Genetic variability and interaction between variables of ornamental interest in a segregating pepper population

Variabilidade genética e interação entre variáveis de interesse ornamental em uma população segregante de pimentos

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Gabriela Cristina Alves Custódio
Master in Plant Production in the Semi-Arid Region
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: gabrielac.agro@gmail.com

Samy Pimenta
PhD in Genetics and Plant Breeding
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: samy.pimenta@unimontes.br

Nelson de Abreu Delvaux Júnior
PhD in Agrochemistry
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: nelson.junior@unimontes.br

Amanda Maria Leal Pimenta
Master in Plant Production in the Semi-Arid Region
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: amandaleal90@hotmail.com

Wellington Silva Gomes
PhD in Genetics and Breeding
Institution: Universidade do Estado de Minas Gerais
Address: Belo Horizonte - Minas Gerais, Brasil
E-mail: wellington.gomes@uemg.br

Hélida Christhine de Freitas Monteiro
PhD in Animal Science
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: helida.monteiro@unimontes.br
Fátima de Souza Gomes
Master in Plant Production in the Semi-Arid Region
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: fatimaagro27@gmail.com

Cíntia dos Santos Bento
PhD in Genetics and Plant Breeding
Institution: Universidade Estadual de Montes Claros
Address: Janaúba - Minas Gerais, Brasil
E-mail: cintia_bento@yahoo.com.br

ABSTRACT
The diversity in the Capsicum genus and the low number of ornamental pepper cultivars available have stimulate the development of breeding programs for this species. The study of segregating populations is important to obtain ornamental pepper cultivars. The objective of this work was to detect the variability in a F2 pepper population through principal component analysis (PCA) considering characters of interest of ornamental peppers and identify which variables affect the height of these ornamental plants through trail analysis. A crossing between two contrasting materials followed by self-fertilization of the F1 population was carried out and seven variables of ornamental interest were evaluated in 333 plants of the F2 generation. The evaluated variables were: mean fruit weight (MFW), mean fruit length (MFL), mean fruit diameter (MFD), mean peduncle length (MPL), mean pericarp thickness (MPT), plant height (PH), and flowering cycle (FC). The results were subjected to principal component analysis and trail analysis using PH as the base characteristic. The two first components explained 90% of the genetic variability of the population; PH and FC together reached 96.9%; and FC alone was responsible for 62.1%. The trail analysis showed that PH is more affected by the FC (24.35%) and MPL (16.6%); increases in these variables may increase PH. MFD presented a low-magnitude negative effect on the base variable (-1.99%). MFW, MFL, and MPT presented low effect on PH, 0.35%, 0.76%, and 7.06%, respectively. A genetic variability that can be explored in breeding programs was detected in the segregating population of ornamental pepper evaluated, mainly for FC and PH. Despite FC and MPL affect positively the plant height, this effect has a low magnitude.

Keywords: ornamental pepper, plant breeding, principal component analysis, flowering cycle.

RESUMO
A diversidade do gênero Capsicum e o baixo número de cultivares de pimenta ornamental disponíveis estimularam o desenvolvimento de programas de melhoramento para essa espécie. O estudo de populações segregantes é importante para a obtenção de cultivares de pimenta ornamental. O objetivo deste trabalho foi detectar a variabilidade em uma população F2 de pimentas por meio da análise de componentes principais (PCA) considerando caracteres de interesse das pimentas ornamentais e identificar quais variáveis afetam a altura dessas plantas ornamentais por meio da análise de trilha. Foi realizado um cruzamento entre dois materiais contrastantes seguido de autofecundação da população F1 e sete variáveis de interesse ornamental foram avaliadas em 333 plantas da geração F2. As variáveis avaliadas foram: peso médio do fruto (PMF), comprimento médio do fruto (CMF), diâmetro médio do fruto (DMF), comprimento médio do pedúnculo (CMP), espessura média do pericarpo (EMP), altura da planta.
(AP) e ciclo de floração (CF). Os resultados foram submetidos à análise de componentes principais e à análise de trilha usando a PH como característica básica. Os dois primeiros componentes explicaram 90% da variabilidade genética da população; o PH e o CF juntos atingiram 96,9%; e o CF sozinho foi responsável por 62,1%. A análise de trilha mostrou que o PH é mais afetado pela CF (24,35%) e pela MPL (16,6%); aumentos nessas variáveis podem aumentar o PH. O MFD apresentou um efeito negativo de baixa magnitude sobre a variável de base (-1,99%). MFW, MFL e MPT apresentaram baixo efeito sobre o pH, 0,35%, 0,76% e 7,06%, respectivamente. Uma variabilidade genética que pode ser explorada em programas de melhoramento foi detectada na população segregante de pimenta ornamental avaliada, principalmente para CF e PH. Apesar de a CF e a MPL afetarem positivamente a altura da planta, esse efeito tem baixa magnitude.

Palavras-chave: pimenta ornamental, melhoramento de plantas, análise de componentes principais, ciclo de floração.

1 INTRODUCTION

Ornamental plants are those grown for esthetic purposes and usually used for gardening or landscaping; they include flowers, shrubs, some tree species, and pot plants, as in the case of ornamental peppers (Capsicum spp.) (Ibraflor, 2018). The demand for these plants has increased in national and international markets, denoting an increasing and continuous appreciation of the consumer market and boosting breeding studies for these species for the development of new cultivars (Guimarães et al., 2020; Rêgo & Rêgo, 2016; Rêgo & Rêgo, 2018).

According to Costa, Silva, Lopes, Carvalho e Gomes (2019), the diversity of the Capsicum genus, combined with the low number of ornamental pepper available in the Brazilian market, stimulates the development of breeding programs for pepper plants. The objective of these breeding programs is the obtaining of cultivars that present desirable characteristics for ornamentation, resistance to pests and diseases, fruit quality, and high production. C. annuum is among the most studied and marketed species of the Capsicum genus and present varieties that are widely grown for food and ornamental purposes, denoting its high potential for industries and variability within the species (Silva, Rodrigues, Bento & Pimenta, 2017; Pimenta et al., 2020; Rêgo & Rêgo, 2016). According to Costa et al. (2019), any pepper plant could be used as an ornamental plant, but not all plants adapt to pot systems. Thus, the development of new ornamental pepper cultivars may follow esthetic and structural precepts to be sold in small pots.

Genetic variability is a basic condition for breeding programs that intend to develop new pepper cultivars. According to Pessoa et al. (2018a), studies on genetic divergence are important
for the selection of superior parents and obtaining of hybrids, which enable the identification of plants with desirable characters for ornamental purposes in their segregating generations through successive cycles of self-fertilization. These studies include genomic selection, finger print of varietal types, identification of genetic connection between genotypes, conservation of genetic resources, and development of non-repetitive nuclear collections (Bhatta, Morgounov, Belamkar, Poland & Baenziger, 2018; Pereira-Dias, Rêgo, Carvalho, Santos & Rêgo, 2019). Therefore, the success of a selection is dependent on the presence of high genetic variability in the species.

Knowing the correlations between ornamental characteristics facilitates the search for new genotypes. It is important when some characteristics are difficult to measure, late expressed in the phenotype, or have low heritability, as in the case of number of pepper fruits per plant (Moreira, Kuhlcamp, Barros, Oliveira & Trindade, 2018; Acevedo et al., 2020) and plant height of ornamental pepper. Plant height present polygenic heritage, mean heritability, and late measurement; thus, other variable with high correlation with it can be used to facilitate the selection. Trail analysis is a tool to assess correlations between these characteristics. It shows a breakdown of the correlations between each pair of variables in a set into direct and indirect effects in a base variable. Thus, it enables to quantify the direct effect of a variable on the other, regardless of other variables, in a cause-and-effect context (Souza et al., 2020).

Principal component analysis (PCA) is another tool to detect genetic variability. This important statistical method enables to easily identify important polygenic characters for breeding programs (Rahevar, Patel, Axatjoshi, Sushilkumar, & Gediya, 2021). According to Singh, Jain & Tiwari (2020), PCA decreases the dimension of a large set of variables to a small set that still contains most information present in the large set.

In this context, the objective of this work was to detect the variability in a F₂ pepper population through PCA considering characters of interest of ornamental peppers and identify which variables affect the height of these ornamental plants through trail analysis.

### 2 MATERIAL AND METHODS

The present experiment was conducted in a greenhouse covered with a 50% shade screen, in the experimental area of the State University of Montes Claros (Unimontes), in Janaúba, MG, Brazil (15°48'09"S, 43°18'32"W, and altitude of 533 m). The study was carried out from June 2019 to January 2021, in three different phases: growth of parents and crossing between them for
the obtaining of F₁ (Step I), growth and self-fertilization of F₁ for the obtaining of F₂ (Step II), and growth of the F₂ population (Step III).

In Step I, two parents with contrasting morphological characteristics were selected; they were from the Capsicum Germplasm Bank of the Unimontes (Pimenta et al., 2020). The seeds of the parents, and the F₁ and F₂ populations were sown in plastic trays filled with a commercial substrate (Biolpant Plus®), using one seed per cell. The trays remained in the greenhouse under daily irrigation. When the seedlings reach four to six definitive leaves, they were transplanted to identified pots filled with a mixture of clayey soil, sand, and bovine manure at the proportion of 1:1:1. The parents and F₁ were grown in 1-liter pots, and the F₂ generation was grown in 3-liter pots containing two plants per pot. Cultural practices over the experiment were according to recommendations for the conventional crops (Filgueira, 2012), with adaptations for the protected environment and pot plants.

Pollen of the parent Uni07 was collected for the crossing between parents, using manual pollination with a paintbrush in the recipient and emasculated flowers of parent Uni01. The pollinated flowers were protected with paper bags for approximately five days. The fruits formed were identified by colored ribbons and were collected when they reached maturation; their seeds were collected to obtain the F₁ generation.

In step II, the floral buttons of F₁ plants were protected using paper bags in the pre-anthesis to ensure the obtaining of self-fertilized fruits. The fruits obtained from the F₁ self-fertilization were harvested, identified, and taken to the Laboratory of Biotechnology of the Unimontes for processing, natural drying, and storage of seeds of F₂ generation. These seeds were stored in paper bags under refrigeration.

In step III, 400 seeds from the F₂ generation were sown, obtained the final 333 plants viable for growth and evaluations. Floral buttons of all plants of this generation were also protected to ensure the obtaining of self-fertilized fruits, for the F₃ generation. One hundred seeds of each genotype (F₂) were stored to compose the following generation.

The variability was detected considering the plants obtained in step III. The quantitative descriptors of fruits evaluated were: mean fruit weight (MFW), measured using a digital balance; mean fruit length (MFL), mean fruit diameter (MFD), mean peduncle length (MPL), and mean pericarp thickness (MPT), measured using a digital caliper; plant height (PH), measured using a tape ruler; and flowering cycle (FC), obtained by the number of days from sowing to the complete
opening of the first flower. All fruit variables were evaluated considering the mean of five fruits per genotype. The genotypes were distributed in a completely randomized design, with five fruits representing the replications of each treatment (genotype).

The data were subjected to tests of normality of data (Lilliefors) and homogeneity of variances (Bartlett), at $p<0.05$. Principal component analysis (PCA) was used to evaluate the genetic diversity between genotypes, considering the number of principal components enough to explain at least 90% of the data variability. A multivariate study was carried out through trail analysis in a single chain diagram, considering plant height as the dependent variable. A multicollinearity test was carried out between the variables, considering the assumptions of the diagnosis of number of condition (NC) that considers the multicollinearity as (NC < 100), moderate to severe (100 ≤ NC ≤ 1.000) and severe (NC > 1.000) (Montgomery & Peck, 1982). In addition, variance inflation factors (VIF) were considered; high VIF (VIF > 10) indicate improper effect of multicollinearity on the results (Kutner, Nachtsheim, Neter & Li, 2005). The statistical analyses were carried out with the aid of the Genes (Cruz, 2016) and Past® (Hammer, Harper & Ryan, 2001) programs.

3 RESULTS AND DISCUSSION

The two first components of the principal component analysis (PCA) explained more than 90% of the genetic variability in the evaluated variables (Table 1). Plant height and flowering cycle reached 96.9%, close to the total variability observed. PCA extracts the main orthogonal contributions that explains the most of the variance of the data matrix; the first principal component (PC1) explains the highest variance of data, followed by the second (PC2), third (PC3) and so on (Maia, Silva & Libânio, 2019). The eigenvalue of a principal component represents the quantity of the variation of a characteristic that is explained by that principal component, which is useful for breeding programs (Rahvar et al., 2021).

Table 1. Values of variance individual and accumulated of the variables evaluated, flowering cycle (FC), plant height (PH), mean fruit length (MFL), mean peduncle length (MPL), mean fruit weight (MFW), mean fruit diameter (MFD) e mean pericarp thickness (MPT), via Principal Component Analysis (PCA). Janaúba-MG, Unimontes, 2021.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Autovalor</th>
<th>Variance (%)</th>
<th>Total variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>42.4305</td>
<td>62.15</td>
<td>62.15</td>
</tr>
<tr>
<td>PH</td>
<td>23.7564</td>
<td>34.80</td>
<td>96.95</td>
</tr>
<tr>
<td>MFL</td>
<td>1.6246</td>
<td>2.38</td>
<td>99.33</td>
</tr>
<tr>
<td>MPL</td>
<td>0.2464</td>
<td>0.36</td>
<td>99.69</td>
</tr>
</tbody>
</table>
The evaluation of the dynamics of the data in the PCA is carried out through the development of graphics. In the graphs, a good result in the interpretation of the genetic variability requires that the two first principal components express more than 80% of the total variance in the data set (Cruz, Carneiro & Regazzi, 2014). The maneuver PC1 vs. PC2 and/or PC3 hinders the graphical analysis, since it is a limited representation, bi or tridimensional, in the case of two in two or three in three principal component analysis, respectively, thus the possibility of simultaneous analysis of at most three principal components should be considered.

Singh et al. (2020), evaluated 10 quantitative characters in C. annuum genotypes using PCA and found that the first five components were responsible for 88.85% of the variability of the data; plant height had the highest variability (36.93%), followed by number of primary branches (22.87%), days until the first flowering (15.62%), days until 50% flowering (7.46%), and days until the first harvest (5.97%). Pessoa, Rêgo, Carvalho, Santos & Rêgo (2018b) evaluated 28 quantitative variables in 16 genotypes of ornamental pepper (C. annuum) and found plant height among eight variables that most contribute to the genetic divergence; this characteristic was efficient to explain the dissimilarity between the genotypes evaluated. Costa (2018) evaluated nine quantitative characters in 12 pepper cultivars with ornamental potential (Capsicum spp.) and found plant height among four variables that presented the highest values in the PC1. These results denote that plant height should be considered, mainly due to its genetic variability. Therefore, this characteristic should be prioritized in studies of divergence in pepper plants.

The flowering cycle alone was responsible for 62.1% (Table 1). The closer the intersection point to the x and y axes, the lower the values obtained as principal components for the variables (Figure 1). According to Costa, Silva, Filha, Santos & Silva (2020), characters connected to plant reproduction cycle are interesting for production and marketing sectors, because precocity is a characteristic of cultivars that result in decreases in costs, since the plants will more rapidly reach the commercial standards. Thus, the genotypes that presented intersections close to the axes are those with lower size and higher precocity that can be selected (Figure 1).
Dispersion graphs can be used strategically for the formation and selection of groups or individual selection of genotypes as candidates for development in breeding programs (Oliveira et al., 2019). The graphical dispersion of genotypes enables the identification of similar and divergent genotypes for the principal components found, assisting breeders in the decisions for selection according to standards established by the market.

The graphical dispersion showed 14 genotypes (4.2%) outside the ellipse \( (p<0.05) \) (Figure 1). These genotypes presented higher values of principal components. Plant height is important for the ornamental plant sector, since the plant architecture defines whether the Capsicum species can be used for ornamental purposes, and can be adapted to a pot plant system. According to the ornamental pepper market in Brazil, pot plants should have 12 to 33 cm (Veiling Holambra, 2019).

Peppers with heights of approximately 30 cm or higher, as in the case of those 14 genotypes, are less targeted by the market, considering the ideotype of ornamental peppers and interest of consumers for plants with low heights (Cunha et al., 2020). This is because ornamental peppers are usually marketed in small pots, which generates a disharmonious architecture between the pot and plants of mean and high sizes.

In Brazil, the standards of the Veiling Holambra Cooperative are used for the marketing of ornamental peppers. This cooperative establishes that the ideal height of these plant is determined according to the pot size used, since the plant height can be 6 to 12 cm in number 06, 08, and 09 pots, 14 to 22 cm in number 11 pots, and 14 to 32 cm in 13, 14, and 15 pots (Veiling Holambra, 2018). This standard is important for ornamental peppers because the ornamental market is not an essential market, such as the food, and ornamental plant consumers buy them only for esthetic purposes, mainly new consumers.
Therefore, considering that the esthetic standard for ornamental purposes should be strictly followed to raise interest of consumers and ensure the marketing success, those 14 genotypes with higher values can be discarded. Information on the importance of variables for genetic divergence enables to discard characteristics that present low contribution to discriminate the evaluated material, which reduces unnecessary factors, such as time, labor, and costs for the experimental unit (Alves, Garcia, Cruz & Figueira, 2003). The individual study is required because the importance of characteristics can vary within a same species (Ferreira, 2016).

Regarding the genotypes inside the ellipse, 95.8% of them presented similarity in the principal components. Considering that these components are dependent on ornamental and production standards required by the market, the results were positive and showed that the population has, in general, good ornamental potential and a genetic variability that can be explored. However, non-genetic factors can also make individuals similar, since shared environments can induce similar characteristics between individuals and develop non-genetic standards of phenotypic variation; and when connected individuals share the same environment, neglecting shared environmental effects can inflate the estimated additive genetic variance ($V_a$) (Thomson, Winney, Oceane & Pujol, 2018). Thus, the sharing of genes is not the single source
of similarity between individuals, shared breeding environments can increase the phenotypic similarity between relatives (Kruuk & Hadfield, 2007).

Regarding the fruit characters, the sum of values of the four components (mean fruit length, mean peduncle length, mean fruit weight, and mean fruit diameter) resulted in only 3.05% of importance for the phenotypic variance (Table 1). The low variability found for these characteristics can be interesting because it shows that the population present a fruit standard, when this standard is adequate for ornamental purposes, making all genotypes good candidates for a new ornamental pepper cultivar. Smaller fruits are, probably, more desirable for ornamental pepper plants, since they show a more harmonious architecture for the plant, creating a better esthetic feature for marketing. According to Luz, Santos & Ambrozoio (2019), fruit length, diameter, and weight are affected by the canopy area and width and the higher the canopy measurements, the lower the fruit measurements.

The last component presented no effect on the variance (Table 1), showing that all genotypes are similar for mean pericarp thickness. Naegele, Mitchell & Hausbeck (2016) evaluated 116 C. annuum genotypes and found susceptibility to fruit rot disease (Phytophthora capsici), with positive correlation to increases in pericarp thickness and fruit length, i.e., peppers with thicker and longer pericarp tend to be more susceptible to P. capsici. This denotes the challenge of breeders in developing resistant materials to fruit rot, since fruits with thicker pericarp are desirable for food and ornamental purposes.

The plant characterization carried out in initial phases of breeding programs enables the breeder to select and discard genotypes that do not present desirable potential, decreasing the number of uninteresting genotypes to be analyzed in future generations, and promoting economy of cost and time. Therefore, the analysis of the obtained results showing low or nonexistent genetic variability for mean fruit length and mean pericarp thickness in a F2 population allows the breeder to understand the tendencies of the phytosanitary dynamics of the population under study and use methods to resolve or improve these dynamics over the breeding program.

The correlations between variables considering direct effects on plant height were higher for flowering cycle (24.35%) and mean peduncle length (16.6%) (Figure 1); the longer the flowering cycle, the higher the plant height. Floral evocation is the time that the meristem is reorganized for flower instead of leaf production. According to Vaz, Santos & Zaidan (2008), during the floral initiation, mitotic activity increases the limits of meristematic region of apical
and/or lateral gems and, then, the mitotic activity and growth practically stops. This decrease in growth when the flowering start can explain the high direct effect of the flowering cycle on plant height.

The mean fruit diameter presented a direct negative effect on the base variable (-1.99%). The mean fruit weight, mean fruit length, and mean pericarp thickness presented low effect on plant height, 0.35%, 0.76%, and 7.06%, respectively. Therefore, these fruit-related characteristics have no or little effect on the height that *C. annuum* L. plants can reach. Luz et al. (2019) found negative correlations between plant height and fruit length, peduncle length, and fruit weight for *Capsicum* spp. Roy, Charttejee, Hossain, Basfore & Karak (2018) evaluated *C. annuum* L. var. *Grossum* plants and found low indirect effects of days until the first flowering (3.5%) and fruit width (1.0%), and fruit weight (-3.9%), fruit length (-9.3%), and pericarp thickness (-0.7%) on plant height. These results corroborate those found in the present study, denoting that these variables do not have significant effect on plant height. Correlations between agronomic characteristics are important for breeding programs, since they contribute to a better selection for production (Soares, Silva, Candido & Vale, 2017).

Low coefficient of determination (9.5%) and high residual effect (95%) were found in the trail analysis. Acevedo et al. (2020) estimated direct and indirect effects of six agronomic characteristics, including number of days for maturation, fruit length and width, and number of fruits per pepper plant (*C. annuum*) and found high residual effect (79%). Salla et al. (2015) found low coefficient of determination (12%) and high residual effect (93%) for the evaluated characteristics of *Plinia cauliflora* plants, with similar values to those found in the present study.

Despite of these low values, the explanatory variables did not need to be totally discarded due to low direct effect on the base variable; thus, an alternative is the use of selection indexes that can provide information on favorable gains in many characteristics (Cruz et al., 2014; Acevedo et al., 2020). Mulamba and Mock (1978) is among these selection indexes; it ranks the genotypes for each characteristic by attributing higher absolutes values to those of better performance and, then, the values attributed to each characteristic are summed, obtaining the sum of the ranks, which determines the genotype classification (Cruz & Regazzi, 2001).
4 CONCLUSIONS

Genetic variability was detected in the evaluated segregating pepper population. The flowering cycle and plant height were the variables that most contributed to the genetic variation. This variability can be explored in breeding programs.

The flowering cycle and mean pericarp length were the variables that most directly affected the plant height, denoting that the longer the cycle, the higher the height of ornamental pepper plant. However, the magnitude of the value of effect on plant height was low.
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