Behavior of the Hottest Chili Peppers in the World Cultivated in Yucatan, Mexico

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Abstract. The Yucatán Peninsula is recognized as the center of genetic diversity of Habanero peppers (Capsicum chinense Jacq.), which can be distinguished from those cultivated in other regions of the world by their aroma, taste, and—most of all—by their pungency. We evaluated three commercial varieties of chili peppers reported as being the hottest in the world: ‘Bhut Jolokia’, ‘Trinidad Moruga Scorpion’, and ‘Carolina Reaper’. The aim of our study was to determine the behavior of the pungency when cultivated under the edaphoclimatic conditions of Yucatan. Our results show that the three varieties registered greater contents in comparison with those reported in other regions of the world. ‘Carolina Reaper’—considered to be the hottest variety in the world, with a pungency of 2,200,000 Scoville heat units (SHU)—when cultivated in Yucatan, had a pungency of 3,006,330 SHU, which was greater than all the other varieties analyzed.

Chili peppers form part of the identity and culture of Mexico. They belong to the genus Capsicum, which originated in America, from Mexico to South America. The word chili derives from the náhuatl chilli or xilli, and in Mexico it is used to refer to any fruit of the genus Capsicum (Long-Solis, 1986). Chili pepper is an extraordinary condiment from the organoleptic test for the pungency of chili peppers. The capsaicinoids are in great demand in the pharmaceutical industry as a result of their application as anti-inflammatory agents (Liu and Nair 2010; Tag et al., 2014), antioxidants (Reddy et al., 2010; Rodriguez-Maturino et al., 2012), and anticancer agents (Jia et al., 2013).

Mexico is one of the centers of domestication and diversity of chili peppers. It is also where the greatest genetic diversity of the genus Capsicum is located (Ibiza et al., 2012). Yucatan is well known for its culinary tradition, which is associated closely with the Habanero pepper, considered to be one of the hottest chili peppers in the world. In Yucatan, the Habanero pepper found particular conditions of soil and climate that allowed it to adapt and diversify in an environment very different to that of its origin. These conditions are associated with the high temperatures of the region, sparse rainfall, and stony soils (basically calcareous with alkaline pH) very poor in organic material (Borges-Gomez et al., 2014). In June 2010, the Designation of Origin was granted to “Habanero pepper of the Peninsula of Yucatan” (Diario Oficial de la Federación, 2010). Subsequently, the Official Mexican Standard (NOM-189-SCFI-2012) was generated (Diario Oficial de la Federación, 2013). However, there are other varieties of the genus Capsicum originating from other regions of the world that have been recognized for their high pungency, such as ‘Bhut Jolokia’, a natural hybrid of C. chinense and C. frutescens, cultivated mainly in Bangladesh and India (Meghvansi et al., 2010). It can be found with other names, such as Bih Jolokia, Borbih Jolokia, Nagahari, Naga Jolokia, Naga Morich, Naga Moresh, Raja Mirchi, and Dorset Naga (Purkayastha et al., 2012). ‘Bhut Jolokia’ was considered one of the hottest in the world; in 2007, it was registered in the Guinness Book of World Records with 1,001,304 SHU. Later, the ‘Trinidad Moruga Scorpion’ variety, originally from Trinidad and Tobago, occupied that place in the Guinness Book of World Records with a pungency of 2,009,231 SHU (Bosland et al., 2012). Currently, the ‘Carolina Reaper’ variety, developed by the U.S. company PuckerButt Pepper in South Carolina, is recognized as the most pungent in the world, with 2.2 million SHU (PuckerButt Pepper Company; 2013), and is registered in the Guinness Book of World Records. Taking into account that the Peninsula of Yucatan is known to distinguish the Habanero pepper for its high level of pungency, our proposal was to evaluate, under the conditions of the region, the pungency behavior of three varieties internationally known for their high level of pungency.

Materials and Methods

Plant material and growth conditions. For this study, three varieties of commercially available chili peppers—‘Trinidad Moruga Scorpion’, ‘Bhut Jolokia’, and ‘Carolina Reaper’—were obtained from different suppliers on the Internet (Spicegarden (n.d.) and PuckerButt Pepper Company (2018), respectively). They were planted from Sept. 2016 to June 2017 in greenhouses located in the seed production unit of the Scientific and Technological Park of Yucatan in Sierra Papacal, Mérida, Yucatán, México, at lat. 21°07′20″N and long. 89°43′41″W, and at an altitude of 9 m a.s.l. During the study period, the average temperature was 18 °C (minimum) and 37 °C (maximum), and relative humidity was 86% (Servicio Meteorológico Nacional, 2018).

The seeds were germinated in polystyrene trays with 200 cavities using commercial substrates called Peat Moss Premier™ (Green Forest, Puebla, Mexico). The plantlets were transplanted to soil after ≥35 d, under greenhouse conditions, at a distance of 30 cm between plants and 100 cm between rows. Plant management and irrigation, pest and disease control, and fertilization were conducted following the established standards applied to the cultivation of Habanero pepper in the region (Tun, 2001). One hundred fifty plants per variety were used, distributed in three rows of 50 plants. Each variety was evaluated based on the descriptors established for Capsicum (IPGRI, 1995). The characteristics evaluated were type of epidermis, fruit color, weight of the fruit (measured in grams), pericarp thickness (measured in millimeters), fruit length (measured in centimeters), fruit width (measured in centimeters), number of fruits per plant, yield per plant (grams per plant).

Standards and solvents. Capsaicin (>99%) and dihydrocapsaicin (>97%) (Sigma Aldrich) were used as external standards, whereas methanol, acetonitrile, and HPLC-grade water were used as solvents (J.T. Baker). The filtrates were acquired using a 0.45-μm membrane filter.
and degasified before use.

Extraction of capsaicinoids. The extraction of capsaicinoids was performed according to the methodology reported by Collins et al. (1995), with slight modifications (100 mg/40 mL, w/v). Mature fruit was picked randomly per row of each variety, cut vertically, and transferred to an aluminum tray to be dried in an oven with a circulation of air for 48 h, after which the dried fruit were ground until a fine homogeneous powder was obtained. The powder was stored at \(-80^\circ\text{C}\) or \(-176^\circ\text{C}\).

To extract the capsaicinoids, three replicates of 100 mg fruit powder were mixed with 40 mL acetonitrile and maintained in a water bath at 80 °C for 4 hours with periodic agitation. The extracts were allowed to cool at room temperature, after which they were centrifuged (Sigma 2-16kl) at 17,968 \(g\) for 10 min at 4 °C. The supernatant was collected and filtered through syringe filters with a polytetrafluoroethylene (PTFE) membrane of 0.45 \(\mu\text{m}\) (no. 721–1345; Thermo Scientific) in 2-mL vials of amber glass and finally stored at 4 °C until the chromatographic analysis was performed.

Analysis of capsaicinoids. Separation and quantification of the capsaicinoids was carried out using HPLC (Agilent series 1200) equipped with an automatic injector and a fluorescence detector. The capsaicinoids were separated by a Zorbax Eclipse Plus C18 (Agilent Technologies, USA) column (4.6 mm i.d. \(\times\) 250 mm) at a temperature of 25 °C and an injection volume of 20 \(\mu\text{L}\). 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 operating conditions of the HPLC for determining total capsaicinoids were a temperature of 25 °C, a flow rate of 1 mL/min, and a runtime of 15 min.

HPLC calibration. The standards of capsaicin and dihydrocapsaicin were used to obtain a calibration curve based on the relationship of the maximum area for the known concentrations of external standards. The stock solution was prepared in 100% (v/v) methanol, and a total of five different concentrations—20, 60, 100, 200, and 300 ppm—were used to generate the calibration curve.

Quantification of capsaicinoids. The concentration of the two major capsaicinoids (capsaicin and dihydrocapsaicin) was estimated using the calibration curve obtained. The quantified concentration of capsaicinoids is reported in milligrams per gram dry weight (DW). The capsaicin contents obtained in the three varieties were converted to SHU to classify them according to their different levels of pungency. This conversion to SHU was performed by multiplying the capsaicin content by the coefficient corresponding to the pungency value for pure capsaicin (\(1.6 \times 10^7\)) (Sanatombi and Sharma, 2008).

Statistical analysis. The experiment was a random block design, with three repetitions. The data obtained are shown as the mean ± SD. To detect significant differences among capsaicinoid profiles of the chili varieties evaluated, an analysis of variance and a Tukey test \((P < 0.05)\) were carried out using the software SPSS (version 22) for Windows.

Table 1. Morphoagronomic characteristics of the three varieties evaluated.

| Varieties      | Type of epidermis | Color of fruit | Length of fruit (cm) | Width of fruit (cm) | Thickness of pericarp (mm) | Wt of fruit (g) | No. of fruits/plant | Yield (g/plant) |
|----------------|-------------------|----------------|----------------------|---------------------|---------------------------|----------------|---------------------|----------------|
| Bhut Jolokia   | Wrinkled          | Red            | 2.42 ± 1.03 b        | 2.01 ± 0.45 b       | 1.46 ± 0.20 b             | 6.75 ± 0.32 b  | 219.7 ± 3.65 a      | 1482.2 ± 9.8 a  |
| Trinidad Moruga Scorpion | Wrinkled          | Red            | 2.97 ± 1.22 a        | 2.51 ± 0.45 a       | 1.65 ± 0.19 a             | 7.77 ± 0.48 a  | 108.7 ± 4.09 b      | 846.7 ± 4.5 b   |
| Carolina Reaper | Wrinkled          | Red            | 2.37 ± 1.71 b        | 1.92 ± 0.45 b       | 1.14 ± 0.10 c             | 3.70 ± 0.45 c  | 98.1 ± 2.76 c       | 362.9 ± 4.3 c   |

The means followed by different letters in the same column are significantly different \((P < 0.05)\) with the Tukey test, the data are shown as the mean ± SD.

Fig. 1. Varieties evaluated include (A) ‘Bhut Jolokia’, (B) ‘Trinidad Moruga Scorpion’, and (C) ‘Carolina Reaper’.

Fig. 2. High-performance liquid chromatography chromatograms of capsaicin (1) and dihydrocapsaicin (2) (A) standard. (B) Sample of ‘Trinidad Moruga Scorpion’.
Table 2. Concentration of capsaicinoids analyzed in milligram per gram of dry weight and pungency of the chili varieties investigated.

| Varieties              | Capsaicin (mg·g⁻¹ DW) | Dihydrocapsaicin (mg·g⁻¹ DW) | Total capsaicinoids (mg·g⁻¹ DW) | Pungency (SHU) |
|------------------------|------------------------|-----------------------------|--------------------------------|----------------|
| Bhut Jolokia           | 92.83 ± 5.53 b         | 27.55 ± 1.79 b              | 120.38 ± 7.33 c                 | 1,938,089 ± 118,030 c |
| Trinidad Moruga Scorpion | 116.40 ± 5.15 a      | 30.71 ± 1.26 b              | 147.11 ± 6.42 b                 | 2,368,534 ± 103,395 b |
| Carolina Reaper        | 123.43 ± 1.77 a        | 63.30 ± 0.97 a              | 186.73 ± 2.75 a                 | 3,006,330 ± 44,384 a  |

*Each value is the average of three samples ± the SD of the mean.

Averages followed by different letters in the same column are significantly different (P < 0.05) with the Tukey test. DW = dry weight; SHU = Scoville heat units.

Results and Discussion

Table 1 shows the results of the analysis of the characteristics evaluated for ‘Bhut Jolokia’, ‘Trinidad Moruga Scorpion’, and ‘Carolina Reaper’. As can be seen, they differ significantly in all characteristics evaluated. ‘Bhut Jolokia’ presented the greatest fruit length (5.28 cm), which differed significantly from ‘Trinidad Moruga Scorpion’ and ‘Carolina Reaper’ (3.63 cm and 2.61 cm, respectively); ‘Trinidad Moruga Scorpion’ outperformed ‘Bhut Jolokia’ and ‘Carolina Reaper’ significantly with regard to fruit weight, fruit width, pericarp thickness, number of fruits per plant, and yield per plant. ‘Carolina Reaper’ had the lowest values for all characteristics evaluated. Figure 1 shows the distinctive characteristics of the fruit analyzed. There are differences among the three cultivars in terms of the shape of the fruit; however, they have in common the rugged nature of the pericarp. Values similar to those of this study have been reported for fruit weight, fruit length, and fruit width in ‘Bhut Jolokia’ (Bhagowati and Changkija, 2009; Kundu et al., 2015).

Identification and quantification of capsaicinoids. To separate the two major capsaicinoids (capsaicin and dihydrocapsaicin) responsible for 90% of the pungency (Manirakiza, 2003) using HPLC, the capsaicinoid content in the chili fruit was determined from the retention time and the peak size for each capsaicinoid present, in relation to the time of retention of the commercial standards, for each compound. The chromatograms shown in Fig. 2 correspond to the standard and the extract of ‘Trinidad Moruga Scorpion’, for which it was possible to identify capsaicin and dihydrocapsaicin, with retention times of 7.46 min and 10.62 min, respectively. The calibration curves showed a linear behavior over the concentration, in a range of 20 to 300 ppm of capsaicin and dihydrocapsaicin; the coefficient of correlation (r) was 0.99912 and 0.99931, respectively.

The data obtained for pungency (Table 2) show the concentration of capsaicin and dihydrocapsaicin, as well as the pungency expressed in SHU of the three cultivars analyzed. The total content of capsaicinoids obtained in this study was within a range of 120.38 to 186.73 mg·g⁻¹ DW, differing significantly from each other.

‘Bhut Jolokia’ has been considered one of the most pungent chili varieties in the world, although this characteristic has shown a high variation depending on the region where it is cultivated. Mathur et al. (2000), in a study conducted with this variety in India, reported a pungency of 855,000 SHU, whereas Bosland and Baral (2007) reported 1,001,304 SHU when it was grown in New Mexico, which allows them to record it in the Guinness Book of World Records as the most pungent variety in the world, displacing ‘Red Savina’ (577,000 SHU). Subsequently, Islam et al. (2015) evaluated the content of capsaicinoids in 92 accessions of ‘Bhut Jolokia’ collections in northwestern India. They found high variability, with a range of 11.95 to 72.05 mg·g⁻¹ DW and a pungency of 191,135 to 1,152,832 SHU. In our study, the content of capsaicinoids registered for ‘Bhut Jolokia’ was 120.38 mg·g⁻¹ DW, which corresponds to a pungency of 1,938,089 SHU, surpassing by 48% that reported by Bosland and Baral (2007) (Fig. 3). In 2012, Bosland et al., working with ‘Trinidad Moruga Scorpion’ cultivated in New Mexico, reported a pungency of 2,009,231 SHU. ‘Trinidad Moruga Scorpion’, cultivated under the conditions of Yucatan, registered a pungency of 2,368,534 SHU (147.11 mg·g⁻¹ DW), which represents a 15% increase in the content of capsaicinoids (Fig. 3). However, the most significant pungency was observed in ‘Carolina Reaper’ cultivated in the state of Yucatan. This cultivar registered 3,006,330 SHU, with a content of 186.73 mg·g⁻¹ DW total capsaicinoids, which represents a 27% increase in the content of capsaicinoids in comparison with those reported in the Guinness Book of World Records (PuckerButt Pepper Company, 2013).

It has been documented by different authors that the accumulation of capsaicinoids and the pungency of the pepper is influenced by different factors, including genotype, environment conditions, nutritional regime, and ripeness of the fruit (Barbero et al., 2014; Zewdie and Bosland, 2000). Tiwari et al. (2005) compared the capsaicin and dihydrocapsaicin content in Bhut Jolokia cultivated in two regions of India—Tezpur (Assam) and Gwalior (Madhya Pradesh)—with different climatic conditions. Their results showed that the pungency of the pepper was reduced by more than 50% in Gwalior, a dry region of India, which demonstrates the effect of environmental conditions on pungency. Duelund and Mourtis (2017) quantified the content of capsaicinoids in ‘Carolina Reaper’ cultivated in Denmark. They obtained a pungency of 1,046,000 SHU, which was low compared to our result (3,006,330 SHU). In a study conducted by Canto-Flick et al. (2008), the content of capsaicinoids from 18 accessions of Habanero pepper collected in the Yucatan, Mexico, was determined. They reported pungency values between 405,228 SHU and 892,719 SHU, which are significantly greater than the reports made for Habanero pepper grown in other regions of Mexico (Orellana-Escobedo et al., 2013). Similarly, our results allow inferring that the capsaicinoids are highly influenced by the environment and that the edaphoclimatic conditions of the Yucatan Peninsula exert a favorable effect for the increase of these compounds in these species.
Conclusions

Given that the Habanero pepper cultivated in the Yucatan Peninsula has been distinguished with the Denomination of Origin in 2010 for its pungency, aroma, and flavor—attributes conferred by the edaphoclimatic conditions of the region—the results of this study corroborate that pungency is greatly affected by the environment, including climate and soil conditions. We analyzed the three pepper cultivars 'Bhut Jolokia', Trinidad Moruga Scorpion, and Carolina Reaper—that have been published in the Guinness Book of World Records, at different times, for being the hottest peppers in the world. When these cultivars were grown in the Yucatan, their capsaicinoid content increased by 48%, 15%, and 27%, respectively. ‘Carolina Reaper’, currently considered the hottest in the world (2,200,000 SHU), when cultivated in Yucatan reached a pungency of 3,006,330 SHU. We consider it important to emphasize that Yucatan is a particularly privileged region in the world for the cultivation of Habanero pepper, which is distinguished by its high level of pungency. Our results provide solid evidence that, not only is capsaicin production stimulated, but also it is potentiated at significantly greater levels, even in cultivars that have been recognized for their high level of pungency.

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