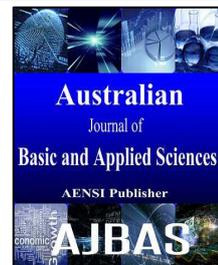




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Agronomic evaluation and morphological characterization of chili peppers (*Capsicum annuum*, Solanaceae) from Brazil

¹Henrique Kuhn Massot Padilha; ¹Carla Vasconcelos Sigales; ²Juliana Castelo Branco Villela; ²Ricardo Alexandre Valgas and ²Rosa Lía Barbieri.

¹Federal University of Pelotas, Crop Science Dept., Capão do Leão, RS - Brazil.

²Embrapa Temperate Agriculture, Genetic Resources Dept., Pelotas, RS - Brazil.

Address For Correspondence:

Henrique Kuhn Massot Padilha, Federal University of Pelotas, Crop Science Dept., Capão do Leão, RS - Brazil.
E-mail: henriquepadilha@gmail.com

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ABSTRACT

There is a large diversity of cultivated peppers in the world. Originated from Americas, *Capsicum* peppers belongs to Solanaceae family. Nowadays, the consumption occurs in different ethnic groups, giving flavor, aroma and color to the food. *Capsicum annuum* is the most important economically specie of this genus and the most cultivated. Genotypes should be properly characterized to allow their use in breeding programs and facilitate their conservation. The value of a genotype preserved in a genebank depends on the information available to promote their use, and consequently, its use becomes a way to ensure their conservation. This study aimed to perform agronomic evaluation and morphological characterization of chili peppers (*Capsicum annuum*) accessions. Twenty accessions from *Capsicum* Genebank of Embrapa Temperate Agriculture (Pelotas – RS, Brazil) were evaluated. The experimental design was a randomized complete block with 20 treatments and three replications. We employed 11 quantitative descriptors for agronomic evaluation and 12 qualitative descriptors for morphological characterization. Results evidenced genetic variability for all evaluated variables. Quantitative data of agronomic evaluation were submitted to analysis of variance (ANOVA) to compare the mean values of accession variables and Tukey test was used for comparison of means. Genotypes analyzed in this work presented early and late reproductive cycles; plant height ranged from 18.57 cm (P22) to 46.63 cm (P266), while canopy diameter ranged from 23.12 cm (P122) to 48.72 cm (P119). According hierarchical clustering (UPGMA) was formed four groups (G1, G2, G3 e G4), grouping those accessions with similar features, such as growth habit, density of leaves, color and fruit shape. Evaluating a unique specie in this work, it was possible find a great genetic variability to such aspects in *Capsicum annuum* accessions from *Capsicum* Genebank of Embrapa Climate Agriculture. These phenotypic differences indicate different purposes for pepper accessions, such as fresh consumption, processed or dried and as an ornamental plant.

INTRODUCTION

There is a large diversity of chili peppers cultivated around the world, and their fruits have different shapes and colors. The uses of these plants are as diverse as the types of fruits found in *Capsicum* (Bosland and Votava, 2012; Albrecht *et al.*, 2012).

Originated from Americas, *Capsicum* peppers belongs to Solanaceae family. Nowadays, the consumption occurs all over the world in different ethnic groups, giving flavor, aroma and color to the food (Ornelas-Paz *et al.*, 2013). What gives pungency to *Capsicum* peppers is a group of capsaicinoids (alkaloids), and among them,

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the most important is the capsaicin (Keyhaninejad *et al.*, 2014). Capsaicin is specific to *Capsicum* genus, unlike piperine, spicy substance of black pepper (*Piper nigrum*), which acts differently in organism (Santos *et al.*, 2013).

There are 30 species in the genus *Capsicum*, of which five are domesticated including the species *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq, *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz et Pav. and approximately 22 are wild and endemic to Americas (Sun *et al.*, 2014).

Among the domesticated species, *Capsicum annuum* is the most widespread popular and important specie. There are many different varieties, such as sweet bell peppers, small pungent types, ornamental varieties, commercial varieties (*jalapeño*, *cayenne*, *serrano*), and other types differing in flower and fruit colour, shape, size and taste (Büttow *et al.*, 2010; González-Zamora *et al.*, 2013).

To increase *Capsicum* plant breeding programs and contribute with genetic resources conservation its necessary a detailed characterization of *Capsicum* diversity existent, including morphological, geographic and molecular information (Hill *et al.*, 2013). Morphological characterization is a process, which uses a descriptive list, proposed by Biodiversity International (IPGRI, 1995), to provide more information about germplasm maintained in working collections and germplasm banks. Thus, it is possible differentiate these genotypes and makes them more useful. The value of a genotype preserved in a genebank depends on the information available to promote their use, and consequently, its use becomes a way to ensure their conservation.

Thus, this study aimed to perform agronomic evaluation and morphological characterization of chili peppers accessions (*Capsicum annuum*) from the *Capsicum* Genebank of Embrapa Temperate Agriculture (Pelotas – RS, Brazil).

MATERIALS AND METHODS

The evaluation was performed by analysis of 20 accessions of *C. annuum* from *Capsicum* Genebank of Embrapa Temperate Agriculture (Table 1). Currently, the genebank maintains 403 accessions, originating from collections, donations from small farmers (landraces), private peppers collectors and fruit acquisition in popular markets.

The sowing was carried in August 2013 in polystyrene trays for seedlings, containing 72 filled cells with commercial substrate. In October, when the plants had five to seven true leaves, they were transplanted to experimental field, spaced 0.5 m between plants and 1.3 m between rows. The rows were covered with black plastic mulching type to make weed control and contribute to maintenance of soil moisture. The plants were irrigated by drip irrigation system.

The experimental design was a randomized complete block with 20 treatments and three replications. The experimental unit was composed of a row with ten plants. In totality, 30 plants of each accession were evaluated.

For agronomic evaluation were utilized 11 quantitative descriptors: elapsed days from sowing to flowering, days elapsed from sowing to the first ripe fruit production, plant height, canopy diameter, number of fruits, fresh weight per fruit, dry matter per fruit, fruit length, fruit diameter, peduncle length and fruit wall thickness.

For morphological characterization, 12 descriptors were employed (IPGRI, 1995): growth habit, branching density, density of leaves, persistence between plant and peduncle, pungency, aroma, fruit surface, immature fruit color, mature fruit color, fruit shape, number of loci and persistence between fruit and peduncle. The fruit descriptors were employed to ten fruits of each plant, totalizing 100 fruits per accession. Data were obtained through the fashion of each accession based on each descriptor.

Plant height and canopy diameter were evaluated utilizing a ruler and fruit descriptors were measured by digital caliper and analytical balance. The ripe fruit from each plant were collected and counted. For each accession, were carried out around five harvesting (depending on accession), due to the staggered fruits ripening. Numbers of fruits collected were summed to obtain total production. Fresh fruit material was determined with scale. For dry matter per plant, ripe fruit from each plant, picked at random, were dried at 50 °C in air circulating oven until constant weight. The samples were weighed out with analytical scale to determination of dry matter weight.

Quantitative data of agronomic evaluation were submitted to analysis of variance (ANOVA) to compare the mean values of accession variables. After confirmation of significant difference between treatments, according to the *p*-value associated with the F test, we evaluated the magnitude of these differences using multiple comparison test. Tukey test was used for comparison of means with 95% confidence. Statistical analyzes were performed using the computer program SAS (SAS, v. 9.2).

Qualitative data were subjected to statistical analysis, by hierarchical clustering, unweighted pair-group method using arithmetic averages (UPGMA) with euclidean distance method utilizing the statistical software R (v.3.1.2). To check the adjustment between the similarity matrix and the dendrogram obtained, we computed the cophenetic correlation coefficient (*r*). To determine the optimal number of groups, the cutoff point in dendrogram was determined according to Mojena's criterion (Mojena, 1977).

Results:

Quantitative and qualitative traits evaluated in *Capsicum annuum* accessions revealed genetic variability (Table 2 and Figure 1). Performing variance analysis by F test, it was evidenced significant differences in averages by Tukey test for all evaluated quantitative traits (Table 2).

The first ripe fruit of each accession were produced in 138 days (P266) to 170 days (P77) from the sowing. Plant height varied from 18.57 cm (P22) to 46.63 cm (P266), while canopy diameter ranged from 23.12 cm (P122) to 48.72 cm (P119).

The P138 accession was featured by producing the highest number of fruits per plant (average production of 890 fruits), followed by P143, which produced an average of 510 fruits per plant (Table 2). The accession that showed higher fresh fruit weight was P202, with 17.33 g per fruit, in contrast to P138, P143 and P302, which had the lowest values for fresh fruit weight with 0.42 g, 0.44 g and 0.56 g, respectively. For variable fresh fruits weight per plant, P266 accession was highlighted, with an average production of 1350 g per plant. The P111 accession also evidenced potential, with average production of 845 g per plant. Fruit length varied from 11.61 mm (P39) to 70.13 mm (P266), these characteristic gathered accessions in nine different groups (Table 2). Fruit width had a variation of 6.95 mm (P302) to 25.62 mm (P202).

The P259 accession had the highest peduncle length (30.07 mm), forming six different groups for this trait. For fruit wall thickness, there was a variation from 0.52 mm (P143) to 3.31 mm (P202), forming five different groups (Table 2). The P111 had the highest value for dry fruit matter (2.8 g). In contrast, P302 showed the lowest value for this character (0.13 g). The P58 e P266 accessions (Figure 1) showed high productivity (Table 2), with large and pungency fruits. Moreover, these accessions exhibited high persistency of fruit and peduncle, branching density and leaves density sparse. However, they had a high persistence of peduncle and plant.

The P111 and P202 accessions (both of *jalapeño* type) showed sparse branching density, smooth fruit surface with striations, elongated fruit shape, high pungency, medium aroma, low persistence between peduncle and plant and high persistence between peduncle and fruit.

According hierarchical clustering (UPGMA) with dissimilarity of 0.65 was formed four groups (G1, G2, G3 e G4) clustering those accessions similar (Figure 2). The cophenetic correlation coefficient obtained between dendrogram and genetic distance matrix was 0.8012. The separation of clusters through the cut-off point in the dendrogram was defined based on Mojena's criterion, with the value $k=1.25$.

The P139 and P259 accessions (Figure 1) were clustered in the same group (G1) (Figure 2). They had yellow ripe fruits, the largest peduncle length and higher persistence of peduncle and plant. Also evidenced the same number of locus and intermediate persistence between peduncle and fruit.

Both groups (G2 and G3) presented accessions with potential features for ornamental plants, such as erect small fruits and multiple colors throughout maturation process. The P58, P111, P202 e P266 accessions were clustered at group four (G4). All of them showed similar characteristics such as two colors of maturation: green unripe fruit and red ripe fruit, large fruits with triangular shape and plant with sparse branching habit.

Regarding persistence of peduncle and fruit, 60% of the accessions showed intermediate persistence and 40% high persistence. None accession showed low persistent. Among evaluated accessions, 75% had fruits with two locules and 25% had fruit with three locules. It was not verified fruits with one or four locules. This character does not seem to be important in plant breeding. Regarding pungency, 65% fruits accessions showed intermediate pungency, 25% low pungency and 10% high pungency.

Table 1: Accession, popular name and origin of evaluated accessions of *Capsicum annuum* from *Capsicum* Genebank of Embrapa Temperate Agriculture. Pelotas, RS – Brazil, 2016.

Accession	Popular name	Origin
P7	pimenta	Renascença – PR
P22	pimentinha vermelha	Renascença – PR
P39	pimenta	Farroupilha – RS
P58	pimenta ornamental	São Lourenço do Sul – RS
P77	pimenta negra	Canoinhas, SC
P111	<i>jalapeño</i>	Turuçu – RS
P119	pimenta ornamental	Rio Grande – RS
P122	pimenta ornamental	Pelotas – RS
P136	pimenta	Vassouras – RJ
P137	pimenta	Vassouras – RJ
P138	pimenta	Vassouras – RJ
P139	pimenta	Vassouras – RJ
P140	pimenta	Vassouras – RJ
P141	pimenta	Vassouras – RJ
P143	pimenta	Vassouras – RJ
P202	<i>jalapeño</i>	Pelotas – RS
P258	pimenta ornamental	Pelotas – RS
P259	pimenta	Pelotas – RS
P266	pimenta	Palhoça – SC
P302	pimenta ornamental	Pelotas – RS

Table 2: Averages of 11 quantitative descriptors of *Capsicum annum* from *Capsicum* Genebank of Embrapa Temperate Agriculture. Pelotas, RS – Brazil, 2016.

AI	DF	DFR	PH (cm)	CD (cm)	FN	FFM (g)	DMF (g)	FL (mm)	LW (mm)	PL (mm)	WT (mm)
P7	80.00 bc	149.00 ab	39.50 abc	45.40 abc	282.63 bcd	2.25 fgh	0.70 ef	23.19 fg	24.32 a	21.62 bc	1.72 bcd
P22	66.67 c	139.00 b	18.57 h	27.58 def	128.53 cd	1.85 ghi	0.42 efgh	30.85 e	11.32 cd	20.50 bcd	1.08 cde
P39	90.00 abc	142.00 ab	29.01 cdefgh	44.23 abc	285.90 bcd	1.01 hij	0.37 efgh	11.61 i	11.45 cd	13.22 f	0.94 de
P58	88.33 abc	146.00 ab	26.30 efgh	29.65 def	49.18 d	12.72 c	2.06 bc	67.82 a	23.52 ab	22.12 bc	1.94 bc
P77	106.67 ab	171.33 a	37.27 abcde	37.62 abcde	164.47 cd	1.60 ghij	0.57 efgh	20.62 g	13.67 bcd	20.95 bc	0.82 de
P111	80.00 bc	153.67 ab	39.50 abc	33.22 cdef	53.50 d	15.74 b	2.80 a	56.80 b	24.77 a	21.54 bc	3.17 a
P119	83.33 abc	148.00 ab	43.57 ab	48.72 a	496.47 b	0.76 ij	0.26 fgh	23.02 fg	7.75 cd	19.53 bcde	0.82 de
P122	75.00 c	161.67 ab	23.60 gh	23.12 f	92.60 d	1.99 fghi	0.50 efgh	28.41 ef	11.77 cd	21.04 bc	0.98 de
P136	86.67 abc	146.00 ab	36.47 abcdef	41.32 abcd	255.53 bcd	1.64 ghij	0.52 efgh	39.83 d	9.38 cd	20.69 bc	0.94 de
P137	83.33 abc	139.67 b	27.61 defgh	38.58 abcde	277.13 bcd	1.42 ghij	0.41 efgh	32.37 e	9.26 cd	19.51 bcde	0.85 de
P138	85.00 abc	140.00 b	34.17 bcdefg	45.67 abc	890.27 a	0.42 j	0.16 gh	12.26 i	8.30 cd	15.73 ef	0.87 de
P139	75.00 c	170.67 a	24.97 fgh	27.18 def	62.97 d	3.15 f	0.75 ef	54.64 bc	11.72 cd	23.77 b	0.90 de
P140	110.00 a	166.00 ab	29.27 cdefgh	36.68 abcdef	399.70 bc	0.84 ij	0.31 efgh	21.63 fg	8.27 cd	19.51 bcde	0.85 de
P141	90.00 abc	140.00 b	29.23 cdefgh	37.59 abcde	217.30 bcd	2.53 fg	0.76 e	27.13 efg	16.86 abcd	19.10 cde	1.15 cde
P143	106.67 ab	153.67 ab	32.31 bcdefg	41.26 abcd	512.90 b	0.44 j	0.15 gh	12.97 hi	7.10 d	16.19 def	0.52e
P202	83.33 abc	161.33 ab	38.66 abcd	33.88 cdef	45.66 d	17.33 a	2.46 ab	64.23 a	25.62 a	22.21 bc	3.31 a
P258	90.00 abc	160.00 ab	30.93 cdefg	34.33 bcdef	152.20 cd	2.46 fg	0.63 efg	22.59 fg	15.93 abcd	22.92 bc	1.14 cde
P259	83.33 abc	156.67 ab	37.63 abcde	45.82 abc	161.57 cd	4.75 e	1.47 d	48.21 c	17.50 abc	30.07 a	1.63 bcd
P266	73.33 c	138.33 b	46.63 a	48.33 ab	141.50 cd	9.45 d	1.65 cd	70.13 a	22.21 ab	23.31 bc	2.56 ab
P302	91.67 abc	151.33 ab	22.53 gh	24.73 ef	219.19 bcd	0.56 j	0.13 h	19.93 gh	6.95 d	18.98 cde	0.89 de

Means followed by the same letter in each column, did not differ significantly by Tukey test at 5% probability. AI: Accession identification; DF: Days to flowering; DFR: Days of the first ripe fruit; PH: Plant height; CD: canopy diameter; FN: fruit number per plant; FFM: Fresh fruits matter; DMF: dry matter per fruit; FL: fruit length; FW: fruit width; PL: peduncle length; WT: Fruit wall thickness.

Discussion:

Accessions exhibited great variation for all evaluated characters, confirming the affirmation of Pickersgill *et al.* (1997): There is a large genetic diversity in *Capsicum* genus and this available variation within various domesticated species has been very little exploited and has certainly not yet been exhausted.

Numbers of days from sowing until the first ripe fruit remained into the expected period. These is an important attribute to monitor the time of seed development, because this period influences directly some traits such as physiological quality and seed viability (Mengarda and Lopes, 2012). All accessions showed different harvest times, evidencing genetic variability for reproductive cycle. This is an important aspect to development of new cultivars with shorter or longer cycles, allowing the farmers a largest number of options to choose in field implantation. Likewise, there was a time that had simultaneously on the same plant: flowers, unripe and ripe fruits. It also has importance in plant ornamental aspects becoming more colorful and attractive to consumers.

Plant height and canopy diameter is an important trait because plant size influences directly their indication of use: cultivation in pots, gardens or fields. In field cultivating, plant height and canopy diameter guide the planning of tillage structure (distance among plants, number of plants per area). There was a great variation among canopy diameters of evaluated accessions, as observed in other studies conducted in *Capsicum* (Sudré *et al.*, 2010; Singh *et al.*, 2014). Furthermore, Pedó *et al.* (2013) commented that canopy diameter is an important attribute to photosynthetic rate, influencing indirectly in plant production.



Fig. 1: Fruits of 20 accessions of *Capsicum annuum* from *Capsicum* Genebank Embrapa Temperate Agriculture evaluated in this work. Pelotas, RS – Brazil, 2016.

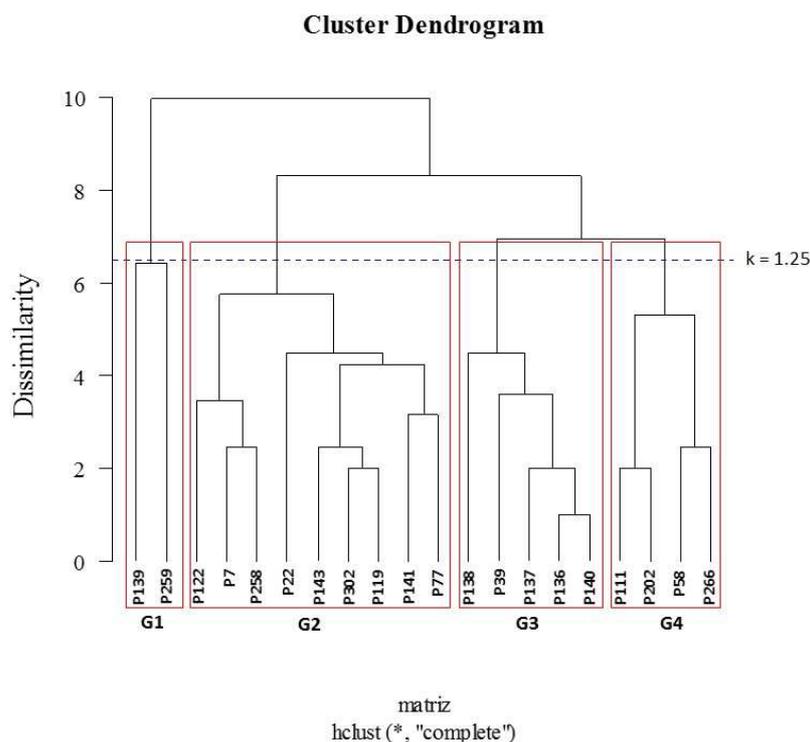


Fig. 2: Dendrogram from analysis of 20 accessions *Capsicum annuum* obtained by clustering UPGMA method based on complete-linkage cluster analysis. Cophenetic correlation coefficient = 0.8012. Pelotas, RS – Brazil, 2016.

Characteristics such as fruit width, fruit wall thickness, plant height and number of fruits per plant were the most influential factors in yield (Cankaya *et al.*, 2010). Ribeiro and Reifshneider (2008) commented that wall thickness is an important attribute in sweet pepper breeding for paprika, because it influences industrial purposes and processing power consumption. These fruit characteristics ranged according to each genotype.

Dry fruit matter is the mass of completely dehydrated fruit. To obtain 1 kg of dried red pepper (*Capsicum baccatum*) it is necessary about 7 kg of fresh pepper fruit (Neitzke, 2012). Find accessions with high value for dry fruit matter is essential for obtaining promising cultivars in this aspect. In other words, this is an important alternative to enhance the performance of dehydrated chili producers.

High persistence of peduncle and plant causes difficulty at harvesting. In general, selections in pepper breeding programs are designed to intermediate persistence of peduncle and plant (to facilitate harvest), and other characteristics such as productivity, plant architecture, earliness and disease resistance (Martins *et al.*, 2010).

The P58 and P266 have potential to *pimenta calabresa* production (fruit dehydrated and subsequently milled thick) or preserves due to large and red ripe fruits. *Jalapeño* peppers presented large fruits with thick wall, strong taste and aromatic flavor. They are appropriate to production of liquid sauces (Ribeiro and Reifshneider, 2008). Furthermore, Cervantes-Paz *et al.* (2014) suggest that fresh or processed *jalapeño* peppers can serve as sources of antioxidants with great potential for food preservation and health promotion.

Regarding hierarchical clustering (UPGMA) with dissimilarity of 0.65 was formed four groups (G1, G2, G3 e G4). The cophenetic correlation measures the fit degree between dissimilarity matrix and resulting matrix simplification due to grouping method. The value (r) was 0.8012 considered a high index. According Bussad (1990) values (r) higher than 0.80 indicate good representability. Milligan and Cooper (1985) reported a detailed investigation of indices for the number of groups and found a satisfactory criterion using k value = 1.25 for Mojena's stopping rule.

To development of new cultivars for industrial dried processing is essential select fruits detached easily from calyx, as opposed to P139 and P259 accessions. The taste of product may change when the calyx is included in grinding (Ribeiro and Reifshneider, 2008). Most wild and semi-domesticated species of *Capsicum* has low persistence between peduncle and fruit, precisely to facilitate the dispersal of their seeds in nature (Murillo-Amador *et al.*, 2015). Furthermore, historically, the selections were performed for pepper varieties with intermediate persistence, allowing fruits to be detached easily, but remaining in the plant during its development.

The P58, P111, P202 e P266 accessions were clustered at group four (G4). These accessions differs from other accessions that presented different colors during maturation process, small fruits and dense or intermediate branching habit, usually common to ornamental genotypes.

None of these accessions produced fruit without pungency, although the most cultivated and more important type of *C. annuum* in Brazil is the bell pepper. According to last agricultural census, Brazil produced 248,767 tons of bell pepper (IBGE, 2006).

Conclusion:

Evaluating a unique specie in this work, it was possible find a great genetic variability to phenotypic aspects in *Capsicum annuum* accessions from *Capsicum* Genebank of Embrapa Climate Agriculture. These accessions are potential source of genes to *Capsicum annuum* breeding programs, both for cultivars development for food consumption, as for ornamental pepper cultivars.

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