

Combining quantitative and qualitative descriptors to predict genetic diversity in *Capsicum*

Mariane Copati^{1*}, Lívia Torres², Françoise Dariva¹, Herika Pessoa¹, Gabriella Almeida¹, Ana Carolina Nascimento³, Moysés Nascimento³, Carlos Nick¹

¹Departamento de Agronomia, Universidade Federal de Viçosa, Viçosa, MG, Brasil

²Departamento de Biologia, Universidade Federal de Viçosa, Viçosa, MG, Brasil

³Departamento de Estatística, Universidade Federal de Viçosa, Viçosa, MG, Brasil

*Corresponding author: marianegonferreira@gmail.com

Abstract

The objective of the present study was to assess the accuracy of analyzing quantitative and qualitative descriptors separately or combined to differentiate *Capsicum* accessions. We assessed 47 *Capsicum* accessions from the UFV Vegetable Germplasm Bank (BGH-UFV) with botanical classification previously known. The experiment was arranged in a randomized block design with four replicates. Variables consisted of 16 morphological descriptors proposed by the IPGRI. Analysis of quantitative descriptors alone was performed by calculating the Mahalanobis distance matrix (D1), while qualitative descriptors alone were analyzed using the simple coincidence index (D2). The joint analysis consisted of the sum of the distance matrices D1 and D2, and the joint calculation of both descriptors using Gower's algorithm. Association between the distance matrices was assessed by Mantel's correlation test, and an accession clustering was performed using the UPGMA method. There was genetic variability amongst *Capsicum* accessions for both quantitative and qualitative traits. Accession clustering was more accurate when performed using the distances obtained by the simple coincidence index (qualitative descriptors alone) and the Gower's algorithm (qualitative and quantitative descriptors combined).

Keywords: pepper; germplasm characterization; germplasm Bank; multivariate analysis; genetic dissimilarity.

Introduction

Pepper (*Capsicum* spp.) is a globally important vegetable crop used as an ornamental plant and in the culinary, pharmaceutical, and cosmetics industries (Gu et al., 2019). According to the latest data from FAO (Food and Agriculture Organization), in 2019, around 38 million and 4.3 million tons of green and dry pepper were produced worldwide respectively, in a cultivated area of about 3.7 million hectares (FAO, 2019). The *Capsicum* genus (Solanaceae family), originated from South and Central America (Nicolaï et al., 2013), is formed by a diverse group of peppers, including sweet peppers and hot peppers. Peppers from this genus contain high levels of vitamin C, besides being a source of vitamins A, B, E, and K (Olatunji and Afolayan, 2020). Main chemical compounds of peppers are capsaicinoids, responsible for fruit pungency, and the carotenoids, capsanthin, and capsorubin, responsible for fruit red color (Albrecht et al., 2012). Brazil is an important diversity center for the *Capsicum* genus, housing domesticated, semi-domesticated, and wild species (Brilhante et al., 2021). The five domesticated species of *Capsicum* already known are *C. annuum*, *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., *C. chinense*, and *C. pubescens* Ruiz et Pav (Gu et al., 2019). A large part of the *Capsicum*'s great genetic variability is preserved in germplasm banks, which are recognized as important sources of genetic

materials for pepper improvement. According to the ONU (2015), accessing existing genetic variability and creating measures to preserve existing genetic resources is a key strategy to develop more sustainable production models, guaranteeing food security and preservation of the genetic resources available. Accession characterization to identify genetic diversity is crucial to explore plant resources more efficiently. Diversity analyses involve both the use of a single methodology or the combination of several methodologies (Moura et al., 2010). Data are numerical measurements, known as quantitative descriptors, and qualitative descriptors, which can be binary or multi-categorical. These two types of descriptors can be analyzed separately as well as combined (Mohammadi and Prasanna, 2003). Several methodologies measuring genetic distance amongst genotypes have already been suggested for diversity studies within the *Capsicum* genus (Sudré et al., 2005; Büttow et al., 2010) as reported by Monteiro et al. (2010) and Neitzke et al. (2010). These authors recommended the use of a dissimilarity matrix based on the number of agreements and disagreements observed between accessions for qualitative variables and the generalized Mahalanobis distance matrix for quantitative variables, respectively. Some works have already used joint analysis of quantitative and qualitative descriptors to discriminate genotypes (Moura et al., 2010;

Lima et al., 2017). For Gonçalves et al. (2008), Sudré et al. (2010), and Quinta et al. (2012), information of quantitative and qualitative variables combined provides more accurate results regarding the identification of contrasting genotypes. More studies involving the accuracy of joint analysis methodologies to predict genetic diversity within accessions however are still needed. Therefore, this study aimed to assess the accuracy of analyzing quantitative and qualitative descriptors alone or combined to cluster *Capsicum* accessions.

Results and discussion

Association between distance matrices

The associations between D1 and D2 and the other distance matrices were low and not significant ($p > 0.05$) (Table 1). According to Mantel's significance test, the best way to represent the quantitative and qualitative data combined is through the matrices D3 and D4 as they showed high and significant associations with the genetic matrices calculated using the quantitative variables (D1) and qualitative variables (D2) alone (0.78 and 0.74, 0.39 and 0.90, respectively).

The association between D1 and D2 was one of the lowest found, which can be justified by the small number of quantitative compared to qualitative variables and the environmental influence that differs between these two types of variables, with the quantitative being more affected. Although the Mantel's coefficient estimates between the matrix D5 (obtained by the sum of the matrices D1 and D2) and D1 was 1.00 and significant, indicating a perfect association between the two matrices, D5 may not be the best option to represent quantitative and qualitative data combined as such result may happen due to a scale effect (different variables were calculated using different scales) which is not desirable in diversity analyses. Cruz et al. (2011) recommend standardization of means before the analysis (i.e., by dividing the means by the standard deviation), as we did in the joint matrix D3 since the variation amplitude in each matrix could vary significantly and hence affect the results.

Despite not being the most appropriate approach to represent the two groups of variables combined, we included D5 in this study for comparison purposes as this type of joint analysis is still used and recommended when all data are on the same scale. The association of D5 with D2 was low and not significant, indicating no agreement between these two dissimilarity matrices.

Genotype clustering

Using the UPGMA method, we formed 4, 8, 5, 7, and 4 clusters for the distance matrices D1, D2, D3, D4, and D5, respectively (Figure 1). Accession clustering by quantitative (D1) and qualitative data (D2) separately disagreed with one another. The highest accuracy (greater number of accessions correctly classified) was observed for the matrix with qualitative data alone, demonstrating higher contribution from qualitative than quantitative variables to accession clustering. This result, however, does not eliminate the need for assessing quantitative traits of crop species as they are essential to breeding programs (Moura et al., 2010). The highest disagreements in accession clustering between the calculated distance matrices and their botanical classification were found for the matrix using quantitative

variables alone (D1), the joint matrix using the sum of distance matrices D1 and D2 (D5), and the joint matrix using the sum of distance standardized means (D3). Therefore, these matrices are not recommended for genetic diversity studies.

Accession clustering using the distance matrices calculated through the simple coincidence index (D1) and the Gower's algorithm (D4) best represented the *a priori* species clustering. Gower's algorithm allows accession clustering using quantitative and qualitative data combined. This joint analysis provides a single dendrogram based on both types of descriptors, facilitating the analysis and use of data, increasing clustering precision (Machado et al., 2015). In addition, it is important to point out that both quantitative and qualitative variables have their advantages and disadvantages in genetic diversity studies which is why they both should be included (Mohammadi and Prasanna, 2003; Lotti et al., 2008; Machado et al., 2015).

The distance obtained from the simple coincidence index (D2) erroneously grouped one *C. baccatum* var. *pendulum*, five *C. annuum* var. *annuum*, and four *C. chinense* accessions. As for the distance obtained from the Gower's algorithm (D4), four *C. baccatum* var. *pendulum*, five *C. annuum* var. *annuum*, and one *C. chinense* accessions were erroneously grouped. All *C. frutescens* accessions were righteously grouped using these two methodologies, and the *C. baccatum* var. *baccatum* was grouped with the other *C. baccatum* var. *pendulum* accessions probably due to the remarkable similarity between them as they are part of the same species.

Clustering consistency

The cophenetic correlation was used to inform clustering consistency. Cophenetic correlations for accession clustering using the distance matrices D1, D2, D3, D4 and D5 were 0.83, 0.88, 0.78, 0.86 and 0.88, respectively. The correlations observed fitted the critical bounds established by Rohlf and Fisher (1968), indicating fine consistency and few disagreements in accession clustering for all methodologies.

Materials and methods

Field trial

To verify the accuracy of analyzing quantitative and qualitative descriptors alone or combined in genotype clustering, we assessed 47 *Capsicum* accessions preserved in the UFV Vegetable Germplasm Bank (BGH-UFV) with botanical classification previously known. The trial was carried out from April 2014 to February 2015 in the Research and Extension Farm Unit *Horta Velha* from the Department of Agriculture at Universidade Federal de Viçosa (20°45'14"; 42°52'53"; 648.74 m of altitude). According to Köppen's classification, the regional climate is Cwb, mesothermic with rainy summers and dry winters. Seventeen accessions belonged to *C. annuum* var. *annuum*, twenty to *C. baccatum* var. *pendulum*, one to *C. baccatum* var. *baccatum*, seven to *C. chinense*, and two to the *C. frutescens* species (Table 2). Pepper seedlings were grown in polystyrene trays of 128 cells. Field transplanting occurred when seedlings reached the 5-6 true leaf stage. Between-row and in-row spacings were 1.0 m and 0.6 m, respectively. Plants were arranged according to a complete randomized block design with four replicates.

Table 1. Significance of the Mantel's test.

	D2	D3	D4	D5
D1	0.16 ^{ns}	0.78*	0.39*	1.00*
D2		0.74*	0.90*	0.16 ^{ns}
D3			0.84*	0.78*
D4				0.40*

D1 – distance matrix based on quantitative variables alone; D2 – distance matrix based on qualitative variables alone; D3 – joint distance matrix using the sum of distance standardized means of each variable group (quantitative and qualitative); D4 – joint distance matrix using the Gower's algorithm; D5 – joint distance matrix using the sum of the matrices D1 and D2. * significant at 0.05; ^{ns} not significant.

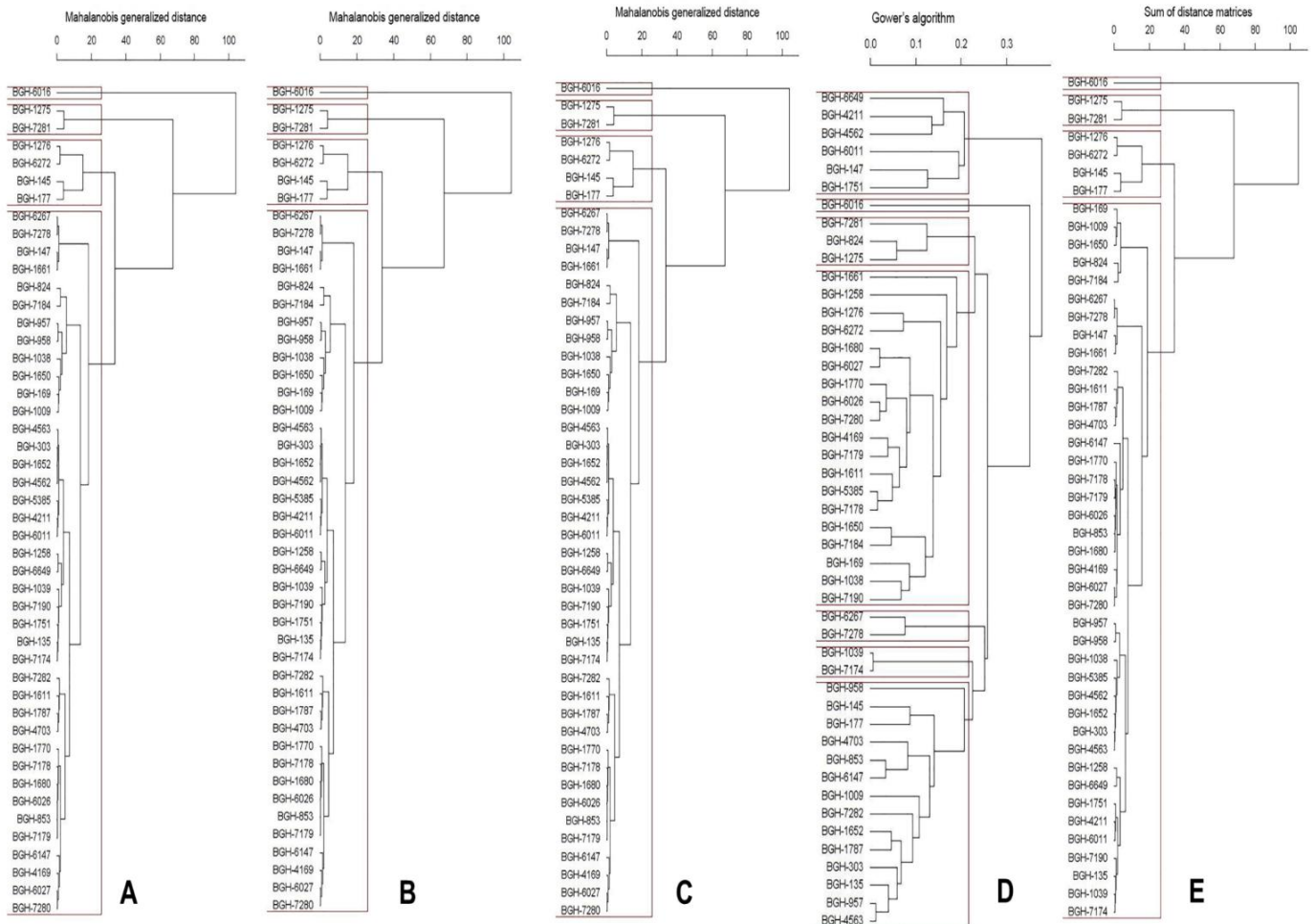


Fig 1. UPGMA clustering using different dissimilarity measures to group 47 *Capsicum* accessions based on quantitative and qualitative descriptors. A) Distance by the simple coincidence index (qualitative variables alone); B) Mahalanobis generalized distance (quantitative distance); C) Joint analysis – distance obtained from the sum of distance standardized means; D) Joint analysis – distance obtained from the Gower's algorithm; E) Joint analysis – distance obtained from the sum of distance matrices of quantitative and qualitative data alone.

The experimental unit consisted of plots with five pepper plants, at which only the central three were phenotyped.

Qualitative and quantitative descriptors

Sixteen descriptors proposed by the International Plant Genetic Resources Institute - IPGRI (1995) for the *Capsicum* genus, were used for phenotype assessment (Table 3).

Statistical analyses

To study the quantitative and qualitative variables separately, we subjected the quantitative data to analyses of variance and, after that, we calculated the generalized

Mahalanobis distance matrix (D1), whereas, for the qualitative data, we calculated the simple coincidence index (D2).

For the joint analysis (qualitative and quantitative variables combined), we calculated three genetic distance matrices: i) for the first matrix, we considered the sum of distance standardized means of each variable group (quantitative and qualitative) to reduce amplitude variation between them (Cruz et al., 2011) (D3); ii) for the second matrix we used the Gower's algorithm (1971) that allowed us to estimate genetic distances between genotypes using both continuum and discrete data.

Table 2. Identification and origin of *Capsicum* accessions preserved in the Vegetable Germplasm Bank from the Universidade Federal de Viçosa (BGH-UFV).

Accessions	Scientific name	Origin
BGH-135	<i>C. annuum</i> var. <i>annuum</i>	Aracaju – SE, Brazil
BGH-145	<i>C. annuum</i> var. <i>annuum</i>	Aracaju – SE, Brazil
BGH-147	<i>C. chinense</i>	Aracaju – SE, Brazil
BGH-169	<i>C. baccatum</i> var. <i>pendulum</i>	Maceió – AL, Brazil
BGH-177	<i>C. annuum</i> var. <i>annuum</i>	Vitória de Santo Antão – PE, Brazil
BGH-303	<i>C. annuum</i> var. <i>annuum</i>	Vitória de Santo Antão – PE, Brazil
BGH-824	<i>C. baccatum</i> var. <i>pendulum</i>	Juiz de Fora – MG, Brazil
BGH-853	<i>C. annuum</i> var. <i>annuum</i>	São José do Rio Pardo – SP, Brazil
BGH-957	<i>C. chinense</i>	Campinas – SP, Brazil
BGH-958	<i>C. annuum</i> var. <i>annuum</i>	Campinas – SP, Brazil
BGH-1009	<i>C. annuum</i> var. <i>annuum</i>	Timbó – SC, Brazil
BGH-1038	<i>C. annuum</i> var. <i>annuum</i>	Petrópolis – RJ, Brazil
BGH-1039	<i>C. annuum</i> var. <i>annuum</i>	Guanabara – RJ, Brazil
BGH-1258	<i>C. baccatum</i> var. <i>pendulum</i>	Marretes – PR, Brazil
BGH-1275	<i>C. baccatum</i> var. <i>pendulum</i>	Raul Soares – MG, Brazil
BGH-1276	<i>C. baccatum</i> var. <i>pendulum</i>	Raul Soares – MG, Brazil
BGH-1611	<i>C. baccatum</i> var. <i>pendulum</i>	Pelotas, colônia – RS, Brazil
BGH-1650	<i>C. baccatum</i> var. <i>pendulum</i>	Viçosa – MG, Brazil
BGH-1652	<i>C. annuum</i> var. <i>annuum</i>	Currais Novos – RN, Brazil
BGH-1661	<i>C. baccatum</i> var. <i>baccatum</i>	General Sampaio – CE, Brazil
BGH-1680	<i>C. baccatum</i> var. <i>pendulum</i>	São Domingos do Prata – MG, Brazil
BGH-1751	<i>C. chinense</i>	Ipeacs, km 47 – RJ, Brazil
BGH-1770	<i>C. baccatum</i> var. <i>pendulum</i>	Cruz Alta – RS, Brazil
BGH-1787	<i>C. chinense</i>	Maceió – AL, Brazil
BGH-4169	<i>C. baccatum</i> var. <i>pendulum</i>	Curitiba – PR, Brazil
BGH-4211	<i>C. chinense</i>	Marabá – PA, Brazil
BGH-4562	<i>C. chinense</i>	Igarapé – MG, Brazil
BGH-4563	<i>C. annuum</i> var. <i>annuum</i>	Igarapé – MG, Brazil
BGH-4703	<i>C. annuum</i> var. <i>annuum</i>	Igarapé – MG, Brazil
BGH-5385	<i>C. annuum</i> var. <i>annuum</i>	Dourados – MT, Brazil
BGH-6011	<i>C. chinense</i>	Belém – PA, Brazil
BGH-6016	<i>C. annuum</i> var. <i>annuum</i>	Belém – PA, Brazil
BGH-6026	<i>C. baccatum</i> var. <i>pendulum</i>	São Paulo, Brazil
BGH-6027	<i>C. baccatum</i> var. <i>pendulum</i>	São Paulo, Brazil
BGH-6147	<i>C. annuum</i> var. <i>annuum</i>	Viçosa – MG, Brazil
BGH-6267	<i>C. frutescens</i>	Univercity Purdue, USA
BGH-6272	<i>C. baccatum</i> var. <i>pendulum</i>	Embrapa, Cenargem, Brasília - DF, Brazil
BGH-6649	<i>C. baccatum</i> var. <i>pendulum</i>	Correntina – BA, Brazil
BGH-7174	<i>C. annuum</i> var. <i>annuum</i>	UENF – RJ, Brazil
BGH-7178	<i>C. baccatum</i> var. <i>pendulum</i>	UENF – RJ, Brazil
BGH-7179	<i>C. baccatum</i> var. <i>pendulum</i>	UENF – RJ, Brazil
BGH-7184	<i>C. baccatum</i> var. <i>pendulum</i>	UENF – RJ, Brazil
BGH-7190	<i>C. baccatum</i> var. <i>pendulum</i>	UENF – RJ, Brazil
BGH-7278	<i>C. frutescens</i>	Natal – RN, Brazil
BGH-7280	<i>C. baccatum</i> var. <i>pendulum</i>	Natal – RN, Brazil
BGH-7281	<i>C. baccatum</i> var. <i>pendulum</i>	Brasília – DF, Brazil
BGH-7282	<i>C. annuum</i> var. <i>annuum</i>	Brasília – DF, Brazil

Table 3. Morphological descriptors for *Capsicum* sp. proposed by the International Plant Genetic Resources Institute - IPGRI (1995).

Plant descriptors	Scoring
Stem color (SC) - recorded on young plants before transplanting	1= green; 2= green with purple stripes; 3= purple; 4= other
Leaf shape (LS)	1= deltoid; 2= ovate; 3= lanceolate
Number of flowers per axil (NFA)	1= one; 2= two; 3= three or more; 4= many flowers in bunches but each in individual axil (fasciculate growth); 5= other (cultivars with two flowers in the first axil and with one only in the other)
Flower position (FP)	1= pendant; 2= intermediate; 3= erect
Corolla color (CC)	1= white; 2= light yellow; 3= yellow; 4= yellow-green; 5= purple with white base; 6= white with purple base; 7= white with purple margin; 8= purple; 9= other
Corolla spot color (CSC)	1= white; 2= yellow; 3= green-yellow; 4= green; 5= purple; 6= other
Anther color (AC)	1= white; 2= yellow; 3= pale blue; 4= blue; 5= purple; 6= other
Calyx annular constriction (CAC) - at junction of calyx and pedicel, and	1= absent; 2= present

observed at mature stage	
Fruit color at intermediate stage (FCIS)	1= white; 2= yellow; 3= green; 4= orange; 5= purple; 6= deep purple; 7= other
Fruit color at mature stage (FCMS)	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= purple; 11= brown; 12= black; 13= other
Fruit shape (FS)	1= elongate; 2= almost round; 3= triangular; 4= campanulate; 5= blocky; 6= other
Fruit shape at pedicel attachment (FSPA)	1= acute; 2= obtuse; 3= truncate; 4= cordate; 5= lobate
Fruit shape at blossom end (FSBE)	1= pointed; 2= blunt; 3= sunken; 4= sunken and pointed
Fruit length (FL): average fruit length of 10 ripe fruits	centimeters
Fruit width (FWi): average fruit width of 10 ripe fruits	centimeters
Fruit weight (FWe): average fruit weight of 10 ripe fruits	grams

This procedure is less complex and has provided reliable results although little explored in genetic diversity studies (Quintal et al., 2012) (D4); and, iii) the third matrix was the sum of the two genetic distance matrices calculated for the quantitative and qualitative variables separately ($D1+D2=D5$), a procedure that is still used in genetic diversity studies (Lima et al., 2017).

The association between the distance matrices was investigated using Mantel's correlation test with 5,000 permutations. Next, we grouped the accessions using the UPGMA method (Unweighted Pair-Group Method Using an Arithmetic Average) (Ferreira, 2018). To determine the number of clusters, we used Mojena's test (1977) with a k value equal to 1.25 as suggested by Milligan and Cooper (1985). Clustering consistency was verified by the cophenetic correlation coefficients estimated. All statistical analyses were performed using the R (<http://www.r-project.org>) and Genes software (Cruz, 2016).

Conclusions

High genetic diversity was observed between the *Capsicum* accessions preserved at BGH-UFV. Although all tested clusterings were consistent by the cophenetic index, the accession clustering using Gower's algorithm and the simple coincidence index best grouped the *Capsicum* accessions according to their botanical classification.

Acknowledgments

This study was financed by "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 001"; "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)" and "Universidade Federal de Viçosa".

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