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*Scientific Article*

# Pre-breeding of a *Capsicum annuum* accession with ornamental potential

# Pré-melhoramento de um acesso de *Capsicum annuum* para uso ornamental

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**ABSTRACT** - *Capsicum annuum* is a domesticated pepper species that exhibits a wide range of fruit size, color, and shape, as well as plant characteristics important for ornamental pepper cultivars, including plant architecture and leaf size and color. This variability can be found in *Capsicum* accessions from germplasm banks and used in breeding programs to develop new ornamental pepper cultivars. The objective of this study was to develop the ornamental characterization and pre-breeding of a segregating *Capsicum annuum* accession from the Active Germplasm Bank of *Capsicum* of the Brazilian Agricultural Research Corporation (Embrapa Temperate Climate). Sixty-seven $F_1$-generation and sixty-two $F_2$-generation plants were characterized based on 16 morphological descriptors: stem color, nodal anthocyanin, plant growth habit, branching density, leaf density, leaf color, number of leaves per axil, flower position, corolla color, calix pigment, immature fruit color, fruit position, mature fruit color, fruit shape, fruit apex shape, and fruit brightness. The relative contribution of each trait to the genetic divergence was determined using the method proposed by Singh (1981). A principal component analysis was carried out. Almost all evaluated variables showed segregation, except for flower position and immature fruit color. Genetic variability was found in both $F_1$ and $F_2$ generations, indicating their potential for developing ornamental *Capsicum annuum* varieties.

**Keywords**: Genetic resources. Morphological characterization. Genetic variability. Peppers.

**RESUMO** - *Capsicum annuum* é uma das espécies de pimentas domesticadas que possui ampla variação de tamanho, cor e formato de frutos, além de caracteres de planta, como arquitetura, porte ecor de folhas, entre outras, que também são muito importantes para cultivares de pimentas ornamentais. Esta variabilidade pode ser encontrada nos acessos de bancos de germoplasma de *Capsicum,* podendo ser utilizada no melhoramento genético para desenvolver novas cultivares de pimentas ornamentais. O objetivo deste trabalho foi realizar a caracterização ornamental e o pré-melhoramento de um acesso segregante de *Capsicum annuum* do Banco Ativo de Germoplasma de *Capsicum* da Embrapa Clima Temperado. Foram caracterizadas 67 plantas da geração $F_1$ e 62 plantas da geração $F_2$, através de 16 descritores morfológicos: cor da haste, antocianina nodal, hábito de crescimento da planta, densidade de ramificação, densidade de folhas, cor da folha, número de flores por axila, posição da flor, cor da corola, pigmento do cálice, cor do fruto imaturo, posição do fruto, cor do fruto maduro, formato do fruto, formato da ponta do fruto e brilho do fruto. A contribuição relativa de cada caráter para a divergência genética foi obtida pelo método proposto por SINGH. Foi realizada análise de componentes principais. Houve segregação para quase todas as variáveis avaliadas, exceto para posição de flor e cor de fruto imaturo. Foi possível observar a variabilidade genética nas gerações $F_1$ e $F_2$, evidenciando seu potencial de uso para o desenvolvimento de variedades ornamentais de *Capsicum annuum*.

**Palavras-chave**: Recursos genéticos. Caracterização morfológica. Variabilidade genética. Pimentas.

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## INTRODUCTION

*Capsicum* is a genus in the family Solanaceae that presents five domesticated species: *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* (PERRY et al., 2007). *Capsicum* crops have shown significant grown in the agribusiness sector in Brazil due to their profitability and employment generation, and mainly because peppers are highly valued as food and can be used in the pharmaceutical industry and as ornamental plants (COSTA et al., 2017). *C. annuum* is the most widely cultivated among these domesticated pepper species and holds high economic importance in Brazil (NEITZKE et al., 2014). This species is characterized by bluish or violet anthers and white corollas, typically bearing one flower per reproduction node (CARVALHO; BIANCHETTI, 2008). Additionally, its fruits exhibit a wide range of size, color, and shape (HERNÁNDEZ-VERDUGO; LUNA-REYES; OYAMA, 2001), and variations in plant traits, including architecture, growth habit, and leaf color. This variability accounts for the numerous pepper types, including sweet pepper, jalapeño, serrano, cayenne, cherry, and ornamental varieties, which typically exhibit small-sized plants.

Genetic variability is a fundamental prerequisite for using a species in breeding programs, as it allows for the selection of accessions with desirable traits as parents (FALEIRO et al., 2008; SANTO; MENEZES; CARMO, 2022). However, despite *Capsicum* germplasm banks in Brazil possess a large collection of accessions with wide genetic variability suitable for the development of new ornamental pepper varieties through breeding programs, cultivars remain scarce in the country (NEITZKE et al., 2010, COSTA et al., 2019). According to Neitzke et al. (2016), desirable traits for ornamental peppers of the genus *Capsicum* include plant architecture; number, shape, color, and position of fruits; color, shape, and density of leaves; easy cultivation; long maintenance of ornamental value in potted plants (durability of fruits and leaves); and continuous fruit production. Genotypes of small-sized plants are primarily desirable for cultivation in flower pots (RIVAS et al., 2023). Growing ornamental pepper plants can be a profitable alternative for family farmers (STUMMEL; BOSLAND, 2007). This indicates the importance of utilizing the available *Capsicum* germplasm in Brazil for the development of varieties that meet the needs of growers and consumers (NEITZKE et al., 2016).

Morphological characterization of accessions from germplasm banks is one of the most important stages in the pre-breeding of *Capsicum* peppers. Accessions identified as promising for ornamental purposes in this characterization can be selected for crosses or direct use after a few cycles of mass selection. This is important for developing populations, lines, and cultivars with desirable traits (AQUINO et al., 2022). Pepper accessions from germplasm banks sometimes exhibit segregation for different traits, which can be an opportunity for pre-breeding. This segregation can be explained by the reproduction type of pepper species, which is predominantly self-pollinating but exhibits an increasing level of cross-pollination depending on the occurrence of wind and pollinating insects. According to Bosland and Votava (2000), the cross-pollination rate in *C. annuum* varies from 2% to 90%. Segregation is a desirable trait in this case; therefore, flowers are not protected, ensuring that fruits are from self-pollination. The segregating accession *Capsicum annuum* P100 in the Active Germplasm Bank of *Capsicum* of the Brazilian Agricultural Research Corporation (Embrapa Temperate Climate) has drawn attention to this study due to its ornamental characteristics, such as compact plant architecture, abundant flowering, and erect and shiny fruits with intense colors. Thus, considering the scarcity of ornamental pepper cultivars and the wide variability of accessions in germplasm banks, the objective of this study was to develop the ornamental characterization and pre-breeding of this segregating *Capsicum annuum* accession from the Active Germplasm Bank of *Capsicum* at the Embrapa Temperate Climate.

## MATERIAL AND METHODS

The accession *Capsicum annuum* P100 from the Active Germplasm Bank of *Capsicum* of the Embrapa Temperate Climate was chosen for evaluation because it is a highly segregating accession with ornamental traits related to color and size of fruits and leaves, as well as variations in plant size and architecture. Segregation for several traits of this accession was previously observed during seed multiplication and morphological characterization.

Sixty-seven $F_1$-generation plants (resulting from self-pollination of plants from the original accession) and sixty-two $F_2$-generation plants (resulting from self-pollination of $F_1$ plants) were characterized. $F_2$ plants were obtained by the single seed descent (SSD) breeding method. $F_1$ seeds were sown in expanded polyethylene trays filled with a sterilized commercial substrate, in October 2018, and maintained in a greenhouse of the Embrapa Temperate Climate, Pelotas, RS, Brazil. The seedlings were transplanted to 7-liter pots containing a commercial substrate when they reached approximately 10 cm height. The pots with the plants were maintained in a greenhouse with anti-aphides screen for ensuring a successfully self-pollination; irrigation was carried out manually. $F_2$ seeds were sown in 2019 under the same conditions as $F_1$ seeds, at the Federal Institute of Rio Grande do Sul, in Bagé, RS. The experimental design was completely randomized. Plants and fruits were evaluated for 16 morphological descriptors (IPGRI, 1995), as shown in Table 1.

**Table 1**. Description of the 16 morphological descriptors used for evaluating *Capsicum annuum* plants of the Active Germplasm Bank of *Capsicum* of the Brazilian Agricultural Research Corporation (Embrapa Temperate Climate).

| Number | Descriptor | Code | Characteristic |
|---|---|---|---|
| 1 | Stem color | 1 | Green |
| | | 2 | Green with violet stripes |
| | | 3 | Violet |
| 2 | Nodal anthocyanin | 1 | Green |
| | | 3 | Light violet |
| | | 5 | Violet |
| | | 7 | Dark violet |
| 3 | Plant growth habit | 3 | Prostrate |
| | | 5 | Intermediate |
| | | 7 | Erect |
| | | 9 | Other |
| 4 | Branching density | 3 | Sparse |
| | | 5 | Intermediate |
| | | 7 | Dense |
| 5 | Leaf density | 3 | Sparse |
| | | 5 | Intermediate |
| | | 7 | Dense |
| 6 | Leaf color | 1 | Yellow |
| | | 2 | Light green |
| | | 3 | Green |
| | | 4 | Dark green |
| | | 5 | Light violet |
| | | 6 | Violet |
| | | 7 | Variegated |
| | | 8 | Green with anthocyanin |

*The color observed in the earliest stage was considered when immature fruits exhibited multiple colors.

**Table 1**. Continuation.

| Number | Descriptor | Code | Characteristic |
|---|---|---|---|
| 7 | Number of leaves per axil | 1 | One |
| | | 2 | Two |
| | | 3 | Three or more |
| | | 4 | Many with short internodes |
| | | 5 | One and two |
| | | 6 | One, two, and three |
| | | 7 | Two and three |
| | | 8 | Two, three, and four |
| 8 | Flower position | 3 | Pendant |
| | | 5 | Intermediate |
| | | 7 | Erect |
| | | 9 | All positions |
| | | 11 | Intermediate and erect |
| | | 13 | Pendant and intermediate |
| 9 | Corolla color | 1 | White |
| | | 2 | Light yellow |
| | | 3 | Yellow |
| | | 4 | Greenish yellow |
| | | 5 | Violet with white base |
| | | 6 | White with violet base |
| | | 7 | White with violet margin |
| | | 8 | Violet |
| | | 9 | Greenish white |
| | | 10 | White with purple spots |
| | | 11 | Greenish white with purple spots |
| 10 | Calix pigment | 0 | Absent |
| | | 1 | Present |
| 11 | Immature fruit color* | 1 | White |
| | | 2 | Yellow |
| | | 3 | Green |
| | | 4 | Orange |
| | | 5 | Violet |
| | | 6 | Dark violet |
| | | 7 | Greenish yellow |
| | | 8 | Yellowish green |
| | | 9 | Yellowish white |
| | | 10 | Brown |
| 12 | Fruit position | 3 | Pendant |
| | | 5 | Intermediate |
| | | 7 | Erect |
| | | 9 | All |
| | | 11 | Pendant and intermediate |
| | | 13 | Pendant and erect |
| | | 15 | Intermediate and erect |
| 13 | Mature fruit color | 1 | White |
| | | 2 | Lemon yellow |
| | | 3 | Pale orange-yellow |
| | | 4 | Orange-yellow |
| | | 5 | Pale orange |
| | | 6 | Orange |
| | | 7 | Light red |
| | | 8 | Red |
| | | 9 | Dark red |
| | | 10 | Violet |
| | | 11 | Brown |
| | | 12 | Black |
| | | 13 | Yellow |
| | | 14 | Pale yellow |
| 14 | Fruit shape | 1 | Elongated |
| | | 2 | Rounded |
| | | 3 | Triangular |
| | | 4 | Bell-shaped |
| | | 5 | Rectangular |
| 15 | Fruit apex shape | 1 | Pointed |
| | | 2 | Truncate |
| | | 3 | Depressed |
| | | 4 | Depressed with a tip |
| 16 | Fruit brightness | 0 | Absent |
| | | 1 | Present |

*The color observed in the earliest stage was considered when immature fruits exhibited multiple colors.

The relative contribution of each trait to genetic divergence was determined using the method proposed by Singh (1981). Principal component analysis (PCA) was performed using the statistical software R Development Core Team (2022) in the Rstudio integrated development environment. The PCA is a widely used analysis for characterization of accessions in germplasm banks, as it identifies the most important traits and their contribution to the total variation between the analyzed individuals, providing indications for removing those that contribute little (DIAS;

KAGEYAMA; CASTRO, 1997; PRIORI et al., 2018).

## RESULTS AND DISCUSSION

Genetic variability was identified for morphological traits in the evaluated progenies. This observed variability is essential for developing new ornamental pepper cultivars. Carvalho et al. (2021), and Jin et al. (2023) reported significant differences between plants, with segregating progenies of the genus *Capsicum*.

The traits that exhibited no variations in $F_1$ plants were: nodal anthocyanin, flower position, and immature fruit color. The following traits exhibited no variation in $F_2$ plants: flower position, immature fruit color, fruit position, branching density, and corolla color. According to the statistical analysis of Singh index, both generations showed no observed variation in flower position and immature fruit color (Figure 1). The absence of genetic variability for a trait can be advantageous in breeding programs when the classification is associated with the standard desired by consumers (GOMES et al., 2022).


**Figure 1**. Traits with no observed variation in $F_1$ and $F_2$ plants, according to the Singh's method (SINGH, 1981): flower position and immature fruit color.

Principal component analysis (PCA) was performed excluding traits with no observed variation through the analysis of Singh (1981). Some other studies have used PCA for estimating genetic diversity in *Capsicum* species. Almeida et al. (2022) evaluated 21 pepper accessions based on 20 morpho-agronomic descriptors and found that the first component explained 77.37% of the data variance. Belay et al. (2019) evaluated 19 morpho-agronomic characteristics in 64 pepper genotypes and found that the first component explained 69.25% of the variance. Manly (2008) pointed out that PCA not always accumulate 80% variance in the two or three first components, as in the case of original variables with low correlation.

In this context, the data obtained in the present study showed high correlation, totaling less than 80% variance. Two principal component analyses were conducted, revealing the factors that most affected the clustering in the two first components, highlighting 31.05% of variables in $F_1$ and 30.37% in $F_2$ plants (Figure 2 and Figure 3).

The PCA for the $F_1$ generation formed a cluster of genotypes in the first quadrant based on their similarity in leaf color, branching density, fruit apex shape, and calix pigment (Figure 2). The second quadrant was formed by genotypes exhibiting similarity in number of leaves per axil, corolla color, and fruit brightness.

The PCA for the $F_2$ generation formed a cluster of genotypes in the first quadrant based on their similarity in leaf color, fruit brightness, fruit apex shape, calix pigment, and leaf density. The second quadrant was formed by a cluster of genotypes exhibiting similarity in number of leaves per axil, corolla color, and nodal anthocyanin (Figure 3).

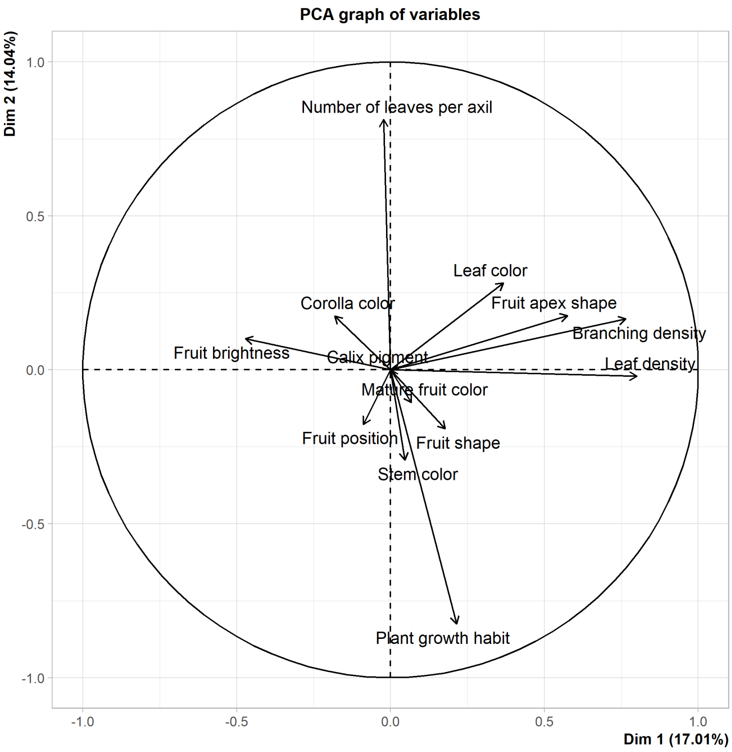


**Figure 2**. Principal component analysis (PCA) plot of 13 traits in 67 $F_1$ plants of a *Capsicum annuum* accession (P100) from the Active Germplasm Bank of *Capsicum* of the Brazilian Agricultural Research Corporation (Embrapa Temperate Climate).

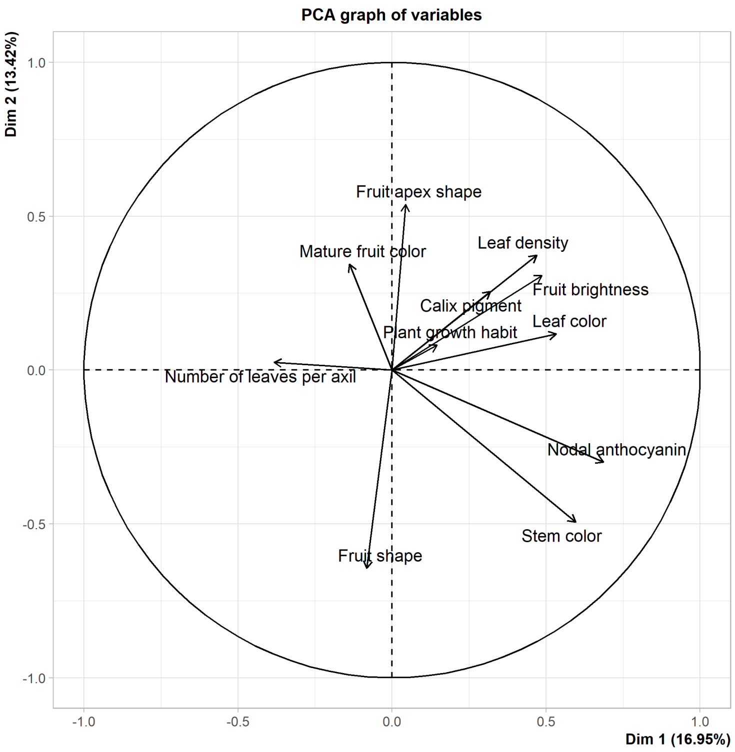


**Figure 3**. Principal component analysis (PCA) plot of 11 traits in 62 $F_2$ plants of a *Capsicum annuum* accession (P100) from the Active Germplasm Bank of *Capsicum* of the Brazilian Agricultural Research Corporation (Embrapa Temperate Climate).

Leaf color, fruit apex shape, and calix pigment were present in the first quadrant for $F_1$ and $F_2$ generations, contributing to genotype clustering. However, leaf density was included in $F_2$. According to Sudré et al. (2010), information on leaf density of plants is important in terms of crop management, as it can aid in defining the spacing for each plant, harvest, and weed control. Additionally, information on this trait is important for ornamental plants, as it is useful for defining plant size. According to Neitzke et al. (2016), the ornamental use of some pepper plants of the genus *Capsicum* is due to the high aesthetic value of some traits, such as plant architecture; number, shape, and position of fruits; and color, shape, and density of leaves.

The number of leaves per axil and corolla color were the traits responsible for clustering genotypes in the second quadrant for $F_1$ and $F_2$ generations; fruit brightness was included only for $F_1$, while nodal anthocyanin was included only for $F_2$ plants. According to Gomes et al. (2022), fruit brightness, along with immature and mature fruit color intensity are important traits, as the selection of these traits is interesting for obtaining populations with no segregation. Additionally, Ari et al. (2016) reported the presence of nodal anthocyanin, or anthocyanin in the entire plant, as an ornamental trait. These data support the potential of the accession *Capsicum annuum* P100 for use in breeding programs with ornamental purposes.

Some traits did not segregate in $F_1$ and $F_2$ generations; one of them was leaf color, which is an important trait, as the contrast between leaf and fruit color is considered an important ornamental attribute in mature or immature fruits (NEITZKE et al., 2016). Regarding the fruit apex shape in $F_1$ plants, 89.05% of them exhibited a pointed shape and 1.49% a depressed shape, whereas 100% of $F_2$ plants displayed a pointed fruit apex. A morphological characterization carried out by Silva et al. (2021) in pepper accessions (*Capsicum* spp.) grown in Maranhão, Brazil, showed different fruit apex shapes: 42.86% pointed, 19.05% truncate, 33.33% depressed, and 4.76% depressed with a tip. Pigment in the calix was another important trait highlighted in $F_1$ plants. According to Costa et al. (2020) and Vasconcelos et al. (2012), flower traits can be used as morphological markers to determine genetic diversity in germplasm banks, with the advantage of being assessable at the onset of the reproduction cycle.

One leaf per axil was observed in 100% of $F_1$ plants, and segregation for this trait was observed in $F_2$ plants, with 53.22% of plants exhibiting three leaves or more per axil, 35.48% of plants with two leaves per axil, and 11.29% of plants with one leaf per axil. The absence of spots on the corolla was observed in both $F_1$ and $F_2$ plants. This information is important for the taxonomic determination of the species, is useful for varietal description and, together with the other descriptors, confirms that the species of the genotypes is *C. annuum* (GOMES et al., 2022). The ornamental aspect of the accession P100 was found in both generations (Figure 1). This accession exhibited a variety of mature fruit colors, which demonstrates its potential as an ornamental plant, considering that fruit color is the most important factor for consumers when choosing ornamental peppers (NEITZKE et al., 2016). The segregating traits of accession P100 still need to advance more generations to obtain one or more homogeneous lines for registering it in the National Registry of Cultivars of the Brazilian Ministry of Agriculture and Livestock as an ornamental plant for use in pots or landscaping. Therefore, the next phases of the breeding program will maintain SSD and selection for at least three cycles of self-pollination of 62 plants/lines.

## CONCLUSION

The genetic variability shown in the $F_1$ and $F_2$ generations of accession P100 (*Capsicum annuum*) can be exploited for the development of ornamental pepper varieties. Segregation existed for traits of ornamental importance, such as leaf color, leaf density, number of leaves per axil, fruit apex shape, calix pigment, corolla color, fruit brightness, and nodal anthocyanin in the evaluated generations.

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