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AGROMORPHOLOGICAL TRAITS AND BIOACTIVE COMPOUNDS OF FOUR MEXICAN CHILI PEPPERS (*Capsicum annuum* var. *annuum* L.)

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ABSTRACT

In Mexico, diverse morphotypes of chili pepper with local recognition are distributed regionally contributing to the *in-situ* conservation of *Capsicum* diversity in the hands of farmers, as is the case of *Huacle* pepper in Oaxaca. The objective of this study was to evaluate the variation among morphotypes of *Huacle*, *Guajillo*, *Ancho* and *Pasilla* chili peppers, based on agromorphological traits, bioactive compounds and antioxidant activity in fruits. The morphotypes were established in greenhouse conditions under a random block design, obtaining fruit at physiological maturity for laboratory analysis. Significant differences ($P \leq 0.01$) were determined in the analysis of variance among and within morphotypes for all the agromorphological characters evaluated, except in plant height at 120 days after transplanting (dat) and specific weight. Different growth patterns were evaluated from 30 to 120 dat, but the final height was similar. Size, weight, shape, pericarp thickness, locules, number and total weight of fruits per plant were used to determine the variation among and within morphotypes. Morphotypes of *Huacle* (CH-4, CH-9 and CH-15) and *Ancho* (AN-R) presented the highest values of fruits, total weight and a low number of fruits per plant. Among morphotypes, significant differences ($P \leq 0.01$) were observed for fruit composition, but not for antioxidant activity. Also, significant differences were seen in vitamin C, flavonoids content, and antioxidant activity. In fruit composition, *Huacle* and *Guajillo* peppers presented a higher vitamin C content ($4.5 \text{ mg acetic acid g}^{-1}$). *Huacle* pepper was also high in total polyphenols ($31.5 \text{ mg GAE g}^{-1}$). *Ancho* pepper showed a higher content of carotenoids ($2.8 \text{ mg } \beta\text{-carotene g}^{-1}$) and total flavonoids (3.3 mg QE g^{-1}). *Pasilla* pepper showed low values in all the evaluated compounds. In conclusion, all morphotypes showed variation in fruit characters (weight, length, width, pericarp thickness, number of locules and fruit length/width ratio). In fruit composition, the variation was in vitamin C, carotenoids, flavonoids and polyphenols, but not in antioxidant activity.

Key words: Morphotypes, *Huacle* pepper, *Ancho* pepper, *Guajillo* peppers, fruit composition, phenolic compounds, vitamin C, antioxidant activity



INTRODUCTION

Peppers are one of the most popular vegetables across the world, and they are consumed raw, cooked, and dried for use as a spice by nearly 25% of the world's population [1]. Chili peppers are ranked as the third-largest vegetable crop globally after legumes and tomatoes [2].

The chile (*Capsicum annum var. annum L.*) is native to Mexico [3]. In the Mexican territory there is a great diversity (in color, form, pungency, aroma and flavor) of chile fruits. These fruits are consumed both fresh and dried. Therefore, the diversity of local morphotypes of dried chile is wide, for example: Ancho, Apaxtleco, Cascabel, Chilcostle, Costeño, De Árbol, De Onza, Guajillo, Huacle, Mirasol, Morita, Mulato, Pasilla, Piquín, Puya, Tabaquero, Taviche, among others [4]. However, some chile morphotypes are in danger of disappearing due to low production in their region of origin [5]. An example of this is the *Huacle* chile, which is the main ingredient for the preparation of mole negro. Black mole is an emblematic dish of Oaxacan cuisine, whose gastronomy is one of the most outstanding in Mexico.

It is necessary to investigate the genetic, chemical and agromorphological variability of local chile morphotypes to support their conservation and sustainable use. Some antecedents of this type of studies in Mexico are: the morphological characterization of Guajillo chile from Zacatecas and Durango [6]; the characterization of Ancho, Loco and Miahuateco chile populations from Puebla [7]; the description of plant and fruit variability of Costeño chile from Oaxaca [8], and the characterization of green and ripe fruits of Agua, *Huacle* and Pasilla chile from Oaxaca [9].

The agromorphological and genetic variability of local chiles needs to be documented. Some areas that should be studied are: the Pacific coastal region of the states of Jalisco, Michoacán, Guerrero, Oaxaca and Chiapas, as well as the Atlantic region in the states of Veracruz, Tabasco and Campeche. Some advances in the evaluation of genetic diversity are: descriptions using genetic markers such as microsatellites [10, 11], RAPDs [12] and genotypifications based on SNPs [13]. The study of the characterization and distribution of *Capsicum* diversity in Mexico has progressively advanced [14], but the diversity of this genus in the tropical and subtropical regions of Campeche, Chiapas, Guerrero, Michoacán, Oaxaca and Veracruz has been scarcely documented.



Chili fruits contribute nutritional compounds to the diet such as vitamin A, E and B, calcium, iron, fiber, protein, carbohydrates, amino acids and functional compounds with antioxidant properties such as ascorbic acid, phenolic compounds, flavonoids, capsaicinoids and carotenoids [15-18]. In relation to the nutritional content of dried chili peppers from Mexico, it has been shown to contain: ash, fiber, fat, protein, polyphenols, and carotenoid content in De arbol, Chipotle, Guajillo, and Morita chiles [19]; pigment profile in Guajillo, Ancho, and Mulato chiles [20]; carotenoid profile in Ancho and Pasilla chiles [21]; the capsaicinoid content in twenty-two populations of Mihuateco, Copi, Mulato and Ancho chiles from Tecamatlán, Puebla [22]; the capsaicinoid content in *Huacle* chiles [23], and the content of vitamin C, anthocyanins, phenols and antioxidant activity in a collection of thirty populations of Guajillo chiles [24].

Advances in the evaluation of the chemical and nutraceutical composition of dried chili peppers are relevant, but have limitations. For example, the Ancho and Guajillo morphotypes have been studied more frequently, but regional types such as Puya, Yahualica from Jalisco, Apaxtleco from Guerrero, Piquín and *Huacle* from Oaxaca, are still marginalized and underexploited, despite the gastronomic importance of the *Huacle* chile mentioned above.

Regional chili peppers or landraces are conserved *in-situ* in farmers' fields, are at risk of loss of variability and genetic erosion, and are not known beyond their regions of distribution [5]. In this context, the objective was to evaluate the variation of eleven morphotypes of *Huacle*, Guajillo, Ancho, and Pasilla chiles, based on agromorphological characters, as well as the content of vitamin C, carotenoids, polyphenols, flavonoids, and antioxidant activity of the fruits, with the purpose of initiating a proposal for genetic improvement of *Huacle* chiles with these morphotypes.

MATERIALS AND METHODS

Biological material, establishment of the experiment and characterization

The evaluation material was made up of six populations of *Huacle* chile (*Capsicum annum* var. *annum* L.) from San Juan Bautista Cuicatlán, Oaxaca (17°47'41.63" to 17° 48'9.87" LN, 96°57'19.44" to 96°57'52.83" LW), and from 600 to 623 m altitude collected from November 2016 to April 2017. The six collections were CH-04, CH-05, CH-06, CH-09, CH-10 and CH-15), and the controls included two collections of Guajillo pepper donated by two farmers from Zacatecas (GU-P and GU-NP), two



collections of Pasilla pepper (PAS-1 and PAS-2) and a red Ancho pepper (AN-R) acquired at the Oaxaca supply center.

The main objective of the biological diversity of cultivated species *ex-situ* evaluation is to know the outstanding characteristics as a gene source for breeding programs for the development of new varieties and hybrids of pepper crops. For this reason, the collection of peppers was cultivated in a greenhouse, conditions different from those of *in-situ* conservation, located in the ex-hacienda of Nazareno, Santa Cruz Xoxocotlán, Oaxaca, in the facilities of the Technological Institute of the Valley of Oaxaca at 1530 m of altitude, sub-warm climate and average annual temperature ranges 17.6 to 22.9 °C.

Transplanting was done on February 25th, 2019 in a randomized complete block design with three replications. Inside the greenhouse, from February to July, the minimum temperature ranged from 10 to 15 °C and maximum from 27 to 38 °C, minimum relative humidity from 25 to 54% and maximum from 78 to 95%. Fertilizer application was carried out through a drip irrigation system using the formulas ultrasol® 15-30-15, 18-18-18, 13-6-40 and calcium nitrate. Pests and diseases control were done through applications of dimetri (1mL/L of water), confidor (1mL/L), neem extracts (*Azadirachta inidica* A. Juus.; 1 mL/L of water), and cupravit (1g/L of water). For characterization and physiological and agromorphological evaluation purposes the height plant (n=5 whit 3 repetitions) was recorded at 30, 60, 90 and 120 days after transplantation (dat), height at the first floral cluster, days elapsed from transplant to flowering, number and total weight fruit per plant, average fruit weight, fruit length and width, fruit length/width ratio, pericarp thickness, number of locules and specific weight (weight/10 fruits volume).

Vitamin C, β -carotene, polyphenols, flavonoids and antioxidant activity evaluation in fruits

Each experimental plot gave 300 g of harvested fruits, which were washed with distilled water, dried and ground in a food processor for 30 seconds. One fraction was separated for immediate vitamin C and β -carotene determinations, and the second fraction was frozen at -20 ° C until phenols, flavonoids and antioxidant activity analysis were performed.

Vitamin C was determined according to the method described by Vera-Guzmán *et al.*

□15□ Extracts absorbance was measured at a wavelength of 520 nm in a UV-vis



spectrophotometer (UV-1800 Shimadzu Corporation Kyoto, Japan) and the vitamin C content was calculated based on the L-ascorbic acid adjusted curve (99% purity; Reg. 84272 Sigma, St. Louis, Missouri, USA). The concentration was reported as mg of ascorbic acid per g of dry base (mg AA g⁻¹ d.b.).

Total carotenoids were determined based on the method described by Vera-Guzmán *et al.* [15]. The extract absorbance was measured at 471 nm on a UV-vis spectrophotometer. Carotenoids were quantified based on a β -carotene adjusted curve (β -carotene with 97.0% purity, Reg. 18174416; Fluka). The concentration was reported as mg of total carotenoids per g of dry base (mg BC g⁻¹ d.b.).

To evaluate total polyphenols, total flavonoids and antioxidant activity content, a different extract was prepared using 1 g of ground sample mixed with 60% ethanol for phenols and flavonoids, and for antioxidant activity, 1 g mixed with 80 % methanol was used. All these cases were homogenized for 30 seconds with a homogenizer (Ultra turrax), centrifuged and the supernatant was filtered for each analysis by spectrophotometry.

Total polyphenols variation was determined by the method reported by Vera-Guzmán *et al.* [15]. The sample extract was evaluated at an absorbance of 750 nm in a UV-vis spectrophotometer. The polyphenols concentration was estimated based on the adjusted curve of a gallic acid standard (3, 4, 5-trihydroxybenzoic acid 97.5% pure; Reg. 2050271 Sigma, St. Louis, Missouri, USA). Results were expressed in milligrams equivalent of gallic acid per g of dry base (mg GAE g⁻¹ d.b.).

Total flavonoid content was determined by the aluminum chloride colorimetric method [15]. The extract absorbance was measured at 425 nm in a UV-vis spectrophotometer. Flavonoid concentration was estimated based on an adjusted curve of the quercetin standard (2- (3, 4-dihydroxyphenyl) -3,5,7-trihydroxy-4H-1-benzopyran-4-one with 98% purity; Reg 317313 Sigma, St. Louis, Missouri, USA). Results were expressed in equivalent mg of quercetin per g of dry base (QE mg g⁻¹ d.b.).

Antioxidant activity was evaluated by DPPH (2,2-Diphenyl-1-picrylhydrazyl) and FRAP (antioxidant power of iron reduction) methods [15]. Absorbances were measured in a UV-vis spectrophotometer at 517 nm, using 80% methanol as target, and results were reported in μ mol equivalent of Trolox g⁻¹ on a dry basis (μ mol ET g⁻¹ d.b.). The activity



by the FRAP method was evaluated as described in Vera-Guzmán *et al.* [15]. Samples absorbances were measured in a spectrophotometer at 593 nm and the results were expressed in $\mu\text{mol ET g}^{-1} \text{ d.b.}$

Statistic analysis

From the variables record and the integration of agromorphological characters and fruits chemical composition databases, analysis of variance, was carried out using the linear model of random blocks with collections into morphotypes grouping (Huacle, Guajillo, Ancho and Pasilla peppers), nesting of collections in morphotypes and nesting of number of plants or laboratory replicas in repetition, and subsequent comparisons of means by the Tukey method ($P \leq 0.05$). All analyzes were performed with the SAS statistical package, version 9 [25].

RESULTS AND DISCUSSION

Agromorphological variation

Analysis of variance shows significant differences ($P \leq 0.01$) among groups of morphotypes and collections for all the agronomic, morphological and physiological characters evaluated, except in plant height 120 days after transplanting (dat) and specific weight, respectively (Table 1). These differences indicate that the Huacle, Pasilla, Guajillo and Ancho peppers morphotypes differ significantly in one or more plant characters, flowering or fruit, and that they are possibly related to their genotypic variation derived from criteria and selection pressures to which they were submitted during its domestication in different regions of Mexico [26].

In physiological and fruit plant characters, differences among Huacle, Guajillo, Ancho and Pasilla chiles stand out. In plant growth at 30, 60 and 90 days, two patterns were integrated, *Huacle* and Guajillo morphotypes showed higher growth in contrast to Ancho and Pasilla with lower height, but finally at 120 days after transplanting (dat) they statistically presented similar height, and indicates that the highest growth rate in initial phases is in favor of the first group. However, the height of the first flower cluster was slightly higher in the Ancho type than in the other morphotypes. In this sense, flowering was intermediate in Guajillo and Ancho (52.5 to 54.8 days), early in *Huacle* (49.5 days) and Pasilla was late at 62.0 days (Table 2). In the case of Ancho, the value here reported differs from that reported by Toledo-Aguilar *et al.* [10] who recorded blooms from 77 to 91 dat in Ancho peppers from Puebla, Mexico, and also markedly different from the Guajillo pepper collections evaluated [6].



Characteristics related to fruit shape, size and density define the variation among and within morphotypes. For example, *Huacle* pepper fruits have a triangular flared shape, block or square with three locules, shorter intermediate width (63.1 mm), intermediate width (40.1 mm), average pericarp thickness and high specific weight (2.2 g/mL). Guajillo pepper is the longest (112 mm), and smaller width (18.7 mm), elongated and narrowest, with less pericarp thickness and low fruit density (1.7 g/mL). Ancho pepper is twice the length (44 mm) of Guajillo and Pasilla, as width with similar Pasilla pepper pericarp thickness, fewer than three locules, and high specific weight similar to *Huacle* pepper. Pasilla pepper is characterized by being elongated (85.9 mm), with less width (24.6 mm), greater pericarp thickness but very low specific weight similar to Guajillo, with low fruit weight (Table 2). Fruit shapes and sizes of each morphotype are due, in part, to a strict selection of fruit made by producers because the consumer demands representative type of fruits even when they are not commercial or improved varieties [27, 23]. In this sense, it could be pointed out that in Oaxaca, there is a direct relationship in the preference for chili peppers consumption and the region of origin where it is grown or produced and influences the *in-situ* preservation of autochthonous or local varieties [27].

In characters associated with performance the variation among and within morphotypes were also evident. In number of fruits per plant, the pattern was *Huacle* > Guajillo > Ancho > Pasilla, averages from 10.5 to 24.3, but in total fruit weight per plant it was Ancho > *Huacle* > Guajillo > Pasilla (160 to 126 g / plant) and in fruit mean weight was Ancho > *Huacle* > Pasilla > Guajillo (12.5 to 34.9 g / fruit) (Table 2). All this make them distinguishable without any problem about their identity, in addition to being part of the Mexican gastronomic culture. It should be noted that the *Huacle* pepper stood out with the number of fruits (24 fruits), surpassing the 12 fruits obtained in yellow, red and black *Huacle* pepper genotypes, with inorganic fertilization and 7 fruits with compost applications [28]. In addition, it exceeds in fruit size (6.3 and 4 cm), compared to the 5.7 cm of polar and equatorial diameter of 3.6 cm, respectively, obtained with three levels of ammoniacal nutrient solution [29].

High agromorphological variability was observed among collections, except in fruit specific weight (g/mL). In plant height 120 dat, variation in *Huacle* pepper was 115.8 to 156.1 cm, in Guajillo pepper was 125.6 to 139.4 cm and in Pasilla pepper was 116.7 to 140.5 cm. In their place of origin, *Huacle* chile plants grown in the open do not exceed 70 cm in height [23]. Outside their place of origin, *Huacle* chile plants grown in



the open reach 145 cm and, in greenhouse 178 cm [29]. In Guajillo and Poblano peppers, plants do not exceed 60 cm [6, 7].

Flowering in *Huacle* pepper ranged from 45.6 to 52.7 dat, from 48.9 to 56.1 dat in Guajillo pepper and from 61.5 to 62.5 dat in Pasilla pepper. This indicates that Pasilla was later and with less variation, and it was reflected with lower number and weight of fruits per plant. Collections of *Huacle* CH-6, CH-9 and CH-15 were promising, early and with a greater number and weight of fruits per plant, similar to the Guajillo GU-P that presented the longest fruit (12.7 cm), but these two late Pasilla collections (PAS-1 and PAS-2) showed the lower number and weight of fruits per plant. In fruit width, CH4 and CH-15 stood out, 49.3 and 53.4 mm, respectively, and of all these collections, the Guajillo GU-NP presented the narrowest with 12.5 mm. Length and width ratio of fruit is an indicator of the shape and type of fruit, Pasilla pepper is more than three times as long as it is wide, but in Guajillo it is more than five times as long as it is wide, Ancho pepper is twice as long as wide but *Huacle* pepper regularly tends to be square, frequently less than twice as long as wide, even CH-15 was wider than long (Table 3). All these plant, flowering and fruit characters were relevant to show the variation among and with in morphotypes, and indicate that *Huacle* is significantly different from the Pasilla, guaje and Ancho morphotypes.

Variation in bioactive compounds and antioxidant activity

Significant differences ($P \leq 0.01$) among morphotypes were shown in laboratory analyzes for all variables except antioxidant activity. Among collections within morphotypes, the variables that did not show significant differences ($P > 0.05$) were carotenoid and polyphenol content (Table 4). Consequently, the Pasilla, Guajillo, Ancho and *Huacle* morphotypes are differentiable in bioactive compounds but not in antioxidant activity evaluated by FRAP and DPPH. However, within each morphotype, collections can be differentiated by vitamin C and total flavonoids content, and antioxidant activity.

There were significant differences in fruit composition among morphotypes. In vitamin C content, Guajillo and *Huacle* peppers had a higher concentration (4.5 mg AA g^{-1}) than Ancho and Pasilla peppers ($<2.2 \text{ mg AA g}^{-1}$). In carotenoids content, Ancho pepper had the higher concentration of all morphotypes. However, the Pasilla pepper, widely used in the preparation of different moles, presented the lowest values in vitamin C, carotenoids, polyphenols and flavonoids. In this study, *Huacle* pepper presented the highest polyphenols concentration and Ancho pepper of flavonoids



(Table 5). In vitamin C content in Guajillo, another study reported a value of 200 mg AA 100 g⁻¹ [24], while in this work it was 450 AA 100 g⁻¹, representing contrasting differences that may be related to genotype, agronomic management, and laboratory estimation methods. Similarly, the estimation of carotenoids by HPLC in Guajillo and Ancho chiles generated by [20] was less than 7.6 mg 100 g⁻¹, lower than that of this work (50 to 280 mg BC 100 g⁻¹) estimated by spectrophotometry.

Within each morphotype, a high variation among collections was quantified, in vitamin C and flavonoid content, and antioxidant activity evaluated by FRAP and DPPH. In vitamin C, Huacle, Guajillo and Pasilla peppers presented a variation from 2.7 to 5.9, 3.0 to 5.9 and 0.6 to 0.7 mg AA g⁻¹, respectively. That is, among morphotypes a pattern is distinguished as follows: *Huacle* ≈ Guajillo > Ancho > Pasilla (Table 6). The variation among collections with respect to carotenoids was 0.4 to 2.8 mg BC g⁻¹ and in polyphenols from 10.9 to 35.8 mg GAE g⁻¹, they did not present significant differences. In terms of flavonoid content, the Ancho pepper collection (AN-R) presented the highest value significantly (3.3 mg QE g⁻¹), followed by the CH-4 *Huacle* collection (2.3 mg QE g⁻¹) and later the other collections where variation was less than 1.9 mg QE g⁻¹, the estimates show that the value of carotenoids in Ancho pepper is more than twice that in Guajillo and Pasilla peppers and close to double in five Guajillo pepper collections. This fact may be an indicator of the high preference of Ancho pepper to prepare various regional stews [21].

In antioxidant activity -evaluated by DPPH and FRAP methods- it was observed that the Guajillo GU-P collection presented the highest antioxidant activity while the Guajillo CH-6 collection had the lowest activity (Table 6). In the first case, GU-P presented higher vitamin C content and intermediate to low values in the other compounds, while CH-6 regularly showed intermediate to low values in all compounds, even though a linear relationship cannot be established between the evaluated compounds and their antioxidant activity.

Huacle pepper is a local and popular morphotype in Oaxacan food to prepare mole negro, and the Pasilla, Ancho and Guajillo morphotypes are widely known in Mexico and are popular in the preparation of different regional moles. Thus, they are all used to prepare moles. Variation among and within morphotypes is due to fruit composition and antioxidant activity. Within the *Huacle* morphotype, fruit chemical composition is an indicator of variation. This pattern was reported among morphotypes of native



Oaxacan chiles [15□ among Guajillo, Morita, Chipotle, and De árbol [19□ and among Ancho, Guajillo, and Mulato [20□

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Among morphotypes of Huacle, Guajillo, Ancho and Pasilla chiles, there were variations in fruit characters (weight, length, width, pericarp thickness, number of locules and fruit length/width ratio). In fruit composition, Ancho, Pasilla, *Huacle* and Guajillo morphotypes showed variations in vitamin C, carotenoids, flavonoids and polyphenols, but not in antioxidant activity. *Huacle* had more than twice the polyphenol content of the other morphotypes. Within each morphotype the variation was in vitamin C, flavonoids and antioxidant activity. The Pasilla morphotypes presented the lowest values in fruit composition.

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Table 1: Mean square of the analysis of variance in plant and fruit characters in Huacle, Guajillo, Ancho and Pasilla chiles, grown in greenhouses

Evaluated Caracters	Morphotype (M)	Collection/M	Rep. (R)	Plant/Rep	Error	C.V (%)
Plant height 30 dat ¹	34.4*	116.4**	259.9**	4.5 ^{ns}	13.4	17.3
Plant height 60 dat	778.2**	1797.5**	2042.8**	67.3 ^{ns}	113.7	17.2
Plant height 90 dat	1143.3*	3536.2**	5568.9**	90.9 ^{ns}	268	14.6
Plant height 120 dat	480.4 ^{ns}	2790.3**	6014.9**	58.2 ^{ns}	252.3	12.1
Days from transplant to flowering	1123.2**	147.1**	779.8**	17.7 ^{ns}	23.9	9.2
Height to first flower cluster	105.2*	632.5**	74.8*	12.3 ^{ns}	20.8	15.3
Total fruit weight	220458**	49248*	158648**	8668 ^{ns}	14114	19.1
Number of fruit	898.8**	242.2*	649.9**	63.1 ^{ns}	73.7	22.3
Average fruit weight	1659.1**	467**	125.8*	62.4 ^{ns}	38.2	16.4
Fruit lenght	61338**	9372**	1319*	216.8 ^{ns}	221.8	18.8
Fruit width	14496**	3462**	386.4**	38.5 ^{ns}	39.9	18.7
Fruit Lenght/width	559.5**	39.3**	5.9**	0.7 ^{ns}	0.8	13.7
Pericarp thickness	10.4**	3**	5.4**	0.2 ^{ns}	0.3	23.6
Number of locules	10.2**	3.7**	3.1**	0.4 ^{ns}	0.3	19.3
Specific weight	0.9**	0.1 ^{ns}	0.1 ^{ns}	0.1 ^{ns}	0.08	14.2

¹dat=days after trasplant; ^{ns}not significant (P > 0.05); *significant to P ≤ 0.05; **significant to P ≤ 0.01; C.V. = coefficient of variation



Table 2: Behavior of Huacle, Guajillo, Ancho and Pasilla peppers grown in a greenhouse, in agromorphological characters

Variables evaluated	<i>Huacle</i> (6)	Guajillo (2)	Ancho (1)	Pasilla (2)
Plant height 30 dat [†] (cm)	21.6 a ^{††}	21.2 ab	18.8 b	20.7 ab
Plant height 60 dat (cm)	64.4 a	63.2 a	53.1 b	57.3 ab
Plant height 90 dat (cm)	116.0 a	111.0 a	105.5 b	105.1 b
Plant height 120 dat (cm)	132.4 a	132.5 a	122.5 a	129.0 a
Days from trasplant to flowering	49.5 c	52.5 bc	54.8 b	62.0 a
Height to first flower cluster (cm)	29.9 b	29.0 b	33.3 a	27.9 b
Fruits total weight/plant (g)	320.4 b	241.9 bc	426.0 a	160.0 c
Number of fruits/plant (average)	24.3 a	17.5 b	12.0 bc	10.5 c
Fruits mean weight (g)	20.0 b	12.5 c	34.9 a	15.5 bc
Fruit lenght (mm)	63.1 d	112.0 a	96.0 b	85.9 c
Fruit width (mm)	40.1 b	18.7 d	44.0 a	24.6 c
Fruit Lenght/width (mm)	1.8 d	6.7 a	2.2 c	3.6 b
Pericarp thickness (mm)	2.2 b	1.8 c	2.6 a	2.5 a
Number of locules	3.0 b	2.6 c	2.6 c	3.3 a
Specific weight (g/mL)	2.2 a	1.7 bc	2.1 ab	1.6 c

[†]dat=days after trasplant; ^{††}Among morphotypes, means with the same letter do not differ significantly (Tukey test, $P \leq 0.05$)

Table 3: Variability in plant and fruit characters between collections of Huacle, Guajillo, Ancho and Pasilla peppers, greenhouse grown

Var.	CH-4†	CH-5	CH-6	CH-9	CH-10	CH-15	GU-P	GU-NP	AN-R	PAS-1	PAS-2
V1††	25.7 a [†]	20.9 acd	16.9 d	23.3 ab	20.2 bcd	22.5 abc	23.3 ab	19.1 bcd	18.8 cd	20.4 bcd	20.9 bcd
V2	85.3 a	57.7 b-e	49.6 e	69.9 b	58.3 b-e	65.7 bc	62.9 bcd	63.5 bc	53.1 cde	50.7 ed	63.6 bc
V3	146.6 a	110.0 bcd	101.4 dc	122.1 b	110.4 bcd	105.7 bcd	106.9 bcd	115.1 bc	105.5 bcd	92.6 d	116.8 bc
V4	156.1 a	128.9 bc	123.0 bc	136.7 b	134.0 bc	115.8 c	139.4 ab	125.6 bc	122.5 bc	116.7 c	140.5 ab
V5	52.7 cde	52.5 c-f	46.8 fg	45.6 g	49.8 d-g	49.7 d-g	48.9 efg	56.1 bc	54.8 cd	61.5 ab	62.5 a
V6	41.1 a	29.9 bcd	20.3 f	27.6 cde	26.8 cde	33.9 b	31.7 bc	26.3 de	33.3 b	23.6 ef	31.9 bc
V7	351.5 ab	236.4 be	305.5 ad	380.7 ab	280.1 be	336.0 ab	317.2 abc	161.1 de	426.0 a	149.3 e	166.9 cde
V8	15.0 bc	17.7 bc	21.9 ab	21.1 ab	18.2 bc	12.2 bc	19.4 abc	29.5 a	12.0 bc	9.8 c	10.9 bc
V9	24.3 bc	14.5 d	15.4 d	17.9 cd	16.4 cd	28.3 ab	18.5 cd	6.1 e	34.9 a	15.5 d	15.5 d
V10	55.0 f	78.2 de	59.4 f	73.6 e	71.7 e	40.4 g	127.0 a	97.0 b	96.0 bc	85.9 cd	85.8 d
V11	49.3 a	27.8 e	35.0 d	39.4 c	35.6 cd	53.4 a	24.9 e	12.5 f	44.0 b	24.0 e	25.1 e
V12	1.1 ef	3.0 c	1.7 de	1.9 d	2.2 d	0.8 f	5.4 b	7.9 a	2.2 d	3.6 c	3.6 c
V13	2.3 abc	1.9 d	2.2 cd	2.3 bc	2.4 abc	2.4 abc	2.1 cd	1.4 e	2.6 ab	2.7 a	2.3 abc
V14	3.2 abc	2.6 e	2.8 ed	3.0bcd	2.9 cde	3.5 a	2.5 e	2.7 de	2.6 e	3.3 ab	3.4 ab
V15	2.5 a	2.3 a	2.1 a	2.3 a	2.1 a	2.1 a	1.9 a	1.4 a	2.1 a	1.5 a	1.6 a

†CH = Huacle, GU = Guajillo, AN-R= Ancho rojo y PAS= Pasilla; ††V1-V4, Plant height to 30, 60, 90 and 120 days after trasplante (dat, cm); V5, Days to flowering; V6, Height to 1st flower cluster (cm); Agronomic characters: V7, fruits total weight (g); V8, number of fruits; V9, fruits weight (g); V10, fruit length (mm); V11, fruit width (mm); V12, fruit length/width; V13, pericarp thickness (mm); V14, number of locules; V15, specific weight (g/vol. 10 fruits). †Between collections, means with the same letter differ significantly (Tukey test, P ≤ 0.05)

Table 4: Mean squares of the analysis of variance of bioactive compounds and antioxidant activity in Huacle, Guajillo, Ancho and Pasilla chile fruits

Variation sources	Vitamin C	Carotenoids	Polyphenols	Flavonoids	Antioxidant activity	
					DPPH	FRAP
Morphotypes (M)	8.5**	2.4**	29.9**	10.2**	34.2 ^{ns}	267.5 ^{ns}
Collection/M	0.1**	0.05 ^{ns}	0.5 ^{ns}	0.3**	234.8*	542.8**
Repetition (R)	0.2 ^{ns}	0.3*	26.5**	1.5**	589.6**	1814.5**
Lab Replica/R	0.004 ^{ns}	0.001 ^{ns}	0.005 ^{ns}	0.01 ^{ns}	3.5 ^{ns}	13.1 ^{ns}
Error	14.2	0.04	0.7	0.04	58.1	99.2
C.V. (%)	24.0	17.0	19.0	11.6	21.7	16.7

^{ns} non significant (P > 0.05); *significant to P ≤ 0.05; **significant to P ≤ 0.01; C.V. = coefficient of variation. DPPH: 2,2-Diphenyl-1-picrylhydrazyl. FRAP: Antioxidant Power of Iron Reduction



Table 5: Divergences and similarities between morphotypes of greenhouse grown chili peppers in compound content and antioxidant activity

Morphotype (n)	Vitamina C (mg AA g ⁻¹)	Carotenoids (mg BC g ⁻¹)	Polyphenols (mg GAE g ⁻¹)	Flavonoids (mg QE g ⁻¹)	DPPH (μ mol TE g ⁻¹)	FRAP (μ mol TE g ⁻¹)
<i>Huacle</i> (6)	4.5 a†	1.8 b	31.5 a	1.9 b	34.3 a	58.4 a
<i>Guajillo</i> (2)	4.5 a	1.4 b	12.0 b	1.4 c	35.9 a	63.3 a
<i>Ancho</i> (1)	2.1 b	2.8 a	15.7 b	3.3 a	37.5 a	65.9 a
<i>Pasilla</i> (2)	0.7 b	0.5 c	11.5 b	1.2 c	35.7 a	57.1 a

†Between morphotypes, means with the same letter do not differ significantly (Tukey test, $P \leq 0.05$)

Table 6: Variation of the bioactive compounds content and antioxidant activity in Huacle, Guajillo, Ancho and Pasilla fruits

Collection†	Vitamin C (mg AA g ⁻¹)	Carotenoids (mg BC g ⁻¹)	Phenols (mg GAEg ⁻¹)	Flavonoids (mg QE g ⁻¹)	DPPH (μ mol TE g ⁻¹)	FRAP (μ mol TE g ⁻¹)
CH-4	5.9 a††	2.0 a	35.7 a	2.3 b	38.0 ab	65.3 ab
CH-5	4.8 abcd	1.7 a	31.2 a	1.8 c	35.5 ab	61.0 ab
CH-6	2.8 bcde	1.5 a	28.3 a	1.8 c	29.7 b	50.5 b
CH-9	2.7 cde	1.8 a	28.2 a	1.7 cd	31.0 ab	53.9 ab
CH-10	5.1 abc	2.0 a	29.5 a	1.8 c	31.8 ab	51.6 b
CH-15	5.6 ab	1.9 a	35.8 a	1.9 c	39.9 ab	68.0 a
GU-P	5.9 a	1.3 a	13.1 a	1.4 ed	42.3 a	69.3 a
GU-NP	3.0 bcde	1.5 a	10.9 a	1.3 e	29.5 b	57.3 ab
AN-R	2.1 de	2.8 a	15.7 a	3.3 a	37.5 ab	65.9 ab
PAS-1	0.7 e	0.6 a	12.0 a	1.3 e	38.5 ab	63.2 ab
PAS-2	0.6 e	0.4 a	10.9 a	1.1 e	32.9 ab	51.0 b

†Prefixes, CH = Huacle; GU = Guajillo; ANR = Ancho rojo; PAS = Pasilla; †† Between collections, means with the same letter do not differ significantly (Tukey test, $P \leq 0.05$)



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