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Breeding Calabrian pepper lines (*Capsicum annuum* L.) for Brazilian agriculture from *sui generis* introduction of germplasm

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Abstract

Few Calabrian pepper cultivars (*C. annuum* and *C. baccatum*) are available in the Brazilian market, and among these, only BRS Mari (*C. baccatum*) was developed in Brazil, by Embrapa. This work aimed to report on the introduction of germplasm in a *sui generis* way and the initial results of Calabrian pepper breeding at Embrapa Vegetables. Original population was obtained through separation of seeds found in dehydrated Calabrian pepper flakes imported from India. The breeding method was individual selection of plants with progeny test and three generations of selection and self-pollination were advanced. Significant differences ($p<0.05$) were noticed among the 14 S_3 lines obtained, for precocity, length and width of the fruit, weight of the fruit, weight and number of fruits per plant. Five S_3 lines were selected based on the following criteria: average fruit weight above 12 g, early flowering (less than 80 days after sowing), fruit length above 13 cm and fruit width around 1.5 cm, dark green color of leaf and sparse or medium pilosity. In the following S_4 generation, the selected lines will be evaluated under field conditions in order to determine yield, disease resistance as well as capsaicinoid concentration in fruits. New genotypes selected may be released as cultivars that meet the growing demand for dehydrated Calabrian pepper flakes.

1. Introduction

The genus *Capsicum*, represented by hot and sweet peppers, originates from the tropical America, is nowadays widely dispersed and grown all over the world. Brazil is an important diversity center of *Capsicum*, holding domesticated, semi domesticated and wild species (Carvalho *et al.,* 2003; Monteiro *et al.,* 2010). The genus consists of about 30 species, only five being considered as domesticated *C. annuum, C. baccatum, C. frutescens, C. chinense* and *C. pubescens,* the latter being not found in Brazil (Moreira *et al.,* 2006).

Currently, India is a great grower and exporter of dehydrated red pepper fruit (*C. annuum*), whole or in form of flakes. In 2013, India produced about 1.4 million metric tons of dehydrated peppers and exported, in 2011, 260 thousand metric tons of dried peppers. Numerous landraces and local population of different kinds of *Capsicum* peppers are grown in India, and most of them belong to *C. annuum* species. Due to natural great variability, India is known as a secondary center of diversity for *Capsicum*.

C. annuum fruits are among the ten most consumed vegetables in Brazil (Rocha *et al.,* 2006; Moreira *et al*., 2006). This species is represented by sweet peppers, peppers for paprika and hot peppers like jalapeño, cayenne, serrano, cherry, among others, besides ornamental varieties (Reifschneider, 2000; Büttow, 2010). *C. annuum* fruits are consumed green and ripe, *in natura*, in sauces or dehydrated. Calabrian pepper, also known as *peperoncino,* generally belongs to this species and is widely used in Italian cuisine.

In Brazil, Calabrian pepper is not a kind of pepper that belongs to a determined species, but rather it is a product from red pepper dehydration in form of flakes, being frequently used as a spice for the food processing industry. Brazilian Calabrian pepper is obtained from red ripe fruits, mainly from *C. baccatum* and *C. annuum*. Fruits are harvested when ripe and dried in the sun on tarps or in dryers with forced hot air circulation. Once dried, the whole fruits are flaked, including the seeds (Ribeiro *et al.,* 2008).

Fungal diseases and low prices are pointed out as the main reasons for the drastic reduction in cultivation area of Calabrian pepper in Turuçu (SCHNEID, LF, personal communication), previously the most important growing region in Brazil.

There are few cultivars of chile pepper (*C. annuum* and *C. baccatum)* available in the Brazilian market that could be used to obtain Calabrian pepper. Out of these cultivars, only 'BRS Mari' (*C. baccatum*) originated from a Brazilian breeding program, implemented by Embrapa Vegetables (Carvalho *et al*., 2009).

The development of new cultivars heavily depends on the genetic resources available, collected and characterized, in collections or germplasm banks – AGB (Nass, 2007). The enrichment of an AGB is normally carried out through germplasm exchange among research institutions, collecting expeditions and acquisition of commercial seeds available in the market. However, in the specific case of Calabrian pepper accession CNPH 50.000, belonging to the *Capsicum* collection of Embrapa Vegetables, the germplasm was introduced in a *sui generis* way: seeds were obtained from a small batch of the commercial product Calabrian pepper (dehydrated red pepper flakes with seeds) from India, evaluated by an agroindustry as excellent quality. The absence of national cultivars of Calabrian pepper (*C. annuum*) adapted to different Brazilian biotic and abiotic conditions motivated the beginning of this research in Embrapa Vegetables. This work aimed to report results of breeding research of Calabrian pepper (*C. annuum*), from the population CNPH 50.000, as well as the identification and selection of plants and lines with promising agronomic characteristics for developing new cultivars of interest to Brazilian agriculture.

2. Material and methods

This work was carried out at Embrapa Vegetables, Brasília, DF, Brazil (15°55'57.31"S, 48°8'11.36"W).

Genotype used: The seeds of *C. annuum* were obtained from a small sample of dehydrated pepper flakes (Calabrian type) imported from India, and registered in the *Capsicum* Germplasm Bank as CNPH 50.000. This batch of pepper in flakes showed capsaicin content of 32,100 Scoville Heat Units (SHU).

Breeding methodology: The breeding method used was individual selection of plants with progeny test. Original seeds were sown and 10 plants obtained (original plants, OP) which were maintained in a screenhouse and self-pollinated. From each plant, 3-5 self-pollinated fruits were harvested and extracted.

For the following cycle, ten S_1 seeds from each of the 10 original self-pollinated plants were used; 87 S_1 plants were obtained, and Open pollinated as well as selfed fruit were obtained from 73 lines. The OP seeds harvested in the screenhouse were also considered S_2.

In the next generation, five seeds from each of the 73 S_1 lines obtained in the previous generation were sown and a total of 327 S_2 plants (4-5 plants/line) were transplanted to a screenhouse, directly in the soil. Fourteen plants were selected among and within S_2 lines. Self-pollinated fruits from selected lines were harvested separately and S_3 seeds of these fruits were extracted. In 2015, 14 S_3 selected lines were taken to the field, 5 plants per plot, in two replications, for a preliminary evaluation of agronomic and processing characteristics of interest (earliness, number of side shoots, color of leaves and unripe fruit, plant height, fruit length and diameter, wall thickness, weight of individual fruit, total number and weight of fruits per plant), and identification of lines adapted to Central Brazil. Concomitantly, three plants from each line were maintained in a screenhouse for controlled self-pollination..

Agronomic and processing characterization: A preliminary characterization of fruits of the original population (CNPH 50.000) as well as of the 73 S2 lines was caried out. Due to great variation in number of side shoots among lines, the number of shoots of each plant was also counted for all S2 lines, which were grouped into five classes.

In addition, a subjective evaluation of agronomic value of S_2 plants was carried out, by at least two evaluators. Based on quantitative and qualitative data obtained three S_2 lines were selected. The three selected lines were also evaluated for number of fruits per plant, total weight of fruits (g), total soluble solids (ºBrix), capsaicin content and color of ripe fruit (5 fruits of each plant).

Parameters fruit length, width and wall thickness were measured using a digital caliper MITUTOYO, model 500-144B. To determine total soluble solids (ºBrix), a digital refractometer ATAGO model PR-1 was used, following standard methodology to clean the equipment and to standardize the samples.

Determination of capsaicin: Determination of capsaicin content of three selected S_2 lines was carried out using AOAC Official Method 995.03 (AOAC, 2006) that can be used to determine capsaicinoids content between 750 and 650.000 Scoville Heat Units (SHU).

Determination of fruit color: For analysis of unripe fruit, one fruit from each plant was harvested, from all lines, and a reading per fruit was carried out. Analysis of ripe fruit color was carried out only for the three selected lines (five fruits per plant and a reading per fruit). Color analysis was carried out by using colorimeter (Minolta Chromometer Model CR-400), standard CIE-L*a*b*. Color measurements were carried out in equatorial region of each fruit.

3. Results and discussion

3.1. Evaluation of original population

Evaluation of characteristics of fruits and plants obtained from the original population (CNPH 50.000) that came from Indian Calabrian pepper showed significant variability among individuals. Significant differences were observed through Scott-Knott test ($p<0.05$) for characteristics fruit diameter and wall thickness, which ranged from 0.8 to 1.2 cm and 0.8 to 1.4 mm, respectively. 'CNPH 50.010' showed significant difference for number of locules per fruit, with average value of 2.3 locules, whereas other genotypes showed two locules per fruit.

Presence of variability in original population is essential for selecting individuals with superior characteristics. Success in development of new cultivars is directly associated to genetic variability of the population to be improved (Cardoso, 2001; Nass, 2007; Ribeiro *et al.*, 2008). Genus *Capsicum* has significant genetic variability (Inoue & Reifschneider, 1989), and among the domesticated species, *C. annuum* has greater diversity, whereas *C. frutescens* has less variability (Casali & Couto, 1984).

3.2. Evaluation of S2 lines

Significant differences among the 73 S_2 lines were observed (Scott-Knott test, $p<0.05$) for all fruit characteristics evaluated. Values of fruit weight ranged from 2.3 g to 10.9 g; fruit length ranged from 7.5 cm to 13.4 cm; fruit diameter ranged from 0.7 cm to 1.4 cm and wall thickness ranged from 0.87 mm to 1.98 mm. Some of these values are close to the most cultivated variety of *C. annuum* pepper grown in India, known as "Pusa Jwala," which presents fruit length ranging from 7 to 13 cm, fruit width from 1 to 1.5 cm and pungency between 30,000-50,000 SHU.

Color of unripe fruits in the 73 S_2 lines ranged from L*= 26.55; a*= -21.62; b*=6.44 to L*= 60.82; a*= -5.86 and b*= 42.19; differences in the intensity of the green color among fruits could be visually verified (light green and dark green). Color of ripe fruits, however, did not show variation noticeable to the naked eye, and due to this fact, they were not evaluated quantitatively. In relation to side shoots (Figure 1), only one line in class 1 (1%) was observed; six lines in class 2 (8%); 26 lines in class 3 (36%); 32 lines in class 4 (44%); and eight lines in class 5 (11%). A fewer number of side shoots requires less labor for thinning and for fruit harvest. Besides, it also allows a higher level of ventilation and decreases humidity in the microclimate formed around the plants, favoring a better phytosanitary condition.

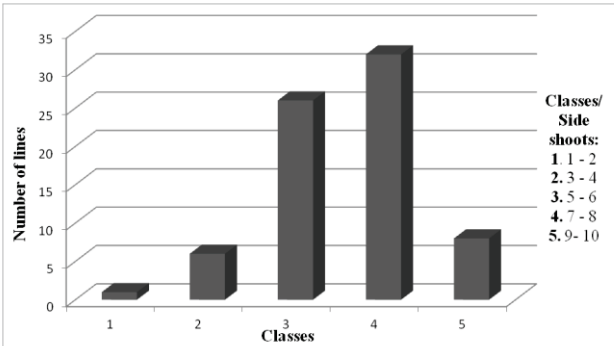

Figure 1. Number of side shoots of 73 S_2 Calabrian pepper lines: average values distributed in shoot number classes. Brasília-DF, Embrapa Vegetables, 2014.

Increase of pruning levels on *C. chinense* pepper plants with consequent decrease of side shoots, resulted in an increase of fruit weight (Jaimez *et al.*, 2002). The authors also suggest that with lower amount of shoots, change in distribution of nutrients assimilated by different parts of the plant may have happened. Alsadon *et al.* (2013) reported a significant increase of size and quality of fruits due to pepper plant pruning, keeping only one branch. Density and number of pruning operations per plant are important factors for *Capsicum* production, both under protected cultivation as well as under field conditions. Dasgan & Abak (2003) concluded that peppers grown in a greenhouse with high plant density and reduced number of side shoots per plant increased significantly the yield per m^2. McCraw & Greig (1986) observed higher yield per plant and fruit weight through pruning peppers grown in field conditions.

3.3. Evaluation of selected S2 lines

In quantitative evaluation, the selected lines CNPH 50.112, CNPH 50.116 and CNPH 50.185 showed significant differences for the characteristics: side shoots, total number and weight of fruits per plant, average fruit weight, length and diameter of the fruit, capsaicin content and color of unripe and ripe fruit (Table 1). The three lines showed fruit length between 9.4 cm and 10.6 cm, fruit diameter between 0.9 cm and 1.2 cm. In Brazil, *C. annuum* cultivars 'Calabrian' peppers (Isla, 2015) and 'Cayenne Dedo-de-Moça' (Feltrin, 2015) present fruit size of 8-12 cm x 1-2 cm and 12 cm x 1 cm, respectively, values close to selected lines. Besides, CNPH 50.112 showed better plant architecture and good fruit yield; CNPH 50.116 showed absence of side shoots and smaller size plants and CNPH 50.185 stood out among others for its excellent fruit yield, color and shape of fruits.

Among the three lines, CNPH 50.185 showed highest average values for total number and weight of fruits per plant, 255 fruits and 1,003 g per plant, respectively. CNPH 50.112 and CNPH 50.116 were less productive, with average of 105 and 86 fruit/plant, respectively.

Table 1. Average values of side shoots, total number of fruits per plant (NTF), average fruit weight (PM), fruit length (CF), diameter (DF), wall thickness (EP), total weight of fruits per plant (PT), unripe fruit color, ripe fruit color, total soluble solids content (ºBrix) and capsaicin (SHU) of three selected S_2 lines. Brasília-DF, Embrapa Vegetables, 2014.

CNPH	Side shoots	Fruits													
		NTF	PM (g)	CF (cm)	DF (cm)	EP (mm)	PT (g)	Unripe color			Ripe Color			ºBrix	SHU
								L*	a*	b*	L*	a*	b*		
50.112	3 b[1]	105 b	6.18 a	10.6 a	1.2 a	1.3 a	647 b	31.83 b	-9.96 b	11.1 b	37.82 a	36.66 b	19.45 b	11.5 a	5246 b
50.116	1 c	86 b	6.42 a	10.5 a	1.1 a	1.4 a	552 b	31.36 b	-8.53 c	9.18 c	37.53 a	36.58 b	19.37 b	10.6 a	5420 b
50.185	6 a	255 a	3.94 b	9.4 b	0.9 b	1.2 a	1003 a	36.49 a	-14.79 a	18.7 a	36.98 a	38.71 a	20.56 a	10.8 a	15727 a
CV(%)	17.1	35.8	13.3	6.4	6.4	10.5	27.4	6.4	13.2	16.8	1.0	2.1	2.9	8.4	

[1]Means followed by the same letter in the column do not differ significantly by the Scott-Knott test ($p < 0.05$)

The large number of fruits showed by CNPH 50.185 can be attributed to its high number of side shoots, since a positive correlation between number of fruits and side shoots can be noticed. Dasgan & Abak (2003) obtained variation of approximately 230% in number of fruits per plant when cultivation with one shoot was carried out (spacing of 80 cm between lines x 15 cm between plants) and four side shoots per plant (spacing of 80 cm x 45 cm), in a greenhouse.

CNPH 50.112 and CNPH 50.116 did not differ statistically for length, diameter and wall thickness of fruits. The three Calabrian pepper selected lines showed fruit length greater than the paprika pepper cultivars and 'BRS Mari' (5.4 cm to 6.3 cm), evaluated by Paulus *et al.* (2015).

Regarding the color, CNPH 50.185 showed the highest values in L*a*b* color space, differing statistically from other selected lines, showing unripe fruits with intense/dark green color and ripe fruits with intense bright red color. According to CIELAB chart for colors and pigments, which considers a* b* values, CNPH 50.185 showed color similar to the pigment *Chromium oxide green* for unripe fruits; and color similar to the pigment *Venetian red* for ripe fruits. Even differing statistically from the lines CNPH 50.112 and CNPH 50.116, for both color of unripe and ripe fruit, the range of values of three lines represented on CIELAB chart was not wide and the color observed in ripe fruits meets the market-required standards. According to Luts & Freitas (2008), colors of pepper fruits come from carotenoid pigments, which for its nutritional value, are among the most important plant pigments. Ripe, red *Capsicum* pepper fruits have 60 times more carotenoids than green fruits, besides having higher concentration of flavonoids and other secondary compounds (Gómez-García & Ochoa-Alejo, 2013).

No significant difference among the three selected lines was observed for soluble solid content, which varied from 10.63 to 11.57 ºBrix. Paulus *et al.* (2015) observed similar results for soluble solids in paprika cultivars (content of 10.30 ºBrix) and BRS Mari (10.2 ºBrix).

CNPH 50.185 presented capsaicin concentration of 15,700 SHU, differing statistically from lines CNPH 50.112 and CNPH 50.116, with contents of 5,200 and 5,400 SHU, respectively. These values are in the range of pungency of Calabrian pepper flakes. Similar values were found by Ziino *et al.* (2009) in Calabrian pepper cultivars 'Amando' (14.700 SHU) and 'Sigaretta' (7.400 SHU).

3.4. Evaluation of S3 lines

Significant differences were detected ($P<5\%$) among the 14 S_3 lines for all parameters evaluated, except for side shoots, plant height and wall thickness. Earliness, determined by number of days until flowering, counted from sowing, ranged from 17 days between the earliest line and the latest line, average fruit length ranged from 9.7 cm to 15.6 cm, average fruit width from 1.2 to 1.5 cm, average fruit weight from 8.1 g to 15.9 g, average fruit weight per plant from 120 g to 730 g and average number of fruits per plant from 17 to 106 fruits. CNPH 50.199 showed low yield due to a severe attack by *Alternaria* sp. observed since transplanting into the field. For color of leaf, average values of parameter b* on CIELAB chart ranged from 18.86 to 20.64 for light green and from 12.55 to 14.79 for dark green and for fruits ranged from 7.47 to 21.25.

Five of 14 S_3 lines (Table 2) were selected based on the following characteristics: average fruit weight above 12 g, less than two side shoots per plant, early flowering less than 80 days after sowing, average fruit length above 13 cm and average fruit diameter around 1.5 cm and dark green leaf; the selected lines did not differ from each other for any of the parameters; and sparse or medium pilosity (CNPH 50.189, 50.192, 50.193, 50.194 and 50.195). It is interesting to highlight that significant increase of fruit size (length and diameter) and average fruit weight of the five selected S_3 lines in relation to the values observed in S_2 lines (Table 1) and OP, from which they derived, was observed. Values of average length and diameter of fruits of selected S_3 lines are close to the highest values observed in Indian local variety "Pusa Jwala" (*C. annuum*), 13 cm and 1.5 cm, respectively. In the next generation (S_4), tests under field conditions will be carried out in order to determine line yield, resistance to diseases as well as analysis of capsaicinoid concentration in fruits.

Limited information, as well as few Calabrian pepper cultivars, are available in the Brazilian market, despite the growing demand for this kind of hot pepper. The results of this work showed that the introduction of germplasm in a *sui generis* way allowed efficient selection of genotypes of interest. The authors found high variability in original population, allowing the selection of new genotypes (*C. annuum*) with superior agronomic and processing characteristics. These materials may be released as cultivars adapted to Brazilian conditions that meet the Brazilian market demand for dehydrated pepper flakes.

Table 2. Average values for fruit weight (PF), length (CF), diameter (DF), days from sowing until flowering (earliness), number of side shoots per plant and stem pilosity observed in five selected S_3 lines and 14 S_3 lines evaluated. Brasília-DF, Embrapa Vegetables, 2015.

Range of average values	PF (g)	CF (cm)	LF (cm)	Earliness	Side shoots	Pilosity
Five selected S_3 lines	12.7 – 15.9	13.3 – 15.6	1.4 – 1.5	70 - 75	1.20 – 1.50	Sparse to medium
14 S_3 lines	8.2 – 15.9	9.7 – 15.6	1.2 – 1.5	70 - 87	1.20 – 1.77	Sparse to medium

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