

## Morphological and anatomical characterization of peduncle, flower and fruit related to easy fruit abscission of *Capsicum chinense* (Solanaceae) genotypes

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**ABSTRACT.** The sweet and chili pepper species of the genus *Capsicum* are originally from America; however, currently they are grown worldwide. Although widely appreciated, especially in the Amazon region, there have been few studies about *C. chinense*. Characterization and classification studies can aid in pepper crop genetic breeding and development of new cultivars with characteristics of agronomic interest. Resistance to fruit abscission makes harvesting difficult, which today is essentially manual, so that development of cultivars with easy fruit abscission, allowing semi-mechanized and mechanized harvest is an important goal. Descriptions of genetic differences based on anatomical characters

are rare in this genus. Along this line, we examined morphological characters and anatomical structures directly related to easy fruit abscission in *C. chinense*, through the evaluation of two genotypes contrasting for this character, maintained by the Embrapa Hortaliças Genetic Breeding Program, as well as an F<sub>1</sub> hybrid. The morphological characterization of flowers and fruits was performed using 13 descriptors, eight defined by the International Plant Genetic Resources Institute and five adapted for this study. The anatomical characterization of the peduncle and flowers was carried out with the elaboration of plant slides to examine structures possibly linked to easy abscission of the fruit. Contrasting morphological polymorphisms were observed in immature fruit color, fruit shape, fruit position on the plant, abscission and fruit firmness, as well as fruit diameter, number of locules and seeds per loculus. Small differences were observed in the anatomical structures of peduncle and flower, such as the shape of the vascular system and lignification of the pericycle, but none were directly related to fruit abscission. Although there was no significant anatomical relationship with fruit abscission, the morphological characters evaluated in this study revealed great genetic variability for these genotypes, demonstrating potential of these genotypes in a breeding program for developing varieties with easy fruit abscission.

**Key words:** *Capsicum chinense*; Abscission; Morphology; Anatomy; Flower; Fruit

## INTRODUCTION

The genus *Capsicum* comprises about 31 species belonging to the Solanaceae family, which is also the botanical family of tomatoes, potatoes, tobacco and petunia. They are named to differentiate them from black pepper (*Piper nigrum*), pink pepper (*Schinus molle*) and Jamaican pepper (*Pimenta officinalis*). Although all of them are called peppers and used as a condiment, they are not closely related and each has specific chemical properties (Carvalho et al., 2006). Among the species of the genus *Capsicum*, five are domesticated (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*) (Moscone et al., 2007). The growing demand for this product in its various forms of consumption has promoted agro-industrial development, generating jobs mainly in family farming and contributing significantly to the economy.

The species *C. chinense* was named after Nikolaus Joseph von Jacquin (1727-1817), a Dutch botanist who mistakenly thought that this species was originally from China, when in fact, all plants of the genus *Capsicum* are originally from America (Bosland and Votava, 1999). Although widely appreciated in the world and in Brazil, mainly in the Amazon region, the sources of information and studies about *C. chinense* peppers are still very rare (Lannes et al., 2007). Morphological and anatomical characterization studies can help to conserve the diversity of the genus, which has great economic importance,

especially for family farmers, as well as for the production of commercial cultivars of interest, through assisted selection by molecular markers.

*Capsicum* species are often described in terms of genetic divergence, considering morphological, agronomic and molecular databases. Sudré et al. (2010) reported that the identification of species of the genus is usually carried out by evaluations of morphological characters, mainly of flowers. However, morphological descriptions of just one plant structure, are not enough to describe and characterize species; studies involving analysis of different parts of the plant are more informative.

Embrapa Hortaliças currently maintains a collection of over 2,000 accesses, being considered the largest Brazilian pepper collection of the genus *Capsicum* (Embrapa Hortaliças, 2007). There is great variability that has not yet been fully explored in the genus, which represents great potential in studies of genetic breeding, mainly in the scope of germplasm banks characterization, identification of species and creation of new cultivars with characteristics of interest. Descriptions of genetic differences based on anatomical characters are rare (Dias et al., 2013).

The pepper fruit can have a strong stalk connection with the fruit in some cultivars, which often causes the fruit to break during manual harvest. Arancibia et al. (2004) reported that the presence of the peduncle and calyx adhered to the fruit at harvest imparted faded color and reduced the quality of sauces. Easy abscission of fruits favors semi-mechanized and mechanized harvests, being a characteristic of great interest for the improvement of pepper.

We examined vegetative (peduncle) and reproductive (flower and fruit) structures of two genotypes: CNPH 4337 (easy fruit abscission from the peduncle) and CNPH 40.001 (difficult fruit abscission from the peduncle) selected in the breeding program of Embrapa Hortaliças (CNPH), contrasting for the characteristics of fruit abscission, as well as an F<sub>1</sub> hybrid CNPH 40.491, resulting from the crossing of these genotypes.

## MATERIAL AND METHODS

The plants used in this study were provided by the Embrapa Hortaliças (CNPH) Breeding Program, which were kept in a greenhouse throughout the development. The analyses were performed at the Plant Genetics Laboratory at EMBRAPA Cenargen.

Three genotypes of *C. chinense* (Parental CNPH 40,001 and 4337 and CNPH 40,491, an F<sub>1</sub> hybrid from the cross CNPH 40,001 X CNPH 4337) were analyzed. These genotypes were collected in the state of Amazonas (Brazil), on properties owned by small farmers who cultivate them. The genotypes were sown in trays of 72 cells filled with the commercial substrate Carolina soil, and the seedlings were transplanted into 10-liter pots filled (three seedlings/pot) with sterilized soil, and maintained by drip irrigation in a automated system, using a Gardena T1030 irrigation controller, with a daily watering shift, ranging from 7 to 12 minutes in duration, following the plant's growth and development and its water demand. Driving vertical growth with staking was performed. Fertilization was carried out one week after transplanting with NPK16-08-12, 2g/pot (Controlled release fertilizer with granules completely covered by elastic polymer. The release of its nutrients occurs through a diffusion process), and 40 days after transplanting with urea (5g/pot), and application of pesticides, preventive and control, against mites and whiteflies, were carried out monthly.

For morphological analysis, the following were considered: three fully open flowers from each of the three plants; three immature and three ripe fruits from each of the three plants. For anatomical analyses, plant cuts were performed on flower buds before anthesis and the peduncle of ripe fruit.

## Morphology

Morphology was evaluated using eight morphological descriptors defined by the IPGRI (International Plant Genetic Resources Institute) for the genus *Capsicum* (IPGRI, 1995) and five descriptors adapted for this study, totaling 13 descriptors, related to the flower and fruit, three quantitative and 10 qualitative results presented below:

**1) Flowers:** Flowers were considered fully open at anthesis.

1. Flower position: pendant (3); intermediate (5); upright (7).

2. Color of the corolla: White (1); Light yellow (2); Yellow (3); Yellow-green (4); Purple with white base (5); White with purple base (6); White with purple margin (7); Purple (8); Other (9).

3. Number of petals per flower.

**2) Fruits:** Descriptors observed for the fruit.

4. Color of the fruit in the immature stage: Yellowish green (1); Light green (2); Medium green (3).

5. Fruit color in the intermediate stage (before ripening): White (1); Yellow (2); Green (3); Orange (4); Purple (5); Deep purple (6); Other (7).

6. Fruit color in the ripe stage: white (1); lemon yellow (2); pale orange-yellow (3); yellow-orange (4); pale orange (5); orange (6); light red (7); red (8); dark red (9); violet (10); brown (11); black (12); yellow (13); pale yellow (14); salmon (15).

7. Fruit position: pendant (3); intermediate (5); upright (7).

8. Fruit shape: Elongate (1); Almost round (2); Triangular (3); Campanulate (4); Blocky (5); Other (6).

9. Fruit diameter (cm).

10. Number of locules (taken from 10 fruits, by means of cross and longitudinal sections): (1); (2); (3); (4); (5)

11. Number of seeds per loculus (manual counting through cross and longitudinal sections).

12. Abscission - Persistence of the ripe fruit in relation to the peduncle (manual force): little persistent (3); intermediate (5); persistent (7).

13. Firmness of the fruit (manual perception of the firmness level when squeezing the fruit between the index and thumb fingers): Soft (1); Intermediate (2); Firm (3).

## Anatomy

The flower buds and fruits were cut immediately after collection and freehand. Cuts were made in longitudinal sections of the flower at an early stage (bud) and perpendicular sections of the fruit stalk.

The sections were clarified with 50% sodium hypochlorite (NaClO) [13% active chlorine] for approximately 5-10 minutes. Then, washing in distilled water was performed for 2-3 minutes. Staining was performed with alcian / safranin blue (4:1) for 10-30 seconds,

and again washed with distilled water to remove excess dye. Subsequently, they were dehydrated with the following alcohol series: ethanol 50% for two minutes, followed by absolute ethanol (98%) for five minutes. Later, the permanent slides were assembled with three drops of stained glass varnish (varnish commonly used in crafts), where the cuts were deposited carefully, followed by the coverslip (Krauss 1997; Paiva et al. 2006). The images were recorded using a photomicroscope associated with a microcomputer with a LAS EZ image capture system. The slides were photographed with a Motic BA300 optical microscope with a digital camera attached.

## RESULTS AND DISCUSSION

### Morphology

Table 1 shows the characteristics evaluated, of flower and fruit, for the three genotypes studied. Variations in the position of the flower were observed between the parents, and the CNPH genotype 40.491 (F<sub>1</sub>) inherited this characteristic from the parent CNPH 4337, and there was no variation in the color of the corolla (petals), which showed white color for the three genotypes. A small variation in the number of petals per flower was observed only in the parental CNPH 4337. Wahua et al., (2014) reported pentameric and hexamer flowers in *C. annuum*.

The genotypes analyzed showed notable differences in the fruit's morphology, being variable in the color of the immature fruit, position of the fruit in the plant, shape and diameter of the fruit, number of locules and seeds per loculus, abscission and firmness, and there was no variation of fruit color at intermediate and ripe stage, which presented orange and red colors respectively (Table 1). Costa (2012) identified six different fruit colors, in intermediate stage of ripeness and six in the ripe stage with five fruit forms in a study of 40 accessions of *Capsicum* spp. and Alves (2009) identified five and seven different colors, respectively, only in a morphotype of *C. chinense* (sweet pepper). Baba et al. (2016) used 14 morphological descriptors of fruit from IPGRI to characterize 71 accessions of *C. chinense* from the genetic bank of the State University of Londrina and some of these descriptors, mainly the shape of the fruits, were essential for the grouping of these accessions.

Theoretically, fruits with soft pulp detach more easily from the peduncle, according to Arancibia et al. (2004), it is the consequence of the disintegration of the fruit tissue. The genotypes CNPH 40.001 and CNPH 4337 showed contrasting characteristics, especially for abscission and firmness of the fruit, while the F<sub>1</sub> hybrid obtained from the crossing of these two genotypes inherited the easy abscission and firmness of soft fruit from the parental CNPH 4337 (Table 1). According to Fenwick et al. (1996), Errington et al. (1997) and Rao and Paran (2003), the enzyme polygalacturonase is one of the most primordial assets, responsible for the aging of the fruit and softening of the cell wall. The normal role of polygalacturonase is to hydrolyze pectins present in the cell wall during fruit ripening, which leads to its softening, a function also demonstrated by Ahmed et al., (2011) using RT-PCR and electron microscopy techniques. Thus, the presence of the polygalacturonase enzyme can be directly related to the firmness of the fruit.

Vilallon et al. (1970) evaluated the fruit-pedicel separation of table tomato varieties for mechanical harvesting, and brought preliminary data, at the time, correlating stages of

maturity, fruit size and shape and separation of the fruit-pedicel with mechanical harvesting, and stated that this latter characteristic was of great importance for studies that made harvest mechanization. Lines of tomatoes with the J2 gene are more susceptible to damage than those that do not have this gene, so it is extensively investigated in studies that evaluate the correlation of fruit size and shape with pedicel separation (Vilallon et al., 1970). In a study of genetic correlation of *Capsicum*, Setiamihardja et al. (1990) concluded that the genes for fruit characters appear to be pleiotropic for pedicel characters and the action of the gene for fruit detachment strength (FDF) was mainly additive, but with considerable dominance and epistasis. Anikulmar et al. (2019), in a genetic inheritance for fruit production study of *C. annuum*, found genes with only additive effects for average fruit weight, fruits per plant and production of green fruits per plant from contrasting parent crosses for fruit orientation (upright / hanging). This is because loci that exhibit dominance, as well as epistasis, also contribute to a variety of additive effects. This means that any segregating locus without dominance, with partial, complete dominance or overdominance contributes to variance of additive effects (Bernardo 2010, 2014). In our study, there was dominance of the loci responsible for FDF or abscission of the fruit from the peduncle, since the F<sub>1</sub> hybrid presented this characteristic equal to its parental CNPH 4337 (Table 1).

**Table 1.** Polymorphic morphological characteristics for the three *Capsicum chinense* genotypes studied (CNPH 40.001; CNPH 4337; CNPH 40.491).

	<b>CNPH 40.001</b>	<b>CNPH 4337</b>	<b>CNPH 40.491 (F<sub>1</sub>)</b>
Flower position	Intermediate (5)	Erect (7)	Erect (7)
Petal color	White (1)	White (1)	White (1)
Petal number per flower	5	5-6	5
Immature fruit color	Yellowish green (1)	Light green (2)	Medium green (3)
Intermediate stage Fruit color	Orange (4)	Orange (4)	Orange (4)
Ripe fruit color	Red (8)	Red (8)	Red (8)
Fruit position	Pendant (3)	Erect (7)	Pendant (3)
Fruit shape	Oval/Flat/ With spout (6)	Almost round (2)	Cylindrical (6)
Fruit diameter	3.2 - 3.6 cm	0.6 - 0.8 cm	1.9 - 2.2 cm
Number of locules	3	5	3
Number of seeds per loculus	4-6	1-2	1-2
Fruit abscission	Persistent (7)	Little persistent (3)	Little persistent (3)
Fruit firmness	Firm (3)	Soft (1)	Soft (1)

The genotype with the largest fruit diameter was parental CNPH 40.001, and the smallest was CNPH 4337, with the resulting F<sub>1</sub> having an intermediate diameter compared to that of the parents (Table 1). Setiamihardja et al. (1990) found additive effects of the gene for continuous pedicel distribution pedicel and fruit diameter, positively correlating the diameter with the force to detach the fruit (FDF), that is, the larger the fruit diameter, the greater the FDF or greater the persistence of the peduncle, in agreement with the data presented in the present study, where the CNPH genotype 40.001 presented the largest fruit diameter and the largest FDF or greater persistence of the fruit to the peduncle.

Figures 1, 2 and 3 show botanical drawings of the genotypes CNPH 40.001, CNPH 4337 and 40.491 (F<sub>1</sub>), respectively, with a high level of detail showing the structures studied and presented previously. It is possible to clearly observe the number of locules of the fruits and the number of seeds per loculus.

Parental CNPH 40.001 had the highest number of seeds per loculus - from 4 to 6 (Figure 1-J<sub>2</sub>), and parental CNPH 4337 had the highest number of locules - 5 (Figure 2-J<sub>2</sub>), in accordance with Gonçalves et al. (2013), who concluded that the number of seeds per fruit is a heterogeneous characteristic within the same repetition, with its variation resulting from the difference in size between the fruits.

Tomato and pepper are two *Solanaceas* fruit crops that present an enormous diversity in the morphology of the fruits (Paran and Van Der Knaap, 2007). Carvalho et al. (2003), stated that there is a great genetic variability expressed in the diversity of colors and shapes of the fruits, especially in the species *C. chinense*. The shape of the fruits showed great variation between the three genotypes. Genotype CNPH 40.001 had an oval shape and a characteristic spout at the bottom end of the fruit, while genotype CNPH 4337 had an almost round fruit shape and genotype CNPH 40.491, a cylindrical shape, apparently intermediate to that of its parents.

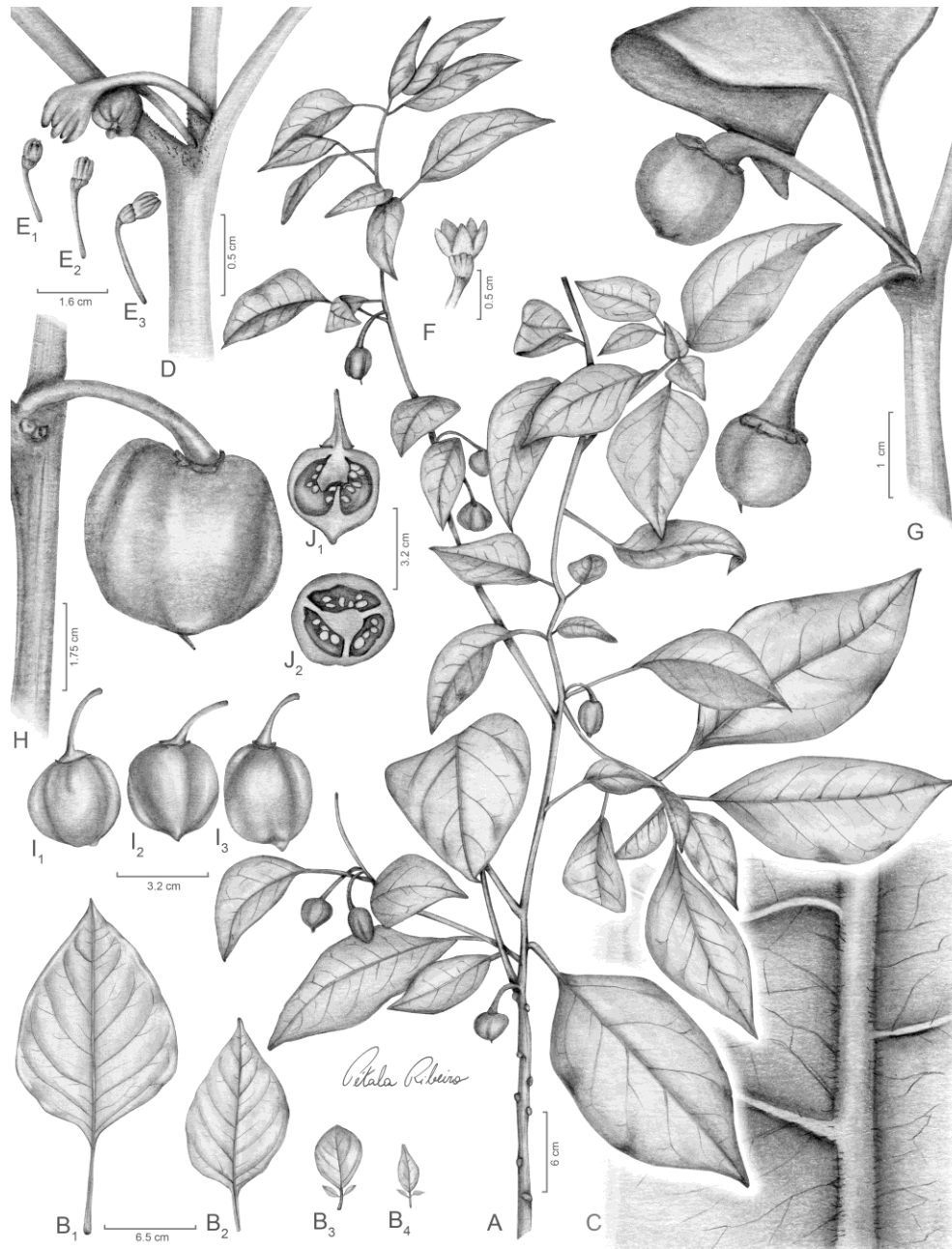
The orientation of the fruit (erect or hanging) is controlled by a single gene (Setiamihardja et al., 1990). The peduncle is clearly pending on parental CNPH 40.001 and erect on parental CNPH 4337, with the genotype CNPH 40.491 (F<sub>1</sub>) inheriting this characteristic (pendant) from CNPH 40.001 (Figures 1-H, 2-G and 3-G, respectively).

Color, shape, position, abscission and diameter of the fruit of the CNPH 4337 genotype, were identical to the study of Carvalho (2014), which used eight morphological descriptors, also from IPGRI (1995) to characterize 30 capsicum accesses from Embrapa Hortaliças germplasm bank, including genotype CNPH 4337 of the present study.

Padilha (2014) morphologically evaluated 20 accessions of *C. annuum* from the Embrapa Clima Temperado Active Germplasm Bank (Pelotas, RS), which maintains approximately 367 conserved accessions of the genus. In this study, 12 qualitative and 14 quantitative descriptors were used, some used in this study: color of the immature and ripe fruit, fruit shape, number of locules, persistence between fruit and peduncle and fruit diameter, and there was great genetic variation in the studied genotypes. Büttow et al. (2010) and Neitzke et al. (2010) also verified the existence of great genetic variability in fruits of *C. annuum* accessions of Embrapa Clima Temperado *Capsicum* Active Germplasm Bank.

The different species and varieties of *Capsicum* peppers can be distinguished by the morphological characteristics of the fruits and, mainly of the flowers (Moreira et al., 2006; Sudré et al., 2010). In this study, there was no difference in the color of the petals, which were white for the three genotypes studied. Only one variation was found in the number of petals per flower of genotype CNPH 4337, shown in Figure 2-F, which presented six petals.

Costa (2012) managed to distinguish three cultivated species of *Capsicum* spp. in 40 accessions in the collection of the Federal University of Amazonas, using 61 morphological descriptors of the IPGRI, of which seven were also used in the present study, and the color of the flower anthers was one of the determining characteristics used to differentiate morphotypes of *C. chinense*, as well as the great variability in the fruits attributes.

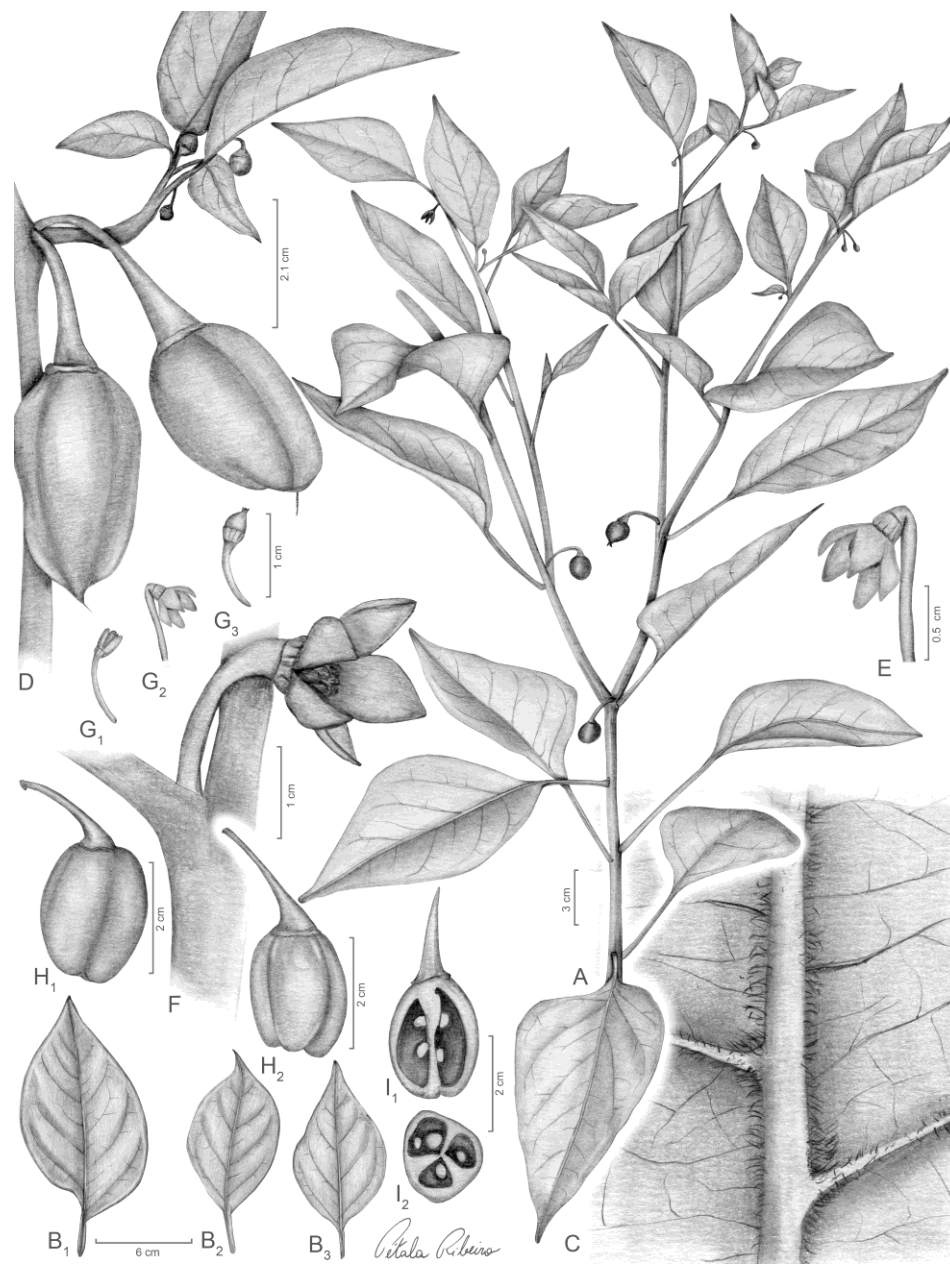


**Figure 1.** A-J. *Capsicum chinense* genotype CNPH 40.001. A. Branch with fruits; B1-4. Leaves on the adaxial face showing size variation; C. Leaf detail on the abaxial face showing the trichomes; D. Nodal portion of the branch showing buds; E1-3. Bud size variations; F. Flower variation with 5 petals; G. Nodal portion of the branch showing the fruit on its pending peduncle; H. Nodal portion of the branch showing the mature fruit; I1-5. Mature fruits and their size variations; J1-2. Fruit in sections - J1. Longitudinal section, J2. Cross section showing the 3 locules. A-J from NAYARA CARVALHO/ CNPH 40.001 (Embrapa CNPH- Brasília, 2020). Illustration by Pétala Ribeiro.





**Figure 2.** A-J. *Capsicum chinense* genotype CNPH 4337. A. Branch with fruits; B<sub>1-6</sub>. Leaves on the adaxial face showing size variation; C. Leaf detail on the abaxial face showing the trichomes; D. Nodal portion of the branch showing leaves, flowers and buds; E<sub>1-3</sub>. Fruit development - E<sub>1</sub>. Flower, E<sub>2</sub>. Emergence of the fruit, E<sub>3</sub>. Immature fruit; F. Flower variation with 6 petals; G. Nodal portion of the branch showing the fruit on its erect peduncle; H. Mature fruit in its largest size; I<sub>1-5</sub>. Mature fruits and their size variations; J<sub>1-2</sub>. Fruit in sections - J<sub>1</sub>. Cross section showing the 5 locules, J<sub>2</sub>. Longitudinal section. A-J from NAYARA CARVALHO/CNPH 4337 (Embrapa CNPH – Brasília, 2020). Illustration by Pétala Ribeiro.



**Figure 3.** A-J. *Capsicum chinense* hybrid CNPH 40.491. A. Branch with buds and fruits; B<sub>1-3</sub>. Leaves on the adaxial face showing size variation; C. Leaf detail on the abaxial face showing the trichomes; D. Nodal portion of the branch showing the fruits on its pending peduncle.; E. Flower with 5 petals; F. Nodal portion of the branch showing flower; G<sub>1-3</sub>. Fruit development - G<sub>1</sub>. Flower, G<sub>2</sub>. Emergence of the fruit, G<sub>3</sub>. Immature fruit; H<sub>1-2</sub>. Mature fruits; I<sub>1-2</sub>. Fruit in sections - I<sub>1</sub> Longitudinal section., I<sub>2</sub>. Cross section showing the 3 locules. A-I from NAYARA CARVALHO / CNPH 40.491 (F<sub>1</sub>) (Embrapa CNPH – Brasília, 2020). Illustration by Pétala Ribeiro.

According to Knapp (2002), pepper fruits are classified as berries, that is, indehiscent fruit with pericarp composed of defined exocarp, mesocarp and endocarp, and contains seeds incorporated into the placenta. In Figure 4, it is possible to observe all the structures mentioned above for the three genotypes, as well as placenta (seed insertion region), seeds and fruit maturation process with insertion of the peduncle. The fruit of genotype CNPH 4337 showed pentalocular conformation, while genotypes CNPH 40.001 and CNPH 40.491 ( $F_1$ ) presented trilocular conformation. In the fruit ripening process, there was a difference in the shade of the green color of the immature fruits, being yellowish green, light green and medium green for the genotypes CNPH 40.001, CNPH 4337 and CNPH 40.491 ( $F_1$ ), respectively (Table 1). There was no variation of the ripe fruits color, the three genotypes showed red color. Yellow fruits are recessive to red in *Capsicum* spp. They lack the red pigments capsanthin and capsorubin, whose formation is catalyzed by the enzyme capsanthin - capsorubin synthase (CCS) (Pickersgill, 2007).



**Figure 4.** *Capsicum chinense*: 1 (CNPH 40.001). A: Trilocular fruit; Cross section; (a1): Exocarp; (a2): Mesocarp; (a3): Endocarp; B: Longitudinal Section (b1): Seeds; (b2): Placenta; C: Maturation Fruit; (c1): Pending peduncle. 2 (CNPH 4337). D: Pentalocular fruit; Cross section; (d1): Exocarp; (d2): Mesocarp; (d3): Endocarp; E: Longitudinal Section (e1): Seeds; (e2): Placenta; F: Maturation Fruit; (f1): Peduncle erect. 3 (CNPH 40.491). G: Trilocular fruit; Cross section; (g1): Exocarp; (g2): Mesocarp; (g3): Endocarp; (g4): Seeds; H: Longitudinal Section (h1): Placenta; I: Maturation Fruit; (i1): Pending peduncle.

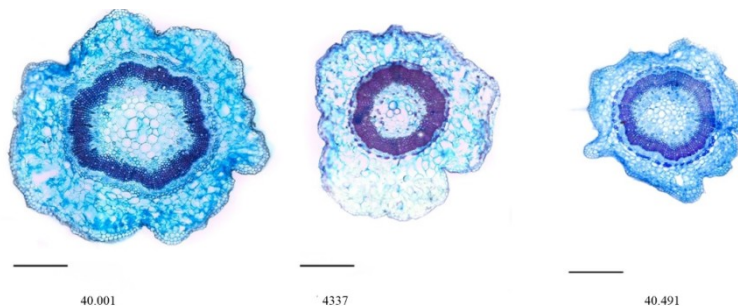
Therefore, there was great variability of the morphological characters evaluated, showing contrasting characteristics in genotypes CNPH 40,001 and CNPH 4337, with the exception of petal color and the fruit color when ripe.

## Anatomy

The methodology for preparing vegetable slides used in this study can be adjusted for other materials of the *Capsicum* genus, since there was difficulty in handling and assembling them. Klein et al. (2004) used a different protocol for visualization in a scanning electron microscope, with fixation, post-fixation and dehydration of the plant material for subsequent packaging in epoxy resin. Therefore, probably the methodology with epoxy resin could have been more effective for making the cuts, providing greater malleability and consequently better visualization of the material in an optical microscope. Wahua et al. (2014) used a protocol with four solutions of alcoholic series for dehydration / rehydration of the material and three solutions of alcohol and chloroform for clarification, using alcian and safranin blue dyes and to assemble the slides, glycerol, and managed to obtain slides as good as those of microtomy (embedded in epoxy).

## Peduncle

Figure 5 shows cross sections of the fruit peduncle of the three genotypes studied, showing epidermis (Ep) and Cortex (Cx), composed of parenchyma; the vascular system (Xylem (Xi) and Phloem (Fl)); uniseriate pericycle (Pe) surrounding the phloem. In the center: medulla (Me) with parenchymal cells.



**Figure 5.** Peduncle of *Capsicum chinense*: Genotype CNPH 40.001: Vascular system (Xi) and (Fl) with hexagonal shape and siphonostele pattern surrounded by the simple uniseriate pericycle (Pe). Simple uniseriate epidermis (Ep), followed by simple uniseriate collenchyma (Co). Marrow (Me) and Cortex (Cx) with parenchymatic tissue. Genotype CNPH 4337: Vascular system (Xi) and (Fl) with ring shape (circular) and siphonostele pattern surrounded by the simple uniseriate pericycle (Pe) with lignified cells. Parenchymatic peduncle marrow (Me). Simple uniseriate epidermis (Ep), followed by simple uniseriate collenchyma (Co). Marrow (Me) and Cortex (Cx) with parenchymatic tissue. Genotype CNPH 40.491 (F<sub>1</sub>): Vascular system (Xi) and (Fl) with ring shape, but slightly hexagonal, and siphonostele pattern surrounded by the pericycle (Pe) simple uniseriate with lignified cells. Simple uniseriate epidermis (Ep), followed by simple uniseriate collenchyma (Co). Marrow (Me) and Cortex (Cx) with parenchymatic tissue.

The vascular system of the peduncle of the three genotypes showed a siphonostele pattern (xylem surrounded by phloem), genotypes 40.001 and 4337, presented hexagonal and circular shapes, respectively, and genotype 40.491 (F<sub>1</sub>), presented an intermediate

shape to that of their parents. Wahua et al., (2014) observed also circular conformation of the vascular system in the stem and root of *C. annuum*. The pericycle of the peduncle of genotype 40.001 did not present lignified cells, in contrast to the other two genotypes (CNPH 4337 and CNPH 40.491). Other structures of the peduncles (epidermis, collenchyma, medulla and cortex) did not differ between the three genotypes. Possibly, a quantitative analysis would provide more details.

Setiamihardja et al. (1990) believed that the force for detaching the fruit (FDF) is mainly due to the genetic system that involves pedicel characters in non-deciduous fruit genotypes, and that pedicel and fruit characters would apparently be involved in FDF. In a histological study with tabasco pepper (*C. frutescens*), Sundberg et al. (2003) concluded that easily detached fruits had elongated peripheral parenchyma cells and large intercellular spaces, characteristics that were not observed in any of the three genotypes studied here.

## Flower

Descriptions of genetic differences based on anatomical characters are rare (Dias et al., 2013), especially in flowers of the species *C. chinense*. Figures 6, 7 and 8 presented below, show anatomical details of the main components of the flower of genotypes CNPH 40.001, CNPH 4337 and CNPH 40.491 (F<sub>1</sub>), respectively.

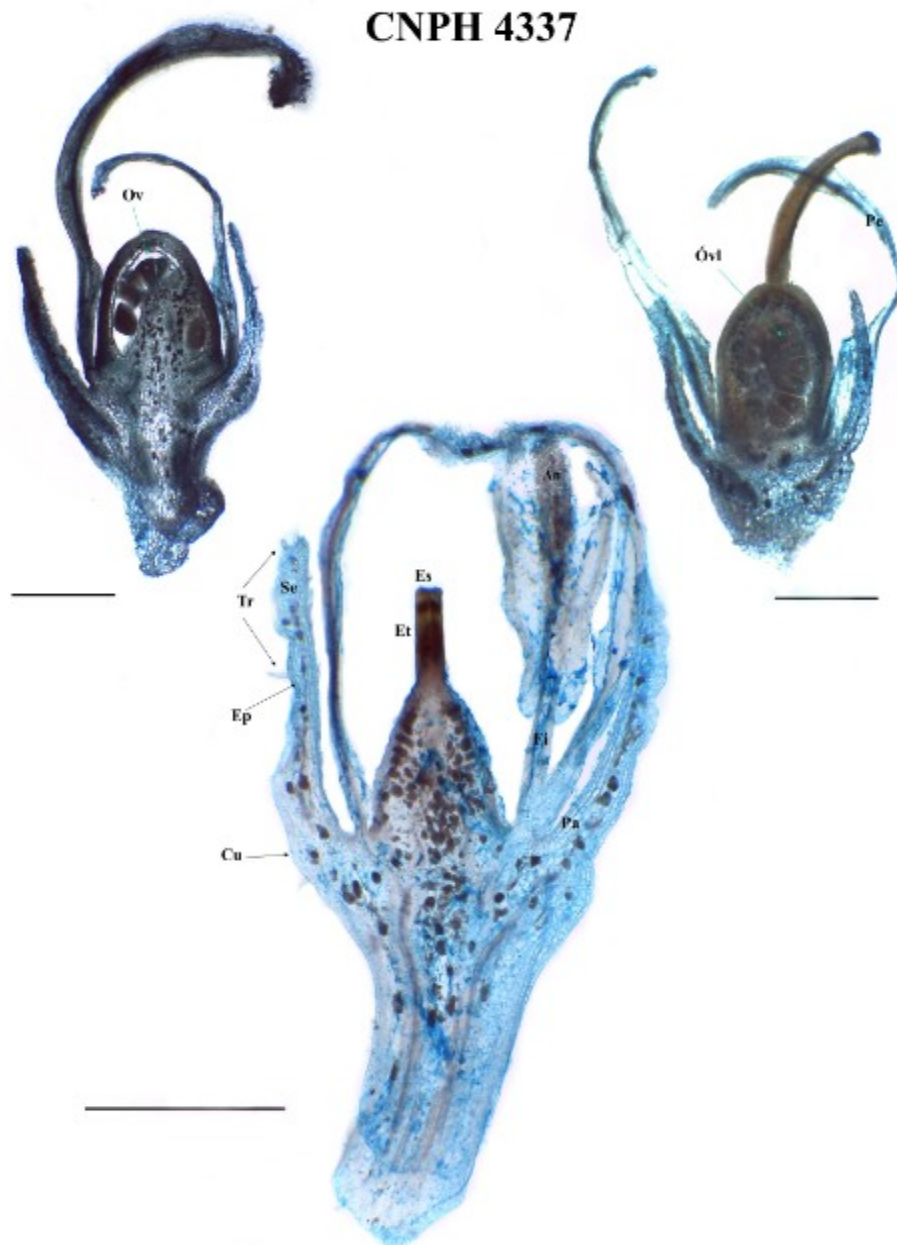
The petals of flowers (Pe) of the genotypes CNPH 40.001 and CNPH 4337 showed uni or biseriate cellular conformation, without much clarity, whereas the genotype CNPH 40.491 (F<sub>1</sub>), presented petals with uniseriate conformation. The cuticle (Cu), which covers the epidermis (Ep), was relatively thin in the parents and thick in hybrid CNPH 40.491 (F<sub>1</sub>).

According to Metcalfe and Chalk (1972), plants of the Solanaceae family can present numerous types of trichomes, which include a considerable diversity of glandular and non-glandular forms, serving in some cases to characterize some botanic families. The presence of glandular trichomes is a characteristic of the species of the family Solanaceae (Maiti et al., 2002). In our study, tector Trichomes (Tr) were observed in the three genotypes, and in hybrid F<sub>1</sub> there was a greater presence of these. Tector trichomes differ from glandular ones because they are formed by a single cell that protrudes out of the epidermis and do not produce secretions, whereas glandular ones produce toxic exudates, that according to Amme et al. (2005), may be related to protection against pathogens.

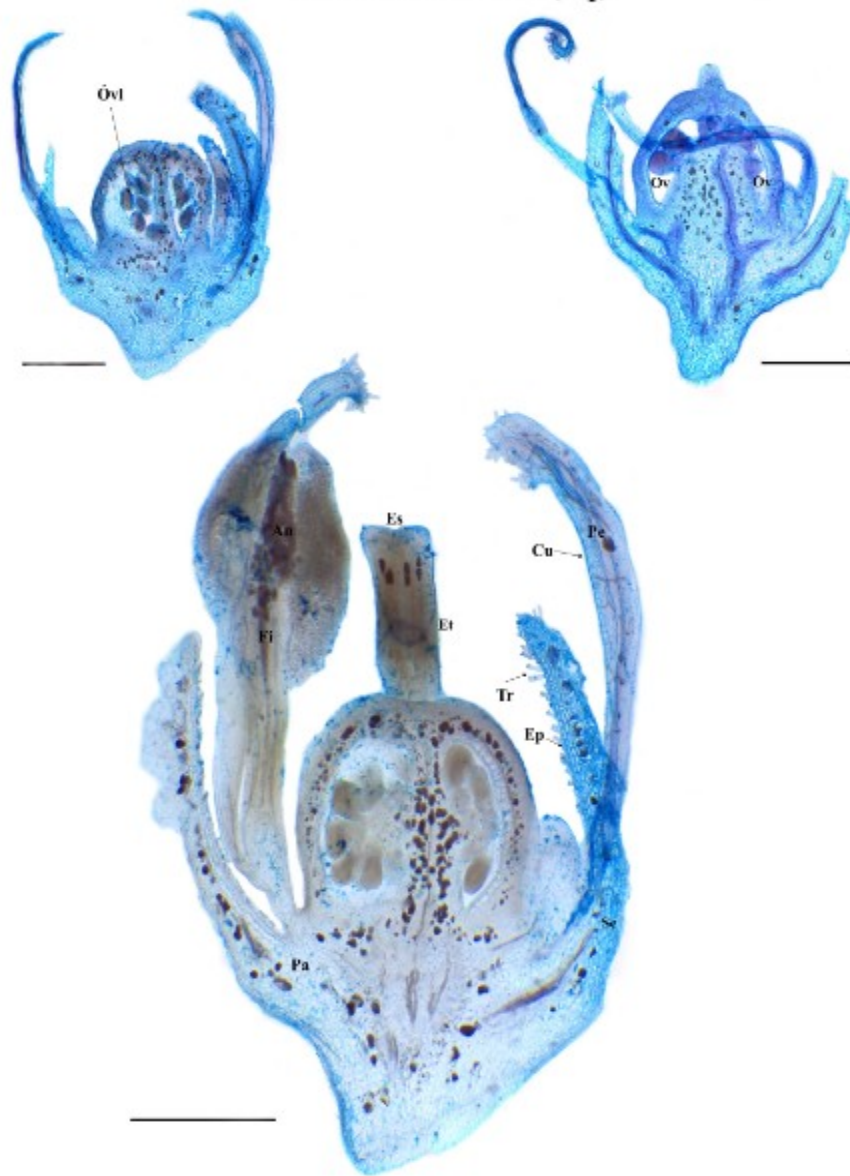
About the reproductive organs, androecium was not observed in genotype CNPH 40.001, probably due to the cut in a lower growth stage, structures already observed in genotypes CNPH 4337 and CNPH 40.491. The gynecium was clearly observed in the three genotypes, differing in the arrangement of carpels, unicarpelar in genotype CNPH 4337 and bicarpelar in the others. Wahua et al. (2014), in a histological study of *C. annuum* observed bilocular conformation. Differences were also found in the shape of the stylet (Et), straight on the sides for genotypes CNPH 4337 and CNPH 40.491 (F<sub>1</sub>), and slightly flat for genotype CNPH 40.001; the stigma showed an apex with receptor cells very evident in the CNPH 40.001 genotype.

**CNPH 40.001**

**Figure 6.** Flower of *Capsicum chinense* genotype CNPH 40.001: Chalice with petals (Pe) with uni or biserial epidermis (Ep), with a relatively thin cuticle (Cu) and six to seven layers of parenchymatic tissue (Pa). Sepals (Se) showing trichomes (Tr). Chloroplasts and starch occasionally present in parenchymal cells. Stigma (Es) enlarged at the apex, with a receptor surface made up of many evident cells. Stylus (Et) slightly flattened on the sides, with three to five cells between the outer surface and the pollen tube. Ovary (Ov) bicarpelar with eggs (Óvl).



**Figure 7.** Flower of *Capsicum chinense* genotype CNPH 4337: Chalice with petals (Pe) with uni- or biserial epidermis (Ep), with a thin cuticle (Cu) and five to six layers of parenchymatic tissue (Pa). Stigma (Es) with apex straight or slightly enlarged, and receptor surface formed by few cells not very evident. Straight (Et) stylus on the sides, with two to four cells between the outer surface and the pollen tube. Ovary (Ov) unicarpelar with ova (Óv). Sepals (Se) showing trichomes (Tr). Fillet (Fi) with anther (An) and pollen grains (Po) inside.

**CNPH 40.491 (F<sub>1</sub>)**

**Figure 8.** Flower of *Capsicum chinense* genotype CNPH 40.491: Chalice petals (Pe) with uniseriate epidermis, with relatively thick cuticle and six to seven layers of parenchymatic tissue (Pa). Tector trichomes (Tr) visibly present, mainly in the inner part of the sepals (Se). Stigma (Es) slightly enlarged at the apex, with receptor surface made up of cells that are not very evident. Straight stylus (Et) on the sides, with four to six cells between the outer surface and the pollen tube. Ovary (Ov) bicarpellary with eggs (Óvl). Fillet (Fi) with anther (An) and pollen grains (Po) inside.



The genetic breeding of *Capsicum* spp. should look for fruits that have persistence of the fruit with an intermediate peduncle, which are not easily knocked down by wind and rain, but they are easily detached during harvest (Fonseca et al., 2008). Thus, it was possible to observe basic stem structures, and small differences in some of them, but none that could be related to easy abscission of fruit. With regard to flowers, it was also possible to observe basic structures, which can help characterization studies for assisted selection in this species.

Dias et al. (2013) examined the anatomy and micromorphology of vegetative and reproductive organs (leaves, fruits and seeds) of four species of *Capsicum* (*C. annuum* var. *annuum*, *C. baccatum* var. *pendulum*, *C. chinense*, and *C. frutescens*) from genetic bank of the State University of Norte Fluminense, with scanning electron microscopy, and found that although the species studied presented a wide morphological and molecular variability, this variability was not reflected in the anatomical characteristics. These results are similar to those presented here, since the morphological analysis was very informative, and the anatomical analysis, despite presenting small variations, were not directly related to the easy abscission of fruits in *C. chinense*. Possibly, a quantitative analysis would provide further details for this purpose. Gersch (1996) used scanning electron microscopy to investigate differences in cell type and cell organization in the fruit receptacle (junction with peduncle) in two cayenne pepper (*C. annuum* L.) genotypes, and he found a distinct region of 15 layers of sclerified cells that extended from the periphery of the fruit into the receptacle, in the genotype of difficult abscission, contrasting with the genotype of easy abscission that presented fewer layers of these same cells. In the methodology, a force meter was used to quantitatively measure (recorded in Kg and converted into Newtons) abscission, a method by which the evaluation becomes less subjective, than the one used in the present study.

The morphological characters evaluated in this study revealed great variation, especially for fruit abscission, demonstrating great genetic potential for using these genotypes in the breeding program, although this variation has not been reflected in the anatomical characteristics. Although the easy abscission of the fruit was contrasting for the parents CNPH 40.001 and CNPH 4337, there was no relationship of the other traits evaluated with it, with the exception of fruit firmness and fruit diameter, which showed direct correlations with easy abscission, in other words, the larger and firmer the fruit, the greater the abscission force for detachment of the peduncle.

Complementary genetic studies that make use of molecular markers for mapping the abscission characteristic of the fruit are very important for the use of these accessions in breeding programs mainly in the context of the development of cultivars that present easy abscission combined with the characteristics of market demand.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

- Ahmed SS, Gong ZH, Khan MA, Yin YX, et al. (2011). Activity and expression of polygalacturonase vary at different fruit ripening stages of sweet pepper cultivars. *Genet. Mol. Res.* 10(4): 3275-3290.
- Alves S (2009). Caracterização e avaliação de genótipos de pimenta-de-cheiro (*Capsicum chinense*). 81p (Masters thesis). Universidade Federal de Amazonas, Manaus.
- Amme S, Rutten T, Melzer M, Sonsmann G, et al. (2005). A proteome approach defines protective functions of tobacco leaf trichomes. *Proteomics.* 5(10): 2508-2518.
- Anilkumar C, Mohan Rao A, Ramesh S, Bhavani B, et al. (2019). Genetics of fruit yield and its component traits under different fruiting habit backgrounds in chili (*Capsicum annum* L.). *J. Genet.* 98: 84.
- Arancibia RA and Molsenbocker CE (2004). Pectin ultra-degradation decreases the force required to detach ripe fruit from the calyx in tabasco pepper. *J. Am. Soc. Hortic. Sci.* 129: 642-648.
- Baba VY, Rocha KR, Gomes GP, Ruas CF, et al. (2016). Genetic diversity of *Capsicum chinense* accessions based on fruit morphological characterization and AFLP markers. *Genet. Resour. Crop Evol.* 63: 1371-1381.
- Bernardo R (2002). *Breeding for quantitative traits in plants* (Vol. 1, p. 369). Woodbury, MN: Stemma press.
- Bernardo R (2014). *Essentials of plant breeding*. Stemma Press.
- Büttow MW, Barbieri RL, Neitzke RS, Heiden G, et al. (2010). Diversidade genética entre acessos de pimentas e pimentões da Embrapa Clima Temperado. *Ciênc. Rural.* 40: 1264-1269.
- Bosland PW, Votava EJ and Votava EM (2012). Peppers: vegetable and spice capsicums. (Vol. 22), Cabi.
- Carvalho SICD (2014). Estudos filogenéticos e de diversidade em *capsicum* e sua aplicação na conservação e uso de recursos genéticos das espécies *C. frutescens* e *C. chinense*. Carvalho SIC, Bianchetti LDB, Bustamante PG, and da Silva DB (2003). Catálogo de germoplasma de pimentas e pimentões (*Capsicum* spp.) da Embrapa Hortaliças. *Embrapa Hortaliças-Documentos (INFOTECA-E)*.
- Carvalho SIC, Bianchetti LB, Ribeiro CSC and Lopes CA (2006). Pimentas do gênero *Capsicum* no Brasil. Brasília: Embrapa Hortaliças, 27p.
- Costa LV (2012). Caracterização morfológica e produtiva de pimentas (*Capsicum* spp). Embrapa Hortaliças, Sistemas de Produção, 2, (2007), Available at: [[https://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Pimenta/Pimenta\\_capsicum\\_spp/index.html](https://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Pimenta/Pimenta_capsicum_spp/index.html)].
- Dias GB, Gomes VM, Moraes TM, Zottich UP, et al. (2013). Characterization of *Capsicum* species using anatomical and molecular data. *Genet. Mol. Res.* 12: 6488-6501.
- Errington N, Mitchell JR and Tucker GA (1997). Changes in the force relaxation and compression responses of tomatoes during ripening: the effect of continual testing and polygalacturonase activity. *Postharvest Biol. Technol.* 11(3): 141-147.
- Fenwick KM, Jarvis MC, Apperly DC, Seymour G, et al. (1996). Polymer mobility in cell walls of transgenic tomatoes with reduced polygalacturonase activity. *Phytochemistry.* 42(2): 301-307.
- Fonseca RM, Lopes R, Barros WS, Lopes MTG et al. (2008). Morphologic characterization and genetic diversity of *Capsicum chinense* Jacq. accessions along the upper Rio Negro – Amazonas. *Crop. Breed. Appl. Biotechnol.* 8: 187-194.
- Gersch KP (1996). Analysis of the Influence of Genotype on Cayenne Pepper Fruit-Receptacle Detachment. Louisiana State University and Agricultural and Mechanical College.
- Gonçalves LGV, Andrade FR, Marimon Junior BH, Schossler TR, et al. (2013). Biometria de frutos e sementes de mangaba (*Hancornia speciosa* Gomes) em vegetação natural na região leste de Mato Grosso, Brasil. *Rev. Bras. Ciênc. Agrár.* 36(1): 31- 40.
- IPGRI (1995). CATIE. Descriptors for Capsicum (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy; the Asian Vegetable Research and Development Center, Taipei, Taiwan, and the Centro Agronómico Tropical de Investigación y Enseñanza. *Turrialba, Costa Rica, 110*.
- Klein DE, Moreira Gomes V, DA Silva-Neto SJ, et al. (2004). The structure of colleters in several species of Simira (Rubiaceae). *Ann. Bot.* 94(5): 733-740.
- Knapp S (2002). Tobacco to tomatoes: A phylogenetic perspective on fruit diversity in the Solanaceae. *J. Exp. Bot.* 53(377): 2001-2022.
- Kraus JE and Arduin M (1997). Manual básico de métodos em morfologia vegetal. Rio de Janeiro: EDUR.
- Lannes, S.M., Finger F, Schuelter AR, Casali VWD, et al. (2007). Growth and quality of Brazilian accessions of *Capsicum chinense* fruits. *Sci. Hort.* 3: 266-270.
- Maiti RK, Villareal L, Trevino A and Valades-Cerda MC (2002). Some aspects on pharmacology of ten species of the family Solanaceae utilized in traditional medicine. *Caldasia.* 24: 317-321.

- Moscone EA, Scaldaferrero MA, Gabriele M, Cechinni NM, et al. (2007). The evolution of chili peppers (*Capsicum* - Solanaceae): a cytogenetic perspective. *Acta Hort.* 745: 137-169.
- Moreira GR, Caliman FRB, Silva DJH and Ribeiro CS (2006). Espécies e variedades de pimenta. Belo Horizonte: EPAMIG, *Informe Agropecuário*. 27(235): 16-29.
- Metcalfe CR and Chalk L (1972). Anatomy of the Dicotyledones: leaves, stem and wood in relation to taxonomy with notes on economic uses. Oxford: Clarendon Press, v.2.
- Neitzke RS, Barbieri RL, Rodrigues WF, Corrêa IV, et al. (2010). Dissimilaridade genética entre acessos de pimenta com potencial ornamental. *Hort. Bras.* 28: 47-53.
- Padilha HKM (2014). Caracterização morfológica, avaliação agrônômica e análise de compostos bioativos em acessos de pimentas (*Capsicum annuum*) (Masters thesis). Universidade Federal de Pelotas, Rio Grande do Sul.
- Paiva JGA de, Fank-de-Carvalho SM, Magalhães MP and Graciano-Ribeiro D (2006). Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. *Acta bot. bras.* 20(2): 257-264.
- Paran I and Van Der Knaap E (2007). Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *J. Exp. Bot.* 58(14): 3841-3852.
- Pickersgill B (2007). Domestication of plants in the Americas: insights from Mendelian and molecular genetics. *Ann. Bot.* 100(5): 925-940.
- Rao GU and Paran I (2003). Polygalacturonase: a candidate gene for the soft flesh and deciduous fruit mutation in *Capsicum*. *Plant Mol. Biol.* 51(1): 135-141.
- Setiamihardja R and Knaeve DE (1990). Association of pedicel length and diameter with fruit length and diameter and ease of fruit detachment in pepper. *J. Am. Soc. Hortic. Sci.* 115(4): 677-681.
- Sundberg M, Molsenbocker CE and Huang Y (2003). Anatomy of fruit detachment in tabasco pepper (*Capsicum frutescens*) Solanaceae. *J. Torrey Bot. Soc.* 130: 231-237.
- Sudré CP, Gonçalves LS, Rodrigues R, do Amaral Júnior AT, et al. (2010). Genetic variability in domesticated *Capsicum* spp as assessed by morphological and agronomic data in mixed statistical analysis. *Genet. Mol. Res.* 9: 283-294.
- Villalon B and Bryan HH (1970). Evaluation of fruit-pedicel separation of fresh market tomato varieties for mechanical harvest. In *Proceedings of the 83rd Annual Meeting of the Florida State Horticultural Society*. (No. 83, pp. 127-130). Miami Beach, USA.
- Wahua C, Okoli BE and Edwin-Wosu NL (2014). Morphological, anatomical, cytological and phytochemical studies on *Capsicum annuum* Linn. (Solanaceae). *J. Exp. Biol.* 4(1): 464-471.