

## Genetic diversity among pepper and chili genotypes by Kohonen's Self-Organizing Maps

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**ABSTRACT.** The study of genetic diversity of a population is one of the pillars to make selection in a breeding program successful. There are several techniques capable of estimating genetic divergence. Among them, those based on multivariate statistics deserve attention. Recently, methodologies based on Artificial Neural Networks (ANN) have been used to study the genetic diversity of a population. One of the strategies within ANN is Kohonen's self-organizing maps (SOMs), which allows the organization of genetic diversity. We estimated and organized the genetic divergence of pepper and chili genotypes (*Capsicum annum*) for selection of diallel crosses. The experiment was conducted in a greenhouse in a completely randomized design with four replications. Nine commercially important genotypes of *C. annum* were evaluated, based on seven quantitative characters of the fruit. Univariate analysis was performed by analysis of variance and cluster mean. Multivariate [unweighted pair group method with arithmetic means

(UPGMA) and Tocher)] and machine-learning techniques [SOMs] were employed. The genotypes showed high genetic variability for all traits. The traits total mass of raw fruit and fresh fruit length contributed the most to genetic diversity. UPGMA and Tocher classified the genotypes into two and four clusters, respectively. Through SOMs, it was observed that the neighborhood pattern between chili and pepper was obtained in only seven of the 12 neurons previously established. Overall, the use of SOM allowed the organization of genetic diversity among the genotypes. Specifically, SOM did not recommend crossing genotypes from the Cascadura Ikeda chili x Giant Ruby chili, Spicy for Pot pepper x Yellow Jamaica pepper, and Volcano pepper x Peter Pepper for variability exploration.

**Key words:** UPGMA; Tocher; Self-organizing maps of Kohonen

## INTRODUCTION

Pepper and chili are important in the fresh vegetable market in Brazil. Worldwide, peppers are important in the condiment, seasoning and canning segments (Guimarães et al., 2020). The *Capsicum* genus belongs to Solanaceae family; there are more than 30 species in this genus. *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* are the cultivated species (Stommel and Bosland, 2006). *C. annuum* is the most widely commercialized species worldwide (Rêgo and Rêgo, 2016, Rêgo et al., 2019). The phenotypic diversity found in *C. annuum* for plant size, leaf and fruit traits could potentially be used in breeding programs for producing fresh fruit flavorings and colorings for the food industry (Stommel, 2008; Mongkolporn and Taylor, 2011; Rêgo et al., 2019; Guimarães et al., 2020). The variability observed in peppers allows the to be used as potted, bedding and garden plants, and cut stems (Stommel and Bosland, 2006; Nascimento et al., 2014; Rêgo et al., 2015; Rêgo and Rêgo, 2018; Pessoa et al., 2019).

Knowledge of the genetic diversity present among accessions has great importance for the management and use of the germplasm in the genetic improvement of species (Bianchi et al., 2016; Pereira-Dias et al., 2019). Variability presented by the individuals constitutes the genetic resources, whose characterization and evaluation are essential for plant breeding projects (Sudré et al., 2005).

Several methods have been proposed to study available phenotypic diversity for pepper improvement (Bianchi et al., 2016). The use of multivariate statistical procedures in germplasm characterization studies is important because one can then consider the correlations among the characters, which in turn allows identifying the sources of genetic variability and the importance of each evaluated character in relation to the total genetic divergence (Moura et al., 1999). This strategy is also important to identify promising parents, targeting the attainment of hybrids with higher heterotic vigor (Cruz et al., 2012).

Recently, artificial neural networks come as more a strategy to assist the breeder in the classification of genotypes in breeding programs. The technique of unsupervised learning machine self-organizing maps (SOM) (Kohonen, 1990 and Kohonen, 2014) unlike other multivariate clusters methods using a conventional statistical approach such as the

method of unweighted pair groups with Arithmetic mean (UPGMA), Tocher and among others, is not capable of organizing genetic diversity (Cruz and Nascimento et al., 2018). The SOM have been used successfully to organize the genetic diversity in rubber tree (Sant'Anna et al., 2021), cotton (Cardoso et al., 2021), sorghum (Silva et al., 2020) and alfalfa (Santos et al., 2019).

We estimated and organized the genetic divergence of the *C. annuum* genotypes using self-organizing Kohonen maps.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse belonging to the Vegetable Crops sector of the Department of Plant Science of the Federal University of Viçosa (UFV), Viçosa, MG, Brazil. The geographical coordinates are 20°45' south latitude and 42°51' W, with an average altitude of 650 m.

The experiment was arranged in a randomized complete block design with nine treatments (genotypes) and four replications. The nine *C. annuum* L. genotypes are a) peppers: (1) Volcano, (2) Cayene, (3) Peter, (4) Spicy for Pot, (5) Yellow Jamaican, and (6) Italian Sweet and b) Chilies: (7) Square, (8) Cascadura Ikeda, (9) Giant Ruby. The quantitative traits were: total fruit weight (TFW g), fruit length (FL mm), width of fruit (WF mm), pericarp thickness (PT mm), number of seeds per fruit (NSF), mass of the total fresh matter of the fruit (MTF, g) and percentage of the mass of the total dry matter of the fruit (% MTD, g). Each experimental unit consisted of a pot with one plant. Sowing was carried out in November due to the requirement of the species for high temperatures. The pepper plant requires high temperatures throughout the cycle. The ideal average monthly temperatures are between 21 and 30°C. With the aid of a datalogger, temperature data ranging from 24 to 32°C were collected during the experiment. The seeds were sown in polystyrene trays, with 200 styrofoam cells, containing commercial substrate (Plantmax®). Two seeds were distributed per cell to ensure that there was no idle cell. The trays were covered with a black cloth to speed up the germination process and kept suspended to favor the natural pruning of the root system by air. In January, seedlings with four pairs of definitive leaves were transplanted into 900-mL pots (13 cm height and 16 cm width) and thinning was performed after one week. Phosphorus (P) and potassium (K) were applied monthly and sprays of insecticides and fungicides biweekly during cultivation, as recommended by Filgueira (2013). At this stage, drip irrigation was automatically applied every three hours. The entire experiment was conducted in a greenhouse. The data were subjected to analysis by univariate analyzes of individual variances and means were compared by the Scott-Knott test ( $P < 0.05$ ) for separating treatment means at different groups, by minimizing the variation and maximizing the variation between groups.

The phenotypes values from each trait were used to estimate the genetic divergence between genotypes was estimated through the distance Mahalanobis. The clustering method used was the method of unweighted pair groups with Arithmetic mean (UPGMA). The criterion Mojena (1977) was used to set the dendrogram optimal number of groups by adopting  $k = 1.25$  (Milligan and Cooper, 1985). To assess the consistency and quality of the cluster, the coefficient of cophenetic correlation (CCC), the distortion between the dissimilarity matrix and the matrix obtained after dendrogram (graphic array) and stress (obtained adjusting accurately the projection matrix dissimilarity in the dendrogram) were

obtained. The CCC was given the correlation between the elements of the dissimilarity matrix and the matrix elements produced by phenogram (cophenetic matrix) (Silva and Dias, 2013).

The genetic divergence of genotypes were organized according to SOM (Kohonen, 1990; Kohonen, 2014). The average for each genotype replication evaluated for all seven variables were used as network inputs. It was used a SOM with two dimensions (3 x 4) with hexagonal grid. The architecture of SOM was of the feedforward type with an input layer and an output layer, called topological map which were divided into three stages according to Cruz and Nascimento (2018), Kohonen (1990), and Kohonen (2014).

The number of iterations was equal to 1000. To perform the analysis the Matlab (Matlab, 2010) and GENES (Cruz, 2012; 2016) software were used.

## RESULTS AND DISCUSSION

The results of the analysis of variance showed significant differences between genotypes *C. annuum* by F-test ( $P < 0.05$ ) for all characters examined (Table 1). The coefficients of variation ranged from 6.47 to 17.72%. Similar results were obtained in other studies *Capsicum spp.* (Ribeiro et al., 2015; Araújo et al., 2018).

**Table 1.** Summary of the analysis of variance for the important traits of pepper and chilli under study.

FV	GL	Mean Square						
		FL	FW	TFW	PT	NSF	MTM	MTD
Genotype	8	60*	17*	4385*	8.3*	4930*	4252*	86*
Residue	27	0.1973	0.086	18.608	0.223	93.331	17.365	2.152
Means	--	6.865	3.014	30.212	2.662	58.820	29.349	15.437
CV(%)	--	6.470	9.753	14.278	17.725	16.424	14.198	9.502

\*significant at 5% significance by the F test; <sup>1</sup>FL: fruit length (cm), FW: fruit width (cm), TFW: total fruit weight (g), PT: pericarp thickness (mm), NSF: number of seeds per fruit, MTM: mass of the total material of the fresh ripe fruit (g), MTD: percentage of mass of the total material of the dry ripe fruit (g).

The cluster analysis between genotypes considering the Scott-Knott criterion provided the formation of four groups to PT, TFW, NSF, MTM, MTD, seven eight groups to the characters FL e FW, respectively (Table 2). Corroborating this result, Nietzke et al. (2010) reported that, the character FL can be considered of greater discrimination. Moreover, the same authors mention that the number of groups highlights the wide variability between genotypes.

Based on the criteria proposed by Singh (1981) in terms of the relative contribution of each character evaluated for genetic divergence between genotypes (Table 3), there was a greater relative contribution to the MTM (86.17%) and FL (7.98%) characters, totaling 94.15%, which are the main determinants in the quantification of genetic divergence. Ferrão et al. (2011) working with *C. annuum* genotypes found similar results, the MTM and FL characters had higher relative contribution, 45.6% and 40.9%, respectively. On the other hand, the low relative importance of pericarp thickness (0%), total fruit weight (0%) and percentage of weight of the total dry fruit matter (0.38%) suggest that these characters may be expendable in future work, reducing hand labor expenses, cost and time.

**Table 2.** Mean obtained for seven agronomic traits in nine *Capsicum annuum* genotypes\*.

Genotype <sup>1</sup>	FL <sup>2</sup>	FW	TFW	PT	NSF	MTM	MTD
1	3.07 f	0.93 f	1.38 d	1.55 d	44.25 c	1.14 d	18.51 b
2	7.78 d	0.94 f	2.07 c	3.64 d	33.79 d	3.20 d	19.03 b
3	6.27 e	1.89 e	1.73 c	7.62 d	53.76 c	6.96 d	20.86 a
4	1.66 h	0.49 g	0.89 d	0.60 d	18.79 d	0.41 d	21.21 a
5	2.35 g	3.79 c	1.58 c	6.67 d	24.76 d	6.30 d	16.17 c
6	13.45 a	2.88 d	3.04 b	36.85 c	49.02 c	35.56 c	11.48 d
7	8.59 c	5.42 a	4.53 a	65.68 b	122.13 a	63.81 b	10.95 d
8	9.93 b	4.89 b	4.66 a	69.43 b	89.69 b	68.09 b	10.72 d
9	8.65 c	5.85 a	4.02 a	79.83 a	93.15 b	78.63 a	9.96 d

\*Means followed by the same letters (in the column) are similar by the Scott-Knott criterion at 5% significance. <sup>1</sup>(1) Volcano pepper (2) Cayene pepper, (3) Peter Pepper, (4) Spicy for Pot pepper, (5) Yellow Jamaica pepper (6) Italian Sweet pepper, (7) Square chili, (8) Cascadura Ikeda chili, (9) Giant Ruby chili. <sup>2</sup> Fruit length (cm), fruit width (cm) (FW), total fruit weight (g) (TFW), pericarp thickness (mm) (PT), number of seeds per fruit (NSF), mass of the total material of the fresh ripe fruit (g) (MTM), percentage of mass of the total material of the dry ripe fruit (g) (MTD) genotypes of nine *C. annuum*.

**Table 3.** Relative contribution (%) of traits to genetic divergence in pepper and chili genotypes, estimated by the method proposed by Singh (1981).

Trait	S <sub>j</sub>	Value (%)
FL	1485.43	7.98
FW	4951.21	2.66
TFW	0.00	0.00
PT	0.00	0.00
NSF	5199.12	2.79
MTM	160,185.68	86.17
MTD	721.01	0.38

FL: fruit length (cm), FW: fruit width (cm), TFW: total fruit weight (g), PT: pericarp thickness (mm), NSF: number of seeds per fruit, MTM: mass of the total material of the fresh ripe fruit (g), MTD: percentage of mass of the total material of the dry ripe fruit (g).

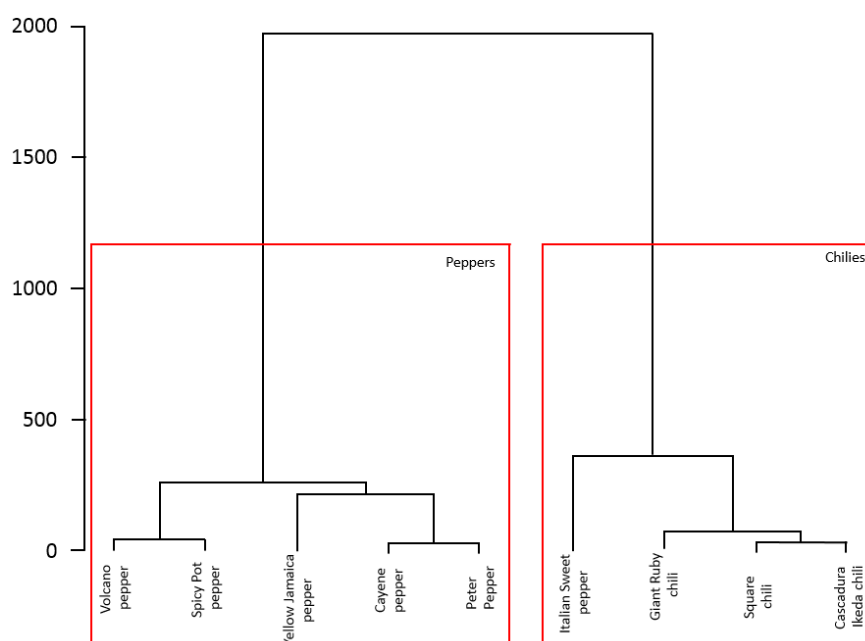
Based on the measures of dissimilarity (Table 4), it can be seen that the lowest dissimilarity was obtained among the Square chili and Cascadura Ikeda chili genotypes ( $D^2 = 28.87$ ), however, the biggest dissimilarity was found between the Spicy for Pot pepper and Giant Ruby chili genotypes ( $D^2 = 3388.47$ ).

**Table 4.** Dissimilarity measures based on the Mahalanobis distance of the nine *Capsicum annuum* genotypes<sup>1</sup> through seven quantitative traits<sup>2</sup>.

	1	2	3	4	5	6	7	8
2	198.32							
3	177.97	25.86						
4	44.88	380.86	359.92					
5	176.46	238.88	180.58	256.75				
6	1776.08	879.65	883.75	2292.24	1554.45			
7	2088.35	1367.04	1221.98	2652.16	1924.71	308.51		
8	2310.58	1480.42	1374.82	288.26	2162.36	262.76	28.87	
9	2787.65	1951.48	1791.82	3388.37	2610.89	508.51	88.38	52.90

<sup>1</sup> (1) Volcano pepper (2) Cayene pepper, (3) Peter Pepper, (4) Spicy for Pot pepper, (5) Yellow Jamaica pepper (6) Italian Sweet pepper, (7) Square chili, (8) Cascadura Ikeda chili, (9) Giant Ruby chili. <sup>2</sup> FL: fruit length (cm), FW: fruit width (cm), TFW: total fruit weight (g), PT: pericarp thickness (mm), NSF: number of seeds per fruit, MTM: mass of the total material of the fresh ripe fruit (g), MTD: percentage of mass of the total material of the dry ripe fruit (g).

Considering the criterion Mojena (1977), two genotype groups were formed by UPGMA methodology. Group I (G1), compound for Italian Sweet pepper, Square chili, Cascadura Ikeda chili and Giant Ruby chili genotypes and group II (G2) by the other genotypes (Figure 1). There was a clear relationship between the cluster allocation and genotype type, as defined according to the types of peppers or chili, and the absence and presence of factor pungency.



**Figure 1.** UPGMA dendrogram by the method based on the genetic Mahalanobis distances for the nine genotypes of *Capsicum annuum* with a cutoff point determined by Mojena (1977).

The Tocher optimization method grouped the 9 *C. annuum* genotypes into four clusters: GI includes pepper Cayene, Peter Pepper, Volcano Pepper, and Pepper Jamaica Yellow., GII includes Square Pepper, Pepper Cascadura Ikeda, and Pepper Ruby Giant., GIII includes sweet Italian pepper, and GIV includes only Spicy to genotype pepper pot genotypes. The Tocher method has greater power of discrimination, enabling the identification of more groups with similar accesses, as has been reported in the literature (Faria et al., 2012; Oliveira et al., 2019).

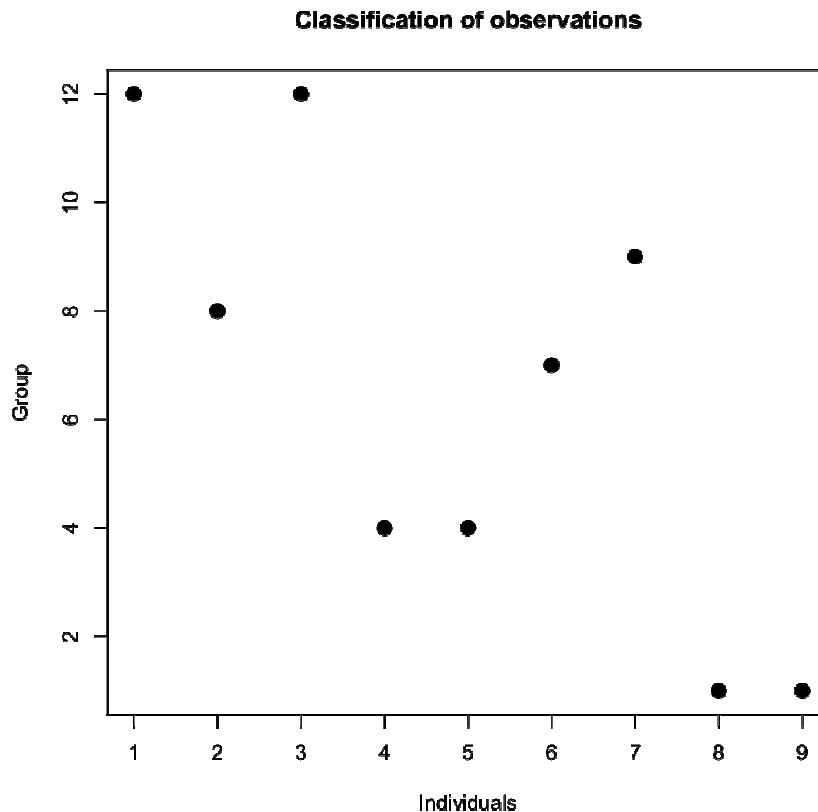
The identification of the genetic relationships and divergence among genetic resources is useful for the selection of parental genotypes in breeding programs (Cruz et al., 2014). The current study was carried out to establish the genetic diversity and relationships among peppers genotypes to identify appropriate parents for hybridization. These results were obtained conformable to Neitzke et al. (2010) and Faria et al. (2012) on what found that the formation of four distinct groups Tocher method to evaluate 17 and 49 access *Capsicum spp.*

Santos et al. (2019) point out that although different clustering methods provide different views of diversity, it is expected that there is an agreement between them. We

believe that the grouping methods can be complementary. When breeders used these together methods have a facility in decision making that genotype must be selected in a particular hybridization breeding program.

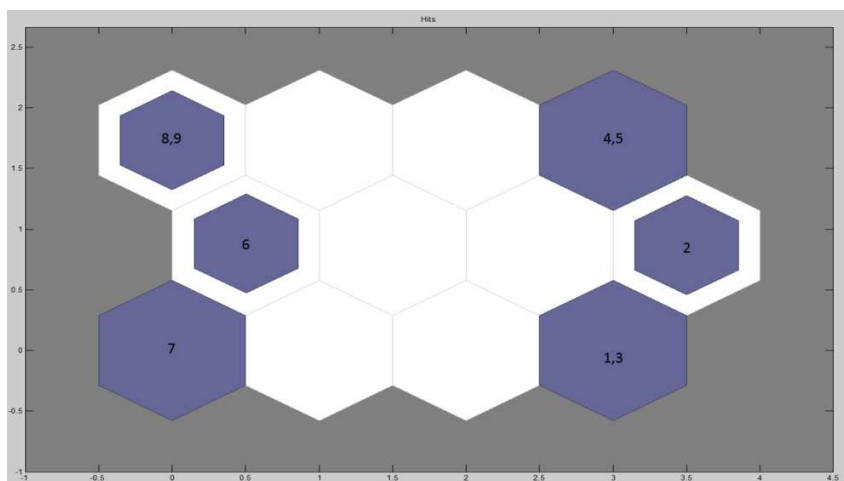
The genetic improvement of important traits in plant breeding depends upon the genetic diversity available within the germoplasm of interest. Here, the genetic diversity of 9 genotypes was studied using traits related to the morphoagronomic descriptors of *C. annuum*, resulting in the identification of distinct groups for the peppers improvement. The genotypes with high levels of genetic variation found in this study are beneficial resources for breeding programs aimed at improving the quality of peppers. The number of defined groups showed agreement between the traditional techniques applied for assessing genetic diversity (UPGMA and Tocher) and the evaluation of SOMs.

Through the scatter plot it is possible to understand the organization of variability (Cruz and Nascimento, 2018). Thus, each cell class is chosen by grouping a class and the result becomes easily interpreted (Figure 2). In other words, there is information that makes it possible to infer the existence of large groups of diversity and the proximity of these groups from the multivariate analysis.



**Figure 2.** Grouping of pepper and chili genotypes in classes to set up by the Kohonen map, considering topological map representing a 3x4 grid. Peppers and Chillies: (1) Volcano pepper (2) Cayene pepper, (3) Peter Pepper, (4) Spicy for Pot pepper, (5) Yellow Jamaica pepper (6) Italian Sweet pepper, (7) Square chili, (8) Cascadura Ikeda chili, (9) Giant Ruby chili.

By means of neural networks designed hexagonal configuration noted that the neighborhood pattern between hot and sweet peppers was obtained in only seven of the twelve neurons previously established (Figure 3). Note that was highlighted splits of the groups with the presence of five non-competitive neurons, forming a corridor separating the hot and sweet peppers. This setting helps the breeder in making decisions on crosses targeting planning. According to Cruz et al. (2012), genetic diversity analysis allows the identification of additional genetically parents, which, when intercrossed populations may lead to greater variability and a higher probability of recovery superior genotypes.



**Figure 3.** Organization of the similarity of *Capsicum annuum* (number(s) inside each hexagons) and self-organizing map of Kohonen method of two-dimensional type with grade 3 x 4 (twelve neurons) hexagonal configuration. Peppers and Chillies: (1) Volcano pepper (2) Cayene pepper, (3) Peter Pepper, (4) Spicy for Pot pepper, (5) Yellow Jamaica pepper (6) Italian Sweet pepper, (7) Square chili, (8) Cascadura Ikeda chili, (9) Giant Ruby chili.

The use of parental genotypes with the greatest possible divergence is important to maximize heterosis in hybrids and increase the genetic base. The most divergent pair of genotypes was Spicy for Pot pepper and Giant Ruby chili ( $D^2=3388$ ), followed by Volcano pepper and Giant Ruby chili ( $D^2=2787$ ). According to the SOM results that organized genetic diversity, do not recommend cross genotypes from the Cascadura Ikeda chili x Giant Ruby chili, Spicy for Pot pepper x Yellow Jamaica pepper, and Volcano pepper x Peter Pepper to variability exploration.

Santos et al. (2019) used the same method in the study of genetic diversity of rice genotypes and found both to the potential of this genetic diversity studies in breeding programs. The SOM method has proved to be an efficient way to identify patterns of similarities, as shown Mwasiagi (2011), who used the technique to distinguish SOUND cotton genotypes. The author concluded that the method was efficient to separate the thin wire thicker, and samples scattered on the map would be disparate, resulting in the irregularity of the fiber. In our case, we found that the method was able to separate peppers mainly by size and poignancy, important characteristics in breeding pepper and chili. Sant'Anna et al. (2021) employed multivariate UPGMA, Tocher, and machine learning



techniques K-means and SOMs to study Genetic diversity associated with natural rubber quality in elite genotypes of the rubber tree. The genotypes in study were classified into six clusters by the UPGMA method. In addition, the results were consistent with those results provide by Tocher, K-means and SOM methodologies. With these methodologies, Sant'Anna et al. (2021) identify clones most divergent (IAC 418 and PB 326; IAC 404 and IAC 56) which can be used to start a breeding program. Cardoso et al. (2021) used ANN in the genetic divergence study of colored cotton plants. These authors observed that SOM was able to organize the similarities between accesses. This method presented a larger number of groups compared to the results obtained using the Mahalanobis matrix. Overall, the authors observed that methods based on computational intelligence proved to be more efficient in detecting similarity.

The ordering of genotypes by the technique of SOM was consistent with the results of UPGMA and Tocher, as maintained the basic structure of the preserved groups, each cluster of maps. According to Silva et al. (2021), the use of artificial neural networks as pattern recognition methods is a promising way, and the SOM network can provide more valuable results than traditional cluster analysis studies on genetic diversity.

## CONCLUSIONS

There is genetic divergence among the pepper, and the methods used allowed the identification of promising groups for diallel crosses. UPGMA and Tocher gave two and four groups, respectively. The SOM has great potential to assist the breeder in decision making in choosing parents for diallel crosses. Specifically, the most divergent pair of genotypes was Spicy for Pot pepper and Giant Ruby chili ( $D^2=3388$ ), followed by Volcano pepper and Giant Ruby chili ( $D^2=2787$ ). According to the SOM results, which organized genetic diversity, crossing genotypes Cascadura Ikeda chili x Giant Ruby chili, Spicy for Pot pepper x Yellow Jamaica pepper, and Volcano pepper x Peter Pepper are not recommended for variability exploration.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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