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# A Computer Vision-Based Methodology to Estimate Fruit Colour Diversity in Ornamental Pepper (*Capsicum* spp.)

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## ABSTRACT

Fruit colour diversity within different ripening stages confers ornamental value for pepper plants. Using images can be helpful in analysing the fruit colour-related genetic diversity and enable selecting accessions for ornamental purposes by avoiding subjectiveness. This study aimed to evaluate the phenotypic diversity among accessions of ornamental pepper using image processing techniques to identify fruit colours. Photos from 40 fruits from each of 12 accessions were captured by a camera from a smartphone. Separate channels and colour indices were extracted from the images to analyse the accessions using both dendrogram and principal components analysis. Dendrogram analysis allowed separating accessions with predominance of dark fruits from those whose fruits showed lighter and more vivid colours, with a cophenetic correlation coefficient of 0.811. The VARI and NGRDI vegetation indices were efficient in discriminating fruits with a predominance of green colour, and the BGI could be used to discriminate accessions with predominantly reddish-brown fruits. The proposed method can be used in small-scale breeding programs for accurately assessing the development of varieties regarding their fruit colour diversity.

## 1 | Introduction

Defined as the light-absorbing and light-reflecting characteristics of an object, colour is one of the most important attributes in agriculture, particularly for fruits and vegetables, as it indicates quality and usually determines their value. In pepper, fruit colour is the characteristic of greatest aesthetic contrast (Dos Santos et al. 2023) and is associated with the presence of pigments related to the nutrition, health and flavour of the fruit (Brand et al. 2014), resulting mainly from the differential accumulation of flavonoids and carotenoids (Delgado-Vargas and Paredes-López 2002; De La Cruz-Ricardez et al. 2020).

When ripe, pepper fruits often acquire a red colour but may diverge with yellow, orange, salmon, brown, green, white, purple, violet, black and intermediate shades during their ripening (De Carvalho and Bianchetti 2009; DeWitt and Bosland 2009), usually occurring together in the same plant. For the ornamental market, pepper plants with the highest value are those with a wide diversity for colour of unripe and ripe fruits, which

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contrasts with the foliage and show an important attribute for the consumer (Neitzke et al. 2016).

Genetic breeding for colour attributes faces difficulties because it is based on visual descriptors that are subjective to human vision, even when colour palettes are used (Chéné et al. 2012; De Jesus et al. 2022). Human detection ability can be influenced by the environment and the evaluator's own emotional factors (Pesante-Santana and Woldstad 2000; Patil, Yadahalli, and Pujari 2011). In the evaluation and selection stages of ornamental pepper plants, the classification of fruit colour is often carried out using the descriptors of International Plant Genetic Resources Institute (IPGRI) (1995). According to the descriptor, the fruit colour is observed in the intermediate and mature stages, items 7.2.2.3 and 7.2.2.6, respectively (IPGRI 1995), disregarding the various ripening stages that occur during the development of the fruit, and not just two. Furthermore, this descriptor does not satisfactorily cover classes that allow for the classification of the great variation and intensity of colours possible in the various ripening stages. In the intermediate stage, the fruits are classified as white, yellow, green, orange, purple, dark purple and others (IPGRI 1995). Intermediate tones and intensities are usually disregarded, as well as the 'Other' category, since considering them leaves more room for subjectivity and variations during the evaluation of germplasm.

Additionally, subjective colour classification also makes parametric data analysis difficult (Fernandes 2022) and also hinders the application of traditional statistical methods, which commonly use classes of numerical objects for analysis, such as multivariate tools. Genetic diversity studies, supported by multivariate clustering methods and principal component analysis, have been used to characterize germplasm and in the selection of target genotypes in breeding programs, including those aimed at ornamental peppers (Costa et al. 2016; Pimenta et al. 2020; Silva and Silva 2021; Albuquerque, Freitas, and Marcelino 2021; Silva and Silva 2021; Pessoa et al. 2025). It is noticeable that in these studies, the colour characteristic is evaluated subjectively and was not always considered in diversity analyses, which is one of the most important descriptors for the ornamental plant market.

Therefore, human vision is not precise and objective, making it difficult to implement a reliable phenotyping intervention (Fahlgren, Gehan, and Baxter 2015; Xiong et al. 2021). Standardization for germplasm evaluation is crucial to eliminate human error and ensure more reliable results (Chacón et al. 2013; Fahlgren, Gehan, and Baxter 2015; Souza et al. 2022). To improve the precision, accuracy and speed of phenotypic evaluation, techniques based on digital images associated with computer vision have been developed as an alternative to existing evaluation methods, as they provide greater reliability to the results obtained (Cortes et al. 2017), helping to identify genetic diversity, which is the raw material for breeding programs.

An image can be defined as a function $f$ (x, y), where x and y are spatial coordinates in a two-dimensional plane, and $f$ is the intensity of the image at a given point (x, y). An RGB (Red, Green, Blue) image consists of three image components, one for each primary colour. The three components are combined to produce a single-colour image (Silva et al. 2019). Using RGB images for colour evaluation is an economical choice to obtain accurate results, especially in small-scale breeding programs or in developing countries. Images are easily obtained from professional cameras and smartphone cameras, a technology accessible to a wide range of users, when compared to multispectral and hyperspectral cameras, which are expensive, require complex calibration and are more difficult to handle (Deng et al. 2018). Additionally, several vegetation indices can be estimated from the RGB channels and associated with the main pigments responsible for colour in plants (Tucker 1979; Khaleghi et al. 2012; Sarkar et al. 2021; Pazhanivelan et al. 2023) and then used in diversity studies for breeding programs.

Within this context, the objective of this study was to evaluate the phenotypic diversity in fruit colours among ornamental pepper accessions, based on a low-cost methodology that uses digital image processing techniques to identify fruit colours, relating for each accession the vegetation indices with the phenotypical expression of fruit colours, and to select promising accessions based on those images and the vegetation indices extracted from them.

## 2 | Material and Methods

### 2.1 | Experimental Site and Plant Material

The experiment was carried out at the Laboratory of Plant Breeding and Data Analysis, Federal University of Piauí (UFPI), Professora Cinobelina Elvas Campus, Bom Jesus, Piauí, Brazil.

A total of 12 accessions of ornamental pepper (*Capsicum* spp.) were used, all of them belonging to the *Capsicum* Germplasm Bank of the Professora Cinobelina Elvas Campus of UFPI. These were selected based on phenotypic variability for fruit colour (Table 1). From each accession, 40 pepper fruits were randomly collected, 20 at each ripening stage (intermediate and mature) classified and defined by IPGRI (1995) (Table 1). Four replicates of 10 fruits were taken, without damage caused by biotic/abiotic factors, from plants grown in pots in a greenhouse.

### 2.2 | Imaging, Segmentation and Data Extraction

Pepper fruits were placed on a white paper surface to facilitate background removal in the following steps. The setup for image acquisition was placed indoors with artificial illumination using fluorescent ceiling lights. Images were captured using a 64-megapixel camera from a Xiaomi Redmi Note 8 Pro smartphone equipped with a Samsung Bright S5KGW1 sensor and stored in JPEG (Joint Photographic Experts Group) format, with a resolution of 4624 × 2136 pixels.

After capturing and storing the images, two analyses were performed. For the colour similarity analysis, histograms of the predominant colours in the images were obtained for each accession. The histograms of the respective accessions were compared by calculating the chi-square distance ($X^2$) between them, based on Equation (1):

$$X^2(H_1, H_2) = \sum \frac{\left(H_1(i) - H_2(i)\right)^2}{H_1(i) + H_2(i)} \tag{1}$$

**TABLE 1** | Accessions of ornamental pepper and their respective predominant fruit colours obtained by characterization according to the descriptors of *Capsicum* spp.

| Accession | Common name | Fruit colour at intermediate stage[a] | Fruit colour at mature stage[a] |
|---|---|---|---|
| Accession 1 | Stromboli laranja | Green | Orange |
| Accession 2 | Espaguetinho | Yellow | Orange |
| Accession 3 | Floribela | Yellow | Orange |
| Accession 6 | Pirâmide | Orange | Red |
| Accession 7 | Pirâmide negra | Dark purple | Orange |
| Accession 9 | Black pearl | Dark purple | Red |
| Accession 14 | Medusa | Orange | Red |
| Accession 18 | NI[b] | Orange | Red |
| Accession 19 | NI[b] | Orange | Red |
| Accession 20 | NI[b] | Orange | Red |
| Accession 21 | NI[b] | Green | Red |
| Accession 22 | NI[b] | Orange | Red |

[a]Classification according to IPGRI (1995).
[b]Common name not identified.

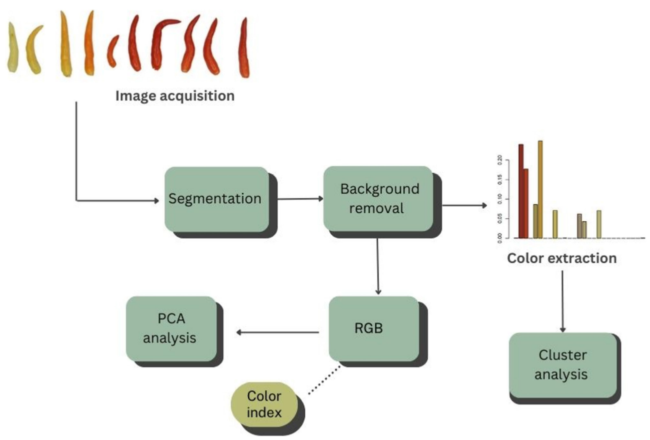


**FIGURE 1** | Process of image acquisition, segmentation and data extraction in *Capsicum* spp. fruits.

where $H_1$ and $H_2$ are the histograms of the images and the sum index $i$ indicates a compartment of the histogram (Pearson 1900; Choudhary, Prasad, and Orso 2012). The distance matrix obtained was used to represent the similarity between the accessions from the hierarchical cluster using the UPGMA method (unweighted pair-group method using arithmetic mean). Cluster fit was checked by estimating the cophenetic correlation coefficient (Sokal and Rohlf 1962).

For the second analysis, colour data were extracted. Initially, the background was removed, by extracting the RGB values, the objects of interest (pepper fruits) and the background, where the BGI was calculated and chosen, with a value of 0.78, which made it possible to generate an image in the 'Stack' format, without the background and with a 'mask'. After removing the background and isolating the fruits, RGB spectral data of the fruits were extracted according to the representative model shown in Figure 1.

In addition to the RGB values, colour values were also extracted in grayscale, calculated from the classical conversion method, using the following expression: $c = 0.29R + 0.59G + 0.11B$, as well as different colour indices (Table 2). Image analysis was performed using R software, version 4.2.2.

Colour data and the indices were then used to perform principal component analysis, processed with the covariance matrix of the original variables, obtaining from it the eigenvalues that built the eigenvectors. The accessions studied were plotted in a biplot for the first two principal components. The biplot was obtained using JMP software version 16.2.

## 3 | Results

From the extraction of the fruit colours, this work made it possible to observe the occurrence of various colour classes in each of the accessions, demonstrating the diversity of colours due to the concomitant existence of fruits at different stages of ripening (Figure 2). Colours and their frequencies vary according to accession, indicating a high phenotypic variation, which enhances the use of this species as an ornamental fruit plant.

Accessions 9 and 21 had a lower occurrence of colours, demonstrated by the presence of few classes (Figure 2f,k). Only two classes for each of these two accessions accumulated more than 70% of the frequency. Red and dark purple fruits were predominant for accession 9 and dark green and red fruits for accession 21. The other accessions had at least 5 colour classes with a relative frequency above 0.05 in the plant. It was possible to identify accessions that evolve from green to orange, such as accession 1 (Figure 2a), and accessions that showed the occurrence of fruit evolving from yellowish-green tones to orange and only then red (Figure 2b,d,i).

**TABLE 2** | Colour indices calculated from RGB (red, green and blue) values.

| Index | Formula | Reference |
|---|---|---|
| SI (Spectral Slop Saturation Index) | $\frac{R-B}{R+B}$ | Escadafal, Belghith, and Ben 1994 |
| HUE (Overall Hue Index) | $atan\left(2\times\frac{(B-G-R)}{30.5\times(G-R)}\right)$ | Escadafal, Belghith, and Ben 1994 |
| VARI (Visible Atmospherically Resistant Index) | $\frac{(G-R)}{(G+R-B)}$ | Gitelson et al. 2002 |
| BI (Bright Index) | $\sqrt{R^2+G^2+\frac{B^2}{3}}$ | Richardson and Wiegand 1977 |
| BGI (Blue Green Pigment Index) | $\frac{B}{G}$ | Zarco-Tejada et al. 2005 |
| NGRDI (Normalized Green Red Difference Index) | $\frac{G-R}{G+R}$ | Tucker 1979 |
| BIM (Brightness Index Modified) | $\sqrt{2R+2G+\frac{2B}{3}}$ | Matias, Caraza-Harter, and Endelman 2020 |
| SCI (Spore Coloration Index) | $\frac{R-G}{R+G}$ | Smith 1983 |

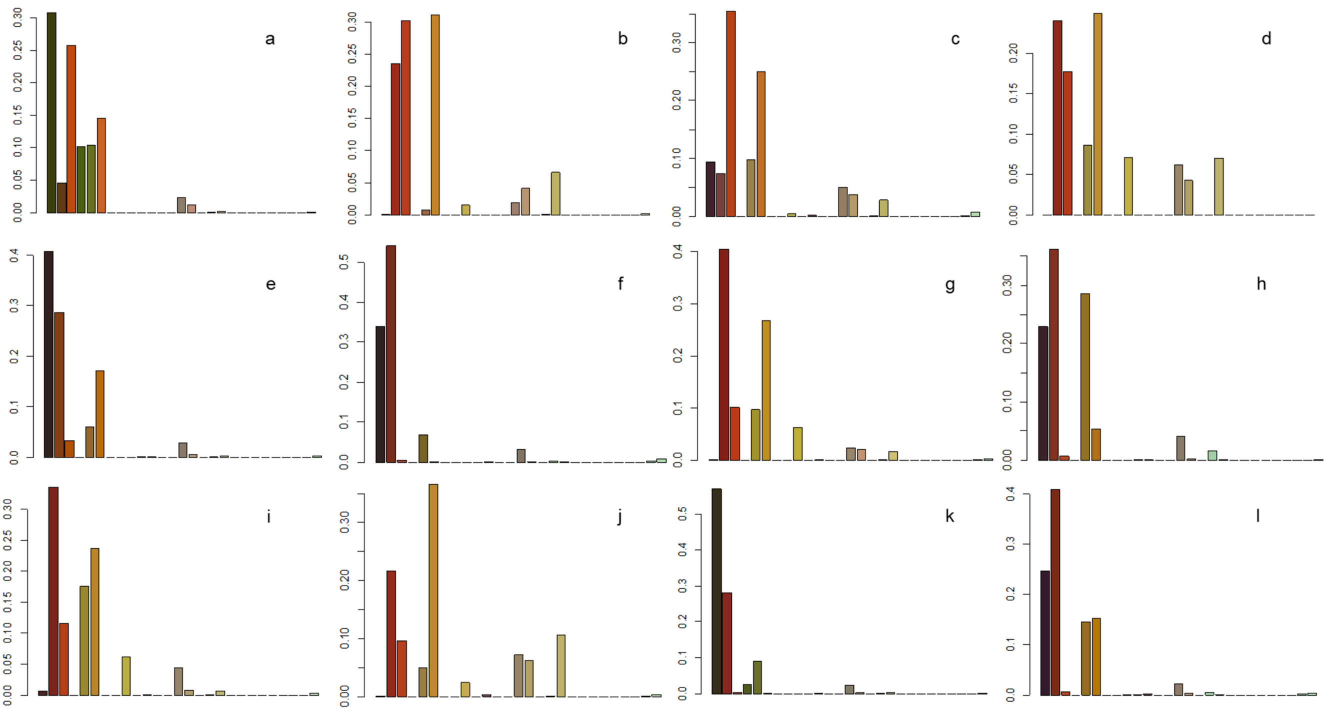


**FIGURE 2** | Relative frequency of colours extracted from images of fruits of different accessions of ornamental pepper (*Capsicum* spp.). (a) Accession 01; (b) Accession 02; (c) Accession 03; (d) Accession 6; (e) Accession 7; (f) Accession 9; (g) Accession 14; (h) Accession 18; (i) Accession 19; (j) Accession 20; (k) Accession 21; and (l) Accession 22.

The dendrogram represented the distance matrix between the accessions of ornamental pepper plants with cophenetic correlation of 0.811, indicating a good fit of the cluster. It was possible to observe the formation of three main clusters (Figure 3), showing variability among the accessions for fruit colour.

Cluster I gathers accessions 18, 22, 7, 21 and 9, which have predominance of fruits with darker shades, passing through brownish shades and red with lower chroma or purity (Figures 2 and 3). Possibly, these accessions show similarities in the colour of fruits during ripening, since accessions belonging to the same cluster are more similar than accessions in different clusters.

The second cluster was composed of accessions 1 and 3 (Figure 2a,c), which have in common fruits with orange shades that evolve to vivid orange but diverge for the presence of an intense green observed only in accession 1 and more pinkish and brown shades, present only in accession 3. Probably, the orange colour in the fruits had a greater discriminatory power in the formation of this cluster.

Accessions 14, 19, 2, 20 and 6, which form the third cluster, are characterized by fruits that predominantly do not contain anthocyanin, that is, more frequent shades of purple (Figure 2b,d,g,i,j, respectively). Fruits of these accessions have lighter shades, which range from light green, with pink shades, yellow and

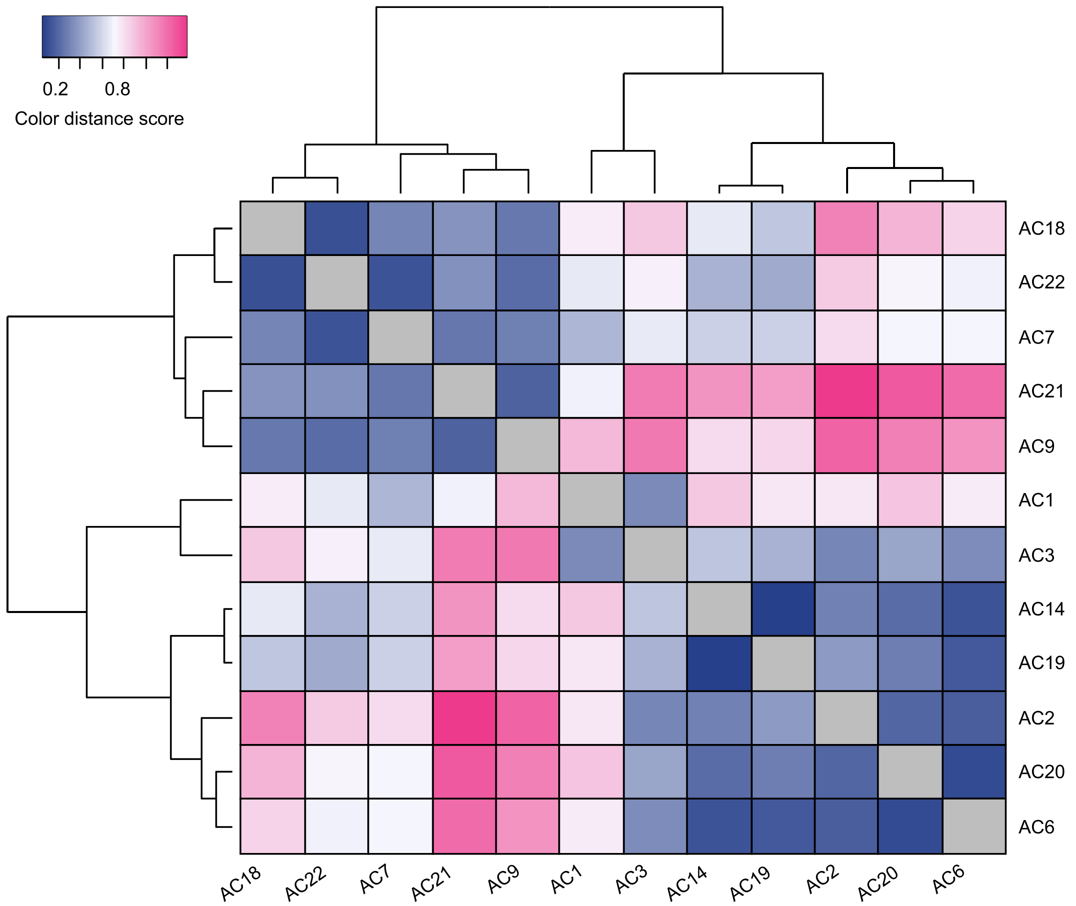


**FIGURE 3** | Dendrogram and heatmap representing the clustering of ornamental pepper (*Capsicum* spp.) accessions.

orange, to more intense and pure shades of red. Pepper fruit colours, obtained by means of images, have discriminatory power in the identification of divergent accessions.

The heatmap reveals that accessions 2 and 21 are the most divergent. Accession 2 (Figure 2b) does not have fruits with dark colours such as brown, purple, or dark green, which is very characteristic of accession 21 (Figure 2k). Both accessions can be selected within the breeding program to meet specific demands of the consumer market.

The conversion of colours into RGB channels, and shades of grey, and the obtaining of the colour indices (Table 2) allowed the accessions to be grouped in the two-dimensional plane according to the first two principal components (Figure 4). These explained 83.2% of the variation found among the pepper accessions studied.

The variables Red, Green, and shades of grey, as well as the BIM and BI indices showed higher absolute values of eigenvectors associated with principal component 1, with association of accessions 2, 6, 14, 19 and 20 (Figure 4). The colour system concentrated in red and green indicates fruits with lighter colour, without the presence of anthocyanin, as shown in Figure 3. On the other hand, the VARI, NGDI, HUE, SCI and BGI indices had a greater weight associated with Component 2. The accessions are more uniform for the SI and Blue parameters, justified by the low contribution of these in the components used.

Accessions 7, 9, 18, 21 and 22 have lower scores for RGB. So the accessions mentioned are characterized by the presence of dark colours. On the other hand, accessions 2, 6 and 20, with the highest scores for RGB, have a predominance of yellow colour (Figure 1). The VARI and NGRDI indices are strongly associated with accession 1, indicating a greater number of classes with different shades of green (Figure 2a). This index can therefore be used in the genetic breeding of ornamental peppers to identify accessions that begin their ripening process and remain green for longer.

In the opposite quadrant, accession 22 stands out with the highest association with the BGI, indicating shades ranging from brown to red. These shades can be observed in Figure 2l for accession 22 and also occur in accessions 7, 9, 18 and 21, also associated with the BGI in the lower left quadrant of the biplot. The BGI can be used to identify pepper fruits with the occurrence of brown and red shades. The other indices are highly correlated with the RGB vectors, used for their estimation, or are not strongly associated with any of the accessions.

## 4 | Discussion

The classification of accessions within the ornamental pepper breeding program considers only the colour of fruits at the intermediate and mature stages identified by a single colour (Table 1), forming only two classes for each accession. It

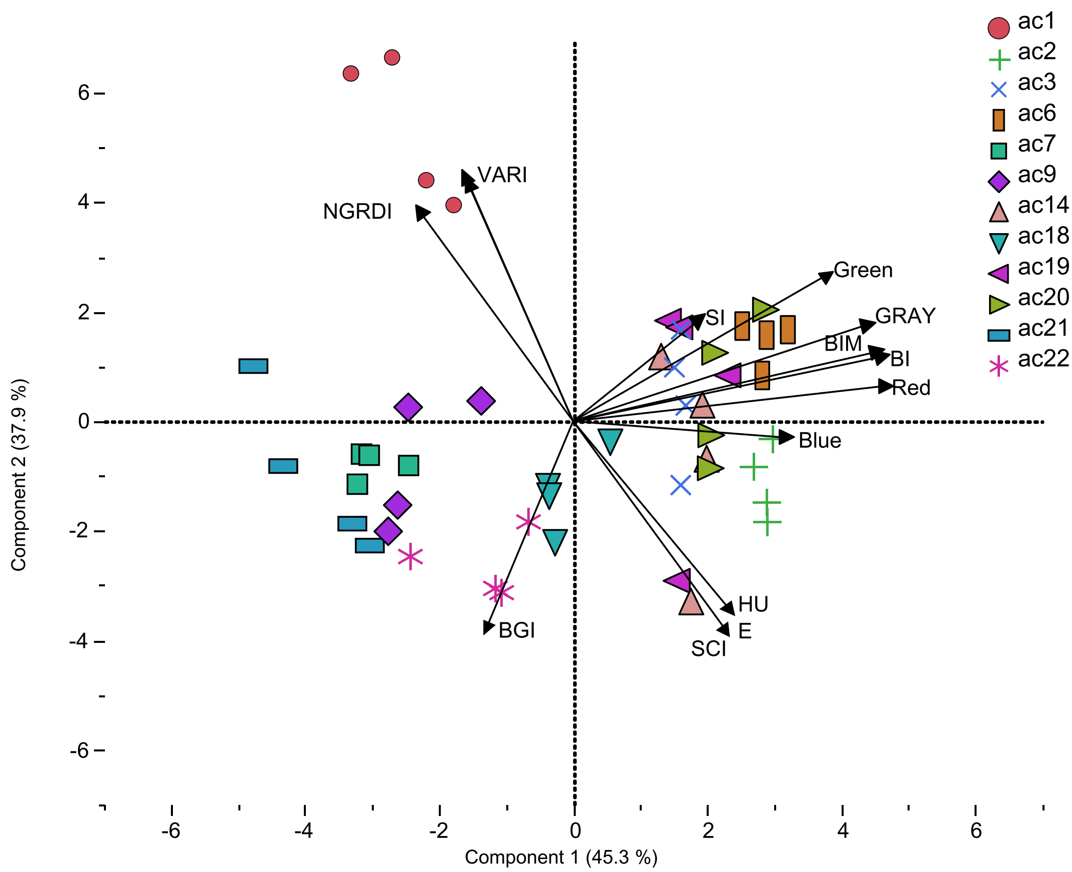


**FIGURE 4** | Biplot with the projection of the accessions in the two principal components referring to the red, green and blue colour system and colour indices of fruits of ornamental pepper (*Capsicum* spp.). BGI: Blue Green Pigment Index; BI: Bright Index; BIM: Brightness Index Modified; HUE: Overall Hue Index; NGRDI: Normalized Green Red Difference Index; SCI: Spore Coloration Index; SI: Spectral Slop Saturation Index; VARI: Visible Atmospherically Resistant Index.

is known that fruits do not show uniform colour during the maturation process, due to the accumulation and differential degradation of pigments (Delgado-Vargas and Paredes-Lopez 2002; De La Cruz-Ricardez et al. 2020). Asynchronous flowering in pepper plants over a longer flowering period (Deyto and Cervancia 2022) allows us to find several stages of development in the same plant, and as a consequence several colour classes, as seen in Figure 2. In this way, the conventional classification adopted in breeding programs using descriptors such as IPGRI, despite being simple and non-destructive (Table 1), does not represent the phenotypic diversity for fruit colour in ornamental pepper plants.

The use of image analysis in fruit colour phenotyping allowed the conversion of qualitative traits into quantitative ones, which provides valuable information for the characterization, recording and identification of variations, eliminating the subjectivity of colour determination (Dantas et al. 2023). Silva et al. (2020) evaluated qualitative traits in 11 genotypes of ornamental pepper plants and reported only three phenotypic classes for fruit colour. In this study, although a smaller number of accessions were used, it was possible to identify several colours in different shades, demonstrating the efficiency of this technique in identifying variability for fruit colour in pepper plants and facilitating the selection of contrasting accessions for genetic breeding.

Accessions that show more colours during ripening may be preferred in the consumer market (Figure 5), so attention is drawn to the accessions with the highest occurrence of classes in different colours and shades (accessions 3, 6, 18, 19 and 22). Accessions 9 and 21 may suit a specific consumer market, with a taste for monochrome (Figure 5a), as they have fewer colour classes and remain on the plant for longer with the fruit of the same colour, with a uniform contrast with the foliage.

Clustering the dendrogram made it possible to identify accessions with similar patterns of colour occurrence. Cluster 1 brought together accessions with purple classes, characteristic of the vast accumulation of anthocyanins in unripe fruits (Lightbourn et al. 2008), which was not observed for the accessions in cluster 3. The frequency of orange colour in at least two classes (Figure 2) contributes to grouping accessions 1 and 3 into cluster 2. Within each group, it is possible to identify subgroups due to the colours that occur less frequently but contribute aesthetically to the ornamental value. Accessions 2 and 19 are in the same cluster characterized by yellow-green, orange and red fruit, but differ in class frequency due to the purity for each of the colours.

The association between the images will help in the selection of accessions of ornamental interest in a shorter period and in a more reliable way, as observed by De Jesus et al. (2022) when


**FIGURE 5** | Phenotypic diversity for fruit colour in ornamental pepper trees and contrast with foliage. (a) Accession 07 shows fewer colour classes and less contrast with foliage; (b and c) Accessions 6 and 22, respectively, with a greater number of classes with different colours and tones contrasting with foliage. Bar: 16 cm.

evaluating passion fruit peel colour by images. The cluster with 99 fruits from the passion fruit germplasm bank showed wide variability, overcoming the simplification of classes normally present in the use of descriptors, with a clear separation between fruits with yellow and purple peel, and the subclusters formed due to the shade of the epicarp. On the other hand, these same authors demonstrated that the analysis based on the descriptors led to the formation of only three clusters, despite the existence of a wide range of peel colours. Possibly, the predominance of fruit colour is considered in the clustering to identify the most similar accessions.

Using the principal components, it was possible to reduce the initial set of data into a few components without any significant loss of information. The first two or three components should explain more than 80% of the total variation in the data evaluated to carry out the selection (Cruz, Regazzi, and Carneiro 2012).

The RGB colour system made it possible to classify the accessions in terms of fruit colour based on three primary components: red (R), green (G) and blue (B). The R, G and B components correspond to the intensity of red, green and blue light, respectively, with values ranging from 0 to 255 (Tsuchiyama and Matsushima 2017). A value of 0 represents darkness (black), while a maximum value indicates high intensity or luminosity (Bueger and Burge 2009; Oliveira et al. 2021). The combination of these three components allows for the formation of various colour possibilities (Rosa et al. 2022), as observed in the diversity of colours in pepper fruits. Dantas et al. (2023) associated the yellow colour of fava landrace seeds with RGB values close to white and seeds with red and black colour with shades of black, demonstrating that the colour parameters of the RGB system are important for genetic divergence studies in breeding programs.

Colour indices take advantage of colour properties to relate to various phenotypic and physiological patterns. Vegetation indices such as VARI and NGRDI, often correlate with the estimates of plant biomass and chlorophyll concentration (Pazhanivelan et al. 2023). In this study, they had a strong association with accession 1 (Figure 4), which has a predominance of green colour classes, which may not be aesthetically interesting because it does not contrast with the foliage. High BGI values indicated a greater occurrence of exposed areas of soil and plant dry matter, thus reflecting the reddish-brown hues (Zarco-Tejada et al. 2005; Oldoni et al. 2013). These shades were present in accession 22, which was strongly associated with the BGI.

The results obtained from the PCA analysis, using all channels and colour indices, when compared to the hierarchical clustering, which only used data from colour extraction, are almost completely in agreement. The three optimal clusters identified by the dendrogram (Figure 3) also tend to group together in the 2D plane defined by the two principal components (Figure 4), with the only exception of accessions 1 and 3. Nevertheless, this fact is in agreement with the small distances of accession 3 to all the accessions in the bottom cluster (14, 19, 2, 20 and 6) that can be observed in Figure 3.

Human identification of colours is quite complex where sensations such as brightness and intensity modify the perception of primary colours and their combinations, which means that in many cases, the definition of colours is a matter of subjective interpretation (López Camelo and Gómez 2004). Subjective interpretation can lead to an erroneous assessment of the diversity of colours existing in a germplasm bank, generally underestimating the colour diversity. More accurate identification of accessions and heterotic groups for colour using computer vision can be used to create commercial cultivars with new patterns to diversify the ornamental market. The methodology used in this study, in addition to being practical and easy, attempted to maintain the quality of the RGB data by using a standardization for images with a white background and indoor environment, since RGB images are easily affected by changes in lighting in the field (Sunoj et al. 2018; Cao et al. 2021). Future studies focusing on identifying colours to study diversity in the plant itself, seeking to avoid increasing identification costs, can benefit from the developed method making it accessible to a greater number of research projects.

## 5 | Conclusion

An RGB image-based low-cost method for objective assessment of fruit colour diversity of accessions has been proposed showing great potential to accurately identify accessions with suitable properties for the ornamental market. Dendrogram analysis made it possible to optimally group accessions into three clusters, with a cophenetic correlation coefficient of 0.811, sharing the accessions within each cluster a very high similarity in patterns of fruit colours. Moreover, using principal components analysis showed that, instead of requiring the use of several vegetations indexes together with RGB channels, the first two components were sufficient to explain up to 83.2% of the variance for the employed dataset. The VARI and NGRDI vegetation indices were efficient in discriminating fruits with a predominance of green colour, and the BGI could be used to discriminate accessions with predominantly reddish-brown fruits. As future line of research following the steps of this study, it seems of importance to evaluate separately the behaviour of fruit colours during ripening process instead of studying the aggregated behaviour along all the ripening stages together.

### Author Contributions

**Marcos Bruno da Costa Santos:** investigation, writing – original draft, data curation. **Raylson de Sá Melo:** data curation, writing – review and editing, formal analysis, validation, visualization. **Victor Eduardo de Carvalho Sousa:** data curation, investigation, writing – original draft. **Artur Mendes Medeiros:** conceptualization, writing – review and editing, supervision. **Angela Maria dos Santos Pessoa:** supervision, writing – review and editing. **Silvokleio da Costa Silva:** conceptualization, writing – original draft. **Antonia Maiara Marques do Nascimento:** writing – review and editing, methodology, supervision. **Priscila Alves Barroso:** conceptualization, funding acquisition, writing – original draft, methodology, formal analysis, project administration, supervision, resources.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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