Enhancing genetic diversity in ornamental chili pepper through interspecific crossing

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Abstract

The observation of morpho-agronomic characteristics of ornamental chili pepper is essential to enhance diversity and get novel cultivars. Interspecific crossing is extremely effective in introducing a desirable character, resulting in a novel cultivar of agronomic relevance. This study aimed to identify the genetic diversity of crossing two open-pollinated ornamental chili pepper cultivars ('Black Pearl'/Capsicum annuum & 'Rawit Prentul'/ Capsicum frutescens) as parents based on twenty morpho-agronomic characters (plant, flower, and fruit). The genetic diversity analysis in interspecific ornamental pepper offspring shows varying results from narrow to broad diversity. The broad genetic diversity in F1 Black Pearl x Rawit Prentul is found in replace with vegetative character, while the reciprocal cross (Rawit Prentul x Black Pearl) is seen in replace with vegetative and fruit characteristics. change The heatmap analysis identified the F1 into three major clusters while the dendogram analysis divided the F1 and F1 reciprocal into four major clusters. In F1 offsprings showed significantly negative and positive correlation between fruit color and morpho-agronomics character.

Keywords: character, genetic, interspecific crossing, morpho-agronomic

Introduction

Ornamental peppers (*Capsicum* spp.) have gained significant popularity and specially cultivated for their aesthetic value, have become increasingly important in landscape design, indoor decoration, and potted plant markets (Stommel & Bosland, 2006).

The development of ornamental chili pepper plants is expected to be an innovative way to provide high-quality, multi-purpose ornamental plants, and, of course, to generate economic value for those who cultivate them. This opportunity for development presents a new challenge for breeders to further increase the genetic diversity of chili plants, particularly in terms of enhancing their beauty, such as fruit color, fruit shape, growth type, and pigmentation on leaves, which are the main attractions of ornamental chili pepper plants ((Stommel & Bosland, 2006). The emergence of new types of ornamental chili pepper plants is expected to

meet consumer preferences and attract farmers' interest in further developing ornamental chili peppers (Wirasti, 2013)

Genetic diversity and heritability are absolute requirements for the success of plant breeding initiatives (Acquaah, 2012). Genetic diversity can increase the likelihood of obtaining better genotypes through selection. Diversity characters and genotypes are useful for determining genotype grouping patterns in a given population based on observed characters and can serve as a foundation for selection activities (Agustina & Waluyo, 2017). The development of novel varieties with intriguing desirable characters necessitates an understanding and utilization of the genetic diversity. Analysis of diversity can be carried out with various types of markers, one of which is the morphology of the plant (Effendy et al., 2018).

The higher the phenotypic diversity in characters unaffected by environmental factors, the greater the The

higher the phenotypic diversity in characters unaffected by environmental factors, the greater the chance of identifying superior genotypes through selection. The diversity of phenotypes in these characters shows the diversity of genetic factors against the encoded characters (Knight, 1979).

This study focused on observing the morphoagronomic characters of F1 offsprings from interspecific crossing as a selection principle for breeding new varieties, breeding activities added with crossing between Openpollinated (OP) cultivars are naturally diverse because their plants aren't genetically identical. When two OP cultivars are crossed, their different genes mix and create a wide range of traits in the offspring and often leads to lots of variation. followed by selection, are commonly conducted to breed ornamental chili peppers (Permadi, 1994). Through this technique, the parent's character of ornamental chili pepper plants can be combined. Plant breeding programs will be successful if there is high diversity and inheritance in the characters to be improved (Bao et al., 2005). It is fundamental to detecting duplicate plants in the germplasm banks (Gonçalves et al., 2008; Laurentin, 2009; Rego et al., 2011). Reliable methods and processes for characterizing germplasm are essential for maximizing available variability (Silva et al., 2017).

Material and Methods

Plant material

The plant material used in this study is two ornamental chili pepper varieties used as parents from different species, namely 'Black Pearl' (Capsicum annuum) denoted as BP, and 'Prentul' (Capsicum frutescens) denoted as PRT, F1 generations from BP x PRT crosses as many as 30 plants and F1 reciprocal as many as 30 plants divided into 10 replication. Seeds of 70 offspring of ornamental chili peppers were sown on expanded polystyrene trays with 72 cells. The transplanting of the seedlings was done, 25-30 days after sown

Phenotypic characters observation

The phenotypic characters diversity was observed using morpho-agronomic characters. Data were recorded on plant height, canopy width, number of leaves, stem diameter, fruit diameter, fruit length, weight per fruit, fruit stalk, anther length, corolla length, pistil length, and fruit color.

Fruit color data was quantitatively observed at each stage of fruit development, there is the immature fruit stage (30-35 days after planting/dap), intermediate fruit stage (45-50 dap), and mature fruit stage (55-65 dap). Each fruit sample was repeated three times. Fruit

color parameters determined by levels of L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) were observed using the Konica Minolta CM-2006 chromameter and calibration was carried out by standard color calibration (Gonçalves et al., 2008).

The qualitative fruit color observation and characterization were carried out at three stages of fruit development using an RHS color chart and Guidelines for Implementing Testing, Uniqueness, Uniformity, and Stability of Chili Peppers (UPOV, 2020). The following variables were measured in the performance of two parental lines and the selected 60 genotypes from the F1 and F1 reciprocal populations. Parental use in this study has been presented in **Figure 1**.

Data analysis

The Analysis of Variance (ANOVA) was applied to the quantitative data to determine the genetic diversity indicated by the value of the Genotypic Coefficient of Diversity (GDC) calculated according to the formula (Singh & Chaudhary, 1985):

$$\sigma^{2}G = \underline{MSg - MSe}$$

$$r$$

$$GDC = \underbrace{(\sqrt{\sigma^{2}G})}_{X} \times 100$$

 $\sigma^2 G$: Genetic variability

MSg: Means Square Genotype
MSe: Means of Square Error

GDC: Genetic Diversity Coefficient

 \overline{X} : Average

Moedjiono and Mejaya (1994) state that the obtained genetic diversity coefficient can be divided into four categories: low diversity (less than 25% of the highest GDC), medium diversity (between 25% and 50% of the highest GDC), high diversity (between 50% and 75% of the highest GDC), and very high diversity (greater than 75% of the highest GDC).

Heat map cluster analysis and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm were used to cluster the data and to visualize the general performance of the F1 offspring. The data were also clustered through dendogram clustering using R-Studio software. The correlogram determines the correlation of twenty morpho-agronomics characters in F1 interspecific offspring.

Results and Discussion

Genetic diversity

Analysis of the variance of the coefficient of genetic diversity (CGD) from 60 offspring of ornamental chili peppers is presented in **Table 1**.



Figure 1. Ornamental chili pepper parents in this study

Table 1. Genetic diversity of morpho-agronomics character in F1 offspring of BP x PRT cross

Character	\overline{X}	σ2 G	CGD (%)	Relative value	Diversity criteria
Plant height (cm)	32.18	54.36	22.70	4%	Narrow
Canopy width (cm)	40.28	500.48	55.56	9%	Narrow
Leaf number (leaf)	94.69	13,062.19	120.70	19%	Narrow
Stem diameter (cm)	0.62	0.08	45.32	7%	Narrow
Fruit diameter (cm)	0.98	0.08	28.67	5%	Narrow
Fruit length (cm)	1.76	1.46	68.67	11%	Narrow
Weight per fruit (gr)	1.26	0.90	75.24	12%	Narrow
Fruit stalk (cm)	1.81	0.28	29.39	5%	Narrow
Anther length (cm)	0.43	0.00	12.81	2%	Narrow
Corolla length (cm)	0.79	0.00	2.83	0%	Narrow
Pistil length (cm)	0.68	-0.01	12.01	2%	Narrow
Immature Lightness	35.43	1,799.36	119.73	19%	Narrow
Immature a*	4.71	822,81	609.01	96%	Broad
Immature b*	6.93	1,946.57	636.65	100%	-
Intermediate Lightness	42.68	6.27	5.87	1%	Narrow
Intermediate a*	33.89	220.05	43.77	7 %	Narrow
Intermediate b*	31.81	168.39	40.79	6 %	Narrow
Mature Lightness	31.65	14.99	12.23	2 %	Narrow
Mature a*	39.14	31.81	14.41	85%	Broad

According to Table 1. BP × PRT crossing, the b* (yellowness-blueness) and a* (redness-greenness) values in the immature stage, as well as the a* value in the mature stage, revealed broad genetic diversity.. According to Permadi (1994) states wide or high genetic variability is indicated by values. When a characteristic's genetic variation value is extremely high, it means that selection may have improved the character. Conversely, the other 17 characters which are plant and flower characters exhibit a narrow genetic diversity.

The F1 genetic diversity from the reciprocal crossing of PRT × BP has diversity in morpho-agronomics character (**Table 2**.). The results of the observation in PRT × BP cross showed broad diversity in plant and fruit character. Plant character is determined by plant height, canopy width, and stem diameter. Meanwhile, fruit character determined by fruit diameter, fruit length,

weight per fruit, fruit stalk, and fruit color chromameter (L*, a*, and b*) at three stages of development (immature, medium, and mature) are presented in Table 2. Genetic diversity fruit length, weight per fruit, fruit stalk, fruit color parameters lightness in the immature stage, and a* in the mature stage revealed a broad genetic diversity.

Analysis of variance in 20 characters from F1 BP × PRT and the F1 reciprocal PRT × BP are higher than environmental variants. This indicates that genetic factors influence diversity more than environmental factors. The parameters influenced by environmental factors are characters that are influenced by many genes (quantitative characters), while parameters that are influenced by genetic factors are influenced by a few genes (qualitative characters). The qualitative characteristics are regulated by one or two genes, slightly influenced by the environment so the selection can be done visually (Mangoendidjojo, 2003).

Table 2. Genetic diversity of morpho-agronomics character in F1, offspring of PRT x BP cross

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Character	\overline{X}	σ2 G	CGD (%)	Relative value	Diversity criteria
Plant height (cm)	25.57	265.32	63.70	73.18%	Broad
Canopy width (cm)	30.34	558.39	77.88	89.48%	Broad
Leaf number (leaf)	47.13	1,683.02	87.05	100%	-
Stem diameter (cm)	0.53	0.11	63.98	73.50%	Broad
Fruit diameter (cm)	0.83	0.04	25.47	29.26%	Narrow
Fruit length (cm)	1.89	2.15	77.52	89.06%	Broad
Weight per fruit (gr)	0.99	0.24	49.45	56.81%	Broad
Fruit stalk (cm)	2.22	1.27	50.79	58.35%	Broad
Anther length (cm)	0.45	0.00	4.82	5.53%	Narrow
Corolla length (cm)	0.78	0.00	3.65	4.19%	Narrow
Pistil length (cm)	0.68	0.00	8.66	9.95%	Narrow
Immature Lightness	52.03	1,531.70	75.22	86.41%	Broad
Immature a*	-4.98	917.75	6.02	6.92%	Narrow
Immature b*	23.66	1,790.57	1.79	2.06%	Narrow
Intermediate Lightness	40.55	27.94	13.04	14.98%	Narrow
Intermediate a*	40.51	71.85	20.92	24.04%	Narrow
Intermediate b*	35.28	34.38	16.62	19.09 %	Narrow
Mature Lightness	30.33	14.99	12.76	14.66 %	Narrow
Mature a*	37.37	2.49	4.22	4.85%	Broad
Mature b*	16.98	43.51	38.85	44.63%	Narrow

F1 : BP × PRT; F1_R : PRT × BP

The results of genetic diversity analysis in plant characters, consisting of plant height, canopy width, number of leaves, and stem diameter showed a broad genetic diversity range from 73,18 - 100% (Table 2). The number of leaves in the PRT × BP cross was very high. Genotypic Diversity Coefficient (GDC) value was determined as a relative value of 100%. Improvement through direct selection is achievable for a character with a very high and high genetic diversity value. The floral attributes exhibited less genetic variation compared to vegetative traits. A characters that have a narrow diversity, selection can be done after the population has multiplied or been raised for several additional generations. At this time, selection aims to increase the frequency of genes that carry superior or adaptive characteristics, especially if the goal is to improve the performance or resistance of plants in certain situations.

High diversity is one requirement for selecting the desired characters because the selection process for these characters will be more efficient. If genetic diversity in a large population indicates that individuals in a diverse population suggest that the opportunity to obtain the expected genotype will be large (Bahar & Zen, 1993). The larger the genetic diversity, the better the chances of success for the breeding effort. The broad phenotypic variability arises from the wide array of genetic diversity and environmental factors and the interplay between genetic and environmental influences (Jamilah et al., 2011). Furthermore, extensive variety might improve selection response since selection reaction is directly proportional to genetic diversity. The larger the genetic diversity, the better the chances of success for

the breeding effort. Furthermore, extensive variety might improve selection response since selection reaction is directly proportional to genetic diversity (Fehr, 1987).

Evaluation of the color character of ornamental chili pepper fruit (Capsicum annuum L.) Based on the rhs color chart

The fruit color changes usually occur at every fruit maturing stage, starting from immature, intermediate, to mature fruits. The Black Pearl Parents go through significant color changes during the immature stage and head toward the intermediate stage. The immature dark purple fruit belongs to the color of Violet group 83A and then turns yellow (Yellow Group 10A) and before going to the maturing stage of the fruit, it turns orange (Orange-Red Group 32A). The purple and black colors in chili peppers express the accumulation of anthocyanins. The purple color of the leaves found in some genotypes is also caused by the same type of anthocyanins present in the fruit. Anthocyanins in fruits accumulate on the outside of the mesocarp and the leaves in the palisade and mesophyll cells (Lightbourn et al., 2008). Based on the data in Table 2. the results of the phenotypic observation of fruit color at 3 stages of development (immature, intermediate, and mature), obtained the result that the color of the immature fruit of the Prentul parents is included in the Yellow-Green Group 145 B. For the F1 generation, the results show various results, some are included in the color category of Violet Group 83 A (11 plants), the same as the Black Pearl parents, and some show a variety of other colors, namely Greyed-Purple N 186A (14 plants) and Green Group 137A (5 plants). There is

an emergence of a variety of new colors in the immature fruit stage, some of which follow the female parents, and some are suspected to be a combination of the colors of the immature fruits of the female and male parents. As for the F1 generation from the reciprocal cross, the results of all its offspring show the same immature fruit color as the parents Prentul (yellowness-greenness).

The results of the observation of the color of the fruit between the two ornamental chili parents and their F1 generation showed that some of the F1 offspring in the BP x PRT cross had the same fruit color as the female parents (Black Pearl), namely passing through two stages of the intermediate fruit color (yellow and orange), meanwhile, some only passed one color in the intermediate fruit stage (orange). For offspring from crosses that only go through one change in the color of the intermediate fruit, it has a grayish-green light fruit color, while for those that show two stages of intermediate fruit color change, it has a dark purple light fruit color. Based on the data in Table 1, the intermediate fruit color for the offspring of the F1 reciprocal shows that all offspring show the same orange color as the parents Prentul (Orange-Red Group N30B) as much as 10% of the population and the remaining 90% are divided into 3 orange color gradations, namely

Germplasm genetic diversity is an important foundation in plant breeding, especially the assembly of superior varieties, and is a key component of germplasm protection and conservation strategies (Thomson et al., 2007; Tilahun et al., 2013). Information related to genetic diversity and relationships between germplasm accession is very useful in understanding the availability of genetic variability and its potential in plant breeding. Genetic variation analysis provides information on the population's genetic diversity and structure, as well as a platform for selecting superior genotypes to develop new cultivars in agricultural improvement breeding projects (Verma et al., 2019).

Morphoagronomic characterization was based on the descriptor list by IPGRI (1995). Twenty quantitative characters were evaluated for plant, flower, and fruit. The evaluated plant characters were: plant height (PH), canopy width (CW), leaf number (LN), and stem diameter (SD). The flower characteristics evaluated were: anther length (AL), corolla length (CL), pistil length (PL). The fruit variables analyzed were: fruit diameter (FD), fruit length (PL), weight per fruit (WPF), and fruit stalk (FS). The data were submitted for analysis of variance. Estimates of heritability, genetic variance, and correlation between

Table 3. The fruit color transition of ornamental chili pepper in three stages of fruit development based on RHS color chart

Genotipe -	Fruit color transition						
Genolipe —	Immature	Intermediate		Mature			
Black Pearl (BP)	Violet Group 83A	Yellow Group 10 A	Orange-Red Group 32A	Red Group 46			
Rawit Prentul (PRT)	Yellow-Green Group 145 B	Orange-Red Group N30B		Red Group 45 A			
	Violet Group 83A	Yellow Group 10 A	Orange-Red Group 32A				
F1	Greyed-Purple N 186A	· .		Red Group 46			
BP × PRT	Green Group 137A						
	Violet Group 83A	Yellow Group 8 B	Orange-Red Group 32A	_			
		Orange-Re	Red Group 45 A				
F1R PRT × BP	Yellow-Green Group 145 B -	Orange-Re					
		Orange-Re					
		Orange-Re					

Note: BP: Black Pearl Parents; PRT: Rawit Prentul Parents; RHS: Royal Horticulture Society Color

Orange-Red Group N30B, N30C, N33A, and N30B. (Table 3)

Based on observation data with the guidance of the RHS color chart, the color of the mature fruit of the two crossed parents shows almost the same red color, for the Black Pearl parents show the red color in the Red Group 46 group and the Prentul parents in the Red Group 45A group., while the F1 offspring are reciprocated, producing offspring with the color of mature fruit following the female parents Prentul (Red Group 45 A).

Heat map and cluster dendogram analysis of all morphoagronomic character in interspecific f1 generation of ornamental chili pepper genetic and environmental coefficients were also estimated.

The observation of morpho-agronomic characters shows the diversity. To find the genetic diversity of the population resulting from crossing both F1 and F1 reciprocal. The findings of the investigation are shown in **Figure 2** as a heatmap. A heatmap is a two-dimensional visual representation of data that employs color changes from hues to darker intensities to express different values. Heatmaps provide three important information pieces: clusters, similarity matrices of columns, and similarity matrices of rows (Fernandez et al., 2017). The similarity matrix of the columns forms a morphological character

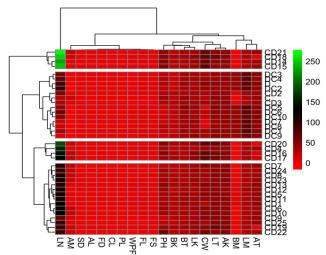


Figure 2. Heatmap clustering parents and F1 interspecific crossing of ornamental peppers

cluster, and the similarity matrix of the rows forms a genotype cluster (Anshori et al., 2018). The results of the visualization of the ornamental chili pepper characters are displayed in the form of a heatmap.

This study used a clustered heatmap to assess the overall performance of twenty observable characteristics among F1 genotypes with two parental lines. Each column represents a certain attribute, and each row represents its measurement. Heatmap created two dendrograms: one vertical indicating the F2 genotypes and parental lines, and one horizontal indicating the qualities that triggered the diffusion. The UPGMA-based heatmap-dendrogram analysis revealed that the chosen F1 genotypes of interspecific hybrids of ornamental pepper were classified into three major groups based on morpho-agronomic features (Figure 2). Another vertical dendrogram displayed the existence of two major meaningful groups (Figure 2). It includes grouping into two: one associated with Leaf Numbers (LN), a* value in the immature stage (AM), Stem Diameter (SD), Anther Length (AL), Fruit Diameter (FD), Corolla Length (CL), Pistil Length (PL), Weight Per Fruit (WPF), Fruit Length (FL), and Fruit Stalk (FS), and the second associated Plant Height (PH), b* value in mature stage (BK), b* value in intermediate stage (BT), Lightness in mature stage (LK), Canopy Width (CW), Lightness in intermediate stage (LT), a* value in mature stage (AK), b* value in immature stage (BM), Lightness in immature stage (LM), and a* value in intermediate stage (AT). These results showed the presence of phenotypic variation in the F1 population concerning morpho-agronomic characteristics for plants, flowers, and fruit (Tiessen et al., 2017).

Research on genetic diversity using genotypeclustering techniques allows for the evaluation of the degree of variety or similarity between genotypes (Alvares, 2011), and as a result, may be a useful tool for breeding improvement (Rego et al., 2003). The island's genetic variety has resulted in a spectrum of genetic variability that might be used for future development, particularly to choose genotypes that could be employed in the ornamental industry and eventually become more environmentally suited.

Morphological indicators can be used as a measure of plant diversity. Diversity in *Capsicum annuum* can occur due to the very high level of viability of pollen, reaching around 90.8%. The results of the cluster analysis on the morpho-agronomic characters of ornamental chili pepper fruits are in **Figure 3**.

Cluster Dendrogram

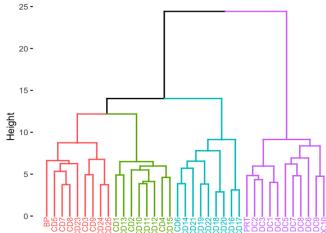


Figure 3. Cluster Dendrogram parents and F1 interspecific crossing of ornamental peppers

Clustering analysis was used to assess the genetic diversity of the F1 ornamental pepper population. Cluster analysis was performed using the Unweighted Paired Group Method with Arithmetic Mean (UPGMA) based on dice coefficient similarity (Figure 3). The dendrogram shows about four main clusters represented by different colors (red, green, blue, and purple). Each color represents a group of data points that were merged earlier, indicating higher similarity among them. The lower the height (y-axis) at which items are joined, the more similar between the offspring.

The cluster analysis revealed that the F1 population and parental lines were divided into two major groups/cluster (Figure 3). The first major cluster had three subclusters: Black Pearl parent and genotypes from BP x PRT cross (CD5, CD7, CD28, CD23, CD3, CD9, CD24, and CD25), while the second subcluster consisted of genotypes from BP x PRT cross (CD1, CD13, CD2, CD10, CD11, CD12, CD4, and CD15), third subcluster BP x PRT offspring (CD6, CD14, CD21, CD19, CD22, CD18,

CD20, CD16, and CD17). Meanwhile, the second cluster consisted of Prentul parent, offspring that resembled the genotype of PRT x BP cross (DC2, DC3, DC1, DC4, DC5, DC7, DC8, DC9, and DC10). The height at which groups merge on the y-axis indicates the distance or dissimilarity between them. Near the top of the dendrogram, all clusters merge around a height of 20, showing a significant difference between the main clusters. This dendrogram could be used to select an optimal number of clusters by cutting the dendrogram at a specific height to separate data points into distinct groups.

The correlation between color parameters in F1 offspring of BP × PRT and PRT × BP cross is shown in Figure 4. and showed varying value. The darker red hue reflects higher positive connections, whereas the darker blue tint reveals stronger negative correlations and white or light-colored cells, show weak or no correlations. There is a strong correlation between lightness (L*) and b* value in the immature fruit stage (0.99), suggesting these two variables move together very closely, the b* value in the immature stage with fruit stalk (0.84), lightness in immature stage with fruit stalk (0.83), a* value and L* value in mature stage (0.82). The leaf number has a positive correlation with canopy width and stem diameter characteristics, indicating increasing the leaf number, canopy width, and stem diameter. High negative correlations (dark blue) may indicate a compensatory relationship, where one variable tends to decrease as the other increases, or they may be opposing characters. The negative correlation level is shown in b* value and a* value in the immature

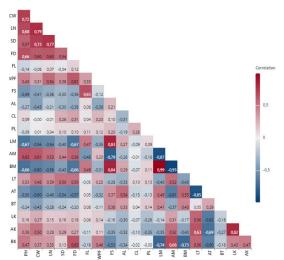


Figure 4. Correlation between twenty morpho-agronomics character of interspecific F1 offsprings of ornamental pepper, **FD**: Fruit Diameter; **FL**: Fruit Length; **WPF**: Weight Per Fruit; **FS**: Fruit Stalk; **LM**: Lightness Immature Color; **αM**: α*: Greenness-Redness Immature Color; **bM**: b*: Blueness-Yellowness Immature; **LT**: Lightness Intermediate Stage, **AT**: α* Greenness-Redness Intermediate Stage; **LK**: Lightness Mature Stage; **AK**: α* Greenness-Redness mature stage and BK: b* Blueness-Yellowness mature stage

stage (-0.93), a* value and lightness in the immature stage (-0.87); a* value and lightness in the intermediate stage (-0.85), indicating that as one of these increases, the other decreases significantly.

Conclusion

Based on phenotypic characters, we concluded that the presence of both phenotypic and genetic diversity occurred in the F1 population and the reciprocal of ornamental pepper originated through interspecific crossing. The UPGMA-based heatmap analysis indicated that the selected F1 genotypes of interspecific hybrids of ornamental pepper were divided into three major groups based on the phenotypic features for morphoagronomic characters. The cluster analysis of the F1 population of ornamental chili pepper (Capsicum annuum) revealed four major groupings based on morpho-agronomic features, each represented by a distinct hue in the dendrogram (red, green, blue, and purple). The correlation between color parameters in F1 offspring of BP × PRT and PRT × BP shows strong positive correlation in some vegetative and fruit characters and also shows strong negative value in fruit color characters.

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