51].

The description of CATIE's *Capsicum* spp. international collection permitted to identify promising materials e.g., after the morphological and participatory characterization, the following accessions were identified as promising: *C. chinense*—10,757 and 17,750; *C. frutescens*—9892; *C. annuum*—15,661 and 7816; and *C. baccatum*—16,209. Also, four accessions were selected with the highest chemical concentration (polyphenols, flavonoids, and capsaicinoids), two of them belong to the species *C. chinense* (17,750 and 10,757), the accession (15,661) belongs to *C. annuum*, and accession 9892 is referred to *C. frutescens.* These selected materials or the collection as a whole could be used by interested scientist as well as farmers.

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Appendix A

Resumen

Con el objetivo de evaluar las potencialidades de 192 accesiones de ***Capsicum*** L., procedentes de 21 países, se realizó la caracterización morfológica y agronómica mediante la aplicación de 57 descriptores cualitativas y cuantitativas. Los análisis multivariados identificaron dos grandes grupos: *C. annuum* (G3, G5, G7 y G8) y *C. frutescens, C. baccatum, C. chinense* y *C. pubescens* (G1, G2, G4, G6 y G9). Los descriptores cualitativos discriminantes fueron color de la corola, color de las anteras y posición de la flor. Las características cuantitativas discriminante fueron longitud, peso y ancho del fruto. La selección participativa identificó 15 materiales por color, olor, textura, sabor, tamaño y grosor de frutos. Los análisis químicos determinaron la mayor concentración de flavonoides en las accesiones 10,757 (16.64 mg/g) y 15,661 (15.77 mg/g). Los contenidos de polifenoles más alto presentaron las accesiones 17,750 (11.68 mg/g) y 10,757 (11.41 mg/g). La mayor concentración de capsaicina se presentó en la accesión 16,209 (55.90 mg/g) y 10,757 (48.80 mg/g). El valor más alto de antioxidante se registró en las accesiones 17,750 (90.85 mg/g) y 15,661 (87.03 mg/g). Todas estas son características importantes con vistas a incrementar el uso industrial y procesos de mejora genética. Los resultados muestran la existencia de una variabilidad genética significativa en *Capsicum*.

Palabras claves: germoplasma; recursos genéticos; accesiones; descriptores.

Appendix B

Table A1. Results of the participatory sensory evaluation for 34 selected accessions of *Capsicum* spp. CATIE. Costa Rica.

Accession	Taste *	Odour *	Texture *	Pulp Thickness *	Colour *	Size *	Total
15,661	5	5	5	5	5	5	30
7818	4	4	4	4	4	4	24
9139	3	3	4	5	5	--**	20
16,460	3	4	3	5	5	--	20
17,151	4	3	5	4	4	--	20
10,886	4	4	4	4	4	--	20
12,911	3	4	4	4	5	--	20
14,757	3	3	4	5	5	--	20
9916b	3	3	3	3	3	3	18
16,462	3	4	3	4	4	--	18
16,454	4	4	3	4	3	--	18
16,458	4	4	4	2	4	--	18
8994	3	3	3	3	3	3	18
10,757	3	3	3	3	3	3	18
16,209	3	3	3	3	3	3	18
22,119	3	3	3	3	3	3	18
17,268	3	3	3	3	3	3	18
9892	3	3	3	3	3	3	18
17,750	3	3	3	3	3	3	18
16,304	3	3	3	3	3	3	18
9902	3	3	3	3	3	3	18
11,204	4	3	3	4	3	--	17
16,450	4	3	3	4	3	--	17
16,457	4	3	3	3	3	--	16
7816	--	3	--	3	4	--	10
9186	--	--	--	5	3	--	8
11,745	--	1	--	2	3	--	6
16,521	--	--	3	--	3	--	6
18,757	--	--	--	2	3	--	5
20,016	1	--	--	2	2	--	5
16,275	--	1	1	--	1	1	4
18,778	1	--	1	1	1	--	4
9183	--	--	1	--	2	--	3
7819	--	--	--	1	--	--	1
Total							510

* Excellent 5; Very Good 4; Good 3; Regular 2; Poor 1; ** Variables that were not of interest.

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