RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (1) 313-323, 2026
http://doi.org/10.54910/sabrao2026.58.1.29
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PHYSIOLOGICAL AND COLOR MORPHOLOGICAL VARIATIONS IN CHILI (*CAPSICUM* SP.) SPECIES UNDER UNCONTROLLED ENVIRONMENT

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SUMMARY

Maintaining the freshness of chili (*Capsicum* sp.) fruits is a major challenge under uncontrolled environmental conditions. This research aimed to investigate the response of various chili species to postharvest physiological and color variations. Chili fruits reached harvest at 80% maturity before storage in an ambient room temperature (25 °C–27 °C) with relative humidity (RH 60%–70%) for 0, 3, 6, 9, and 12 days. For assessing fruit quality, the evaluation of fruit weight loss (%), texture (Kgf), total soluble solids (%), and pericarp thickness (mm) ensued. The color variation determination comprised values of lightness, red-green coordinate, yellow-blue coordinate, hue angle, and chroma value. The genetic makeup of genotypes influenced the physiological quality and color of chili fruit during storage. Genotypes with high adaptation to the existing environment showed a smaller reduction in fruit quality traits. The stored ripe chili revealed a change in color brightness and sharpness. The chili genotype Katokon appeared with the highest level of adaptation to an uncontrollable environment, while the genotype Pesona was the least adapted. These two chili genotypes would be ideal for genetic studies related to long shelf life. The next challenge could relate to the interspecific crossing between the genotypes Katokon (*C. chinense*) and Pesona (*C. annuum*).

Keywords: Chili (*Capsicum* sp.), species, fruit freshness, fruit quality traits, weight loss, firmness, pericarp thickness, total soluble solids, color variations

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: June 14, 2025; Accepted: December 07, 2025.

Citation: Perdani AY, Wahyuni S, Heryanto R, Syukur M, Wahyuni Y, Ritonga AW, Maharijaya A, Hartati (2026). Physiological and color morphological variations in chili (*Capsicum* sp.) species under uncontrolled environment. *SABRAO J. Breed. Genet. 58 (1) 313-323. http://doi.org/10.54910/sabrao2026.58.1.29*.

Key findings: In chili (*Capsicum* sp.) genotypes, the use of physiological and color traits is rare to evaluate their diversity. Instead, using the phenotypic markers, such as plant height and fruit yield, is common. Determining the level of adaptation in chili fruits to the environment may be possible using physiological and color traits as an alternative method.

INTRODUCTION

Chili (*Capsicum* sp.) growing prevails in tropical, subtropical, and temperate regions worldwide, and in Indonesia, chili is also a major spice crop. The chili demand is increasing in both local and international markets (Glodjinon *et al*., 2021). However, maintaining its quality, freshness, and shelf life after harvest is difficult in tropical countries like Indonesia. The quality's deterioration leads to several types of postharvest damage, including aging, dehydration, physiological disorders, mechanical injury, and microbial infection, which may occur at various stages, from harvest to consumption (Sharma *et al*., 2024). Chili pepper sales are usually indirect to consumers, as they require being transported first from production areas to distant vegetable markets (Kaban *et al*., 2021). During this transportation process, many possibilities may occur, such as temperature fluctuations, vibration, prolonged handling, and physical damage, all of which can further reduce fruit quality.

The farmer-wholesaler-retailer-consumer market chain is the most common in chili peppers. However, the lengthy process is solvable during the peak production stage when farmers decide to store their vegetable harvest for short periods to avoid selling out. Long-distance transport also causes weight loss and loss of freshness, firmness, and discoloration, and storage at 22 °C accelerates these quality trait variations over 12 days (Al-Dairi *et al*., 2021). During storage and transportation, the freshly harvested crops require low temperatures and high relative humidity (Cao *et al*., 2019). Hence, it is vital to reduce temperature and increase relative humidity to maintain the product's quality during storage and further transportation. However, most cooling machines are not affordable for smallholding farmers. The shelf life of fresh chili peppers has an estimate of 2–3 days (Kassim *et al*., 2020). Fresh chili peppers lose much water content fast after harvest and appear to shrink and change fruit color within a few days (Glodjinon *et al*., 2021). Although the pungency of chili peppers is primarily identifiable by genetically controlled capsaicinoids levels, studies have shown that high storage temperatures can gradually reduce capsaicin levels during long-term storage (Schweiggert *et al*., 2006). Evidence on the effects of short-term storage and relative humidity on capsaicin levels in fresh chili peppers is scarce; however, inappropriate temperature and humidity conditions may indirectly affect consumer perception of pungency by accelerating tissue dehydration and degradation, rather than directly reducing capsaicin.

Fresh chilies are highly perishable horticultural products and are naturally susceptible to microbial spoilage, as well as physiological and biochemical damage, which together contribute to their shorter shelf life (Sharma *et al*., 2024). During storage, the damage to chili peppers can result from respiration, and the said process continues until harvest. The respiration metabolism can cause loss in moisture content and weight, as well as a decline in chili fruit vigor. The respiration rate and the proportion of different respiration pathways affect the shelf life of postharvest horticultural crops, including chilies (Bovi *et al*., 2018; Lufu *et al*., 2024). Increased temperature also eventually influences metabolic reactions. Increased temperature causes aging due to faster respiration, enzymatic processes, and changes in sensory properties such as hardness and fruit color (Liu *et al*., 2024). Relative humidity (RH) is a major factor in the transpiration rate, and the RH increased from 76% to 96% during storage leading to a decrease in transpiration (83.5%) at 5 °C (Caleb *et al*., 2014).

The chili fruits' shelf life mostly receives alterations from genetic factors, which can be visible from the fruits' morphological and physiological characteristics. Various factors contribute to fruit deterioration during postharvest handling and can reduce the shelf life of chili peppers intended for fresh consumption. These include mechanical damage, the presence of insects and diseases, loss of moisture content, exposure to extreme temperatures, physiological disorders, and loss in dry matter caused by respiration (Bovi *et al*., 2018; Lufu *et al*., 2024). Therefore, identification of the factors that cause fruit deterioration is necessary to ensure shelf life. These factors are important to understand what causes a decrease in fruit weight, which can be due to loss of moisture content, which makes the chili fruits wilt and shrink. These environmental conditions can also affect the color and brightness of the fruits. Color is an essential factor for consumers when choosing a product, and changes in color can alter their preferences. Therefore, understanding how different chili species respond visually and physiologically after harvest is crucial for improving postharvest handling and supply chain management. The presented study aimed to evaluate the postharvest physiological and color changes of different *Capsicum* species under short-term storage. The goal is to identify which chili pepper species maintain better quality and longer shelf life in response to postharvest physiological and color changes.

MATERIALS AND METHODS

Genetic material

The experiment commenced at the Greenhouse Research Lab of the Research and Innovation Agency-BRIN, Bogor, Indonesia, where environmental conditions' monitoring could take place throughout the study. The genetic material comprised 23 chili (*Capsicum* spp.) genotypes belonging to four different species, i.e., *C. frutescens* (two genotypes), *C. chinense* (six genotypes), *C. baccatum* (two genotypes), and *C. annuum* (13 genotypes), as described in Table 1. The arrangement of genotype treatments and storage time levels transpired in a randomized complete block design (RCBD) with factorial arrangement and three replications. The first factor was the 23 chili genotypes, while the second factor was storage time levels (0, 3, 6, 9, and 12 days). Harvesting continued when the chili fruits reached 80% maturity. The basis of chili fruit selection relied on size uniformity and health, with no damages. The cleaned fruits obtained regular arrangement on plastic trays before their storage at ambient temperatures (26 °C–27 °C) and relative humidity (60%–70%). Daily recording of temperature and relative humidity used a calibrated digital thermo-hygrometer placed inside the storage room.

Physiological variation analysis

Fruit weight loss

In each chili genotype, fruits arranged on a plastic tray totaled 10. The fruits sustained storage at room temperature (26 °C–27 °C) and RH (60%–70%). The measurement of fruit weight loss ensued by weighing fruit samples at different storage periods (0, 3, 6, 9, and 12 days) using a digital balance with an accuracy of ±0.01. Moreover, calculating the fruit weight loss followed the formula of Haile (2018).

$$\text{Fruit weight loss (\%)} = \frac{\text{Initial Weight-Final Weight}}{\text{Initial Weight}} \text{x } 100$$

Fruit texture

The chili fruits' texture and hardness assessment continued using the Penethrometer Force Gauge PCE-FM20 tool following the manufacturer's protocol with a maximum load of 2 kg. The hardness test measurement depended on the level of product resistance to the piercing needle of the tool. Three chili fruit samples attained random selection in each treatment. Each chili measurement had three points: the basal, center, and tip of the fruit. Expressing the measurement results was in Kgf (kilogram-force), where 1 Kgf = 9.81 N (Newton) and conversely 1 N = 0.10197 Kgf. The thickness of the chili fruits' pericarp

Table 1. Genetic material used in the study.

No.	Genotype	Species	Category	S.No.	Genotype	Species	Category
G1	Bonita	*C. frutescens*	Cayenne	G13	Hot Banana	*C. annuum*	Bell pepper
G2	ORI 212	*C. frutescens*	Cayenne	G14	Viola	*C. annuum*	Cayenne
G3	Red Bhut Jolokia	*C. chinense*	Cayenne	G15	Arisa	*C. annuum*	Chili pepper
G4	Peach Chupetinho	*C. chinense*	Ornamental	G16	Adelina	*C. annuum*	Chili pepper
G5	Red Chupetinho	*C. chinense*	Ornamental	G17	Fish Paper	*C. annuum*	Chili pepper
G6	Habanero Fransia	*C. chinense*	Bell pepper	G18	Anies	*C. annuum*	Chili pepper
G7	Red Habanero	*C. chinense*	Bell pepper	G19	SSP	*C. annuum*	Chili pepper
G8	Katokon	*C. chinense*	Bell pepper	G20	Pesona	*C. annuum*	Chili pepper
G9	Bishop Crown	*C. baccatum*	Chili pepper	G21	Seloka	*C. annuum*	Chili pepper
G10	Lemon Drop	*C. baccatum*	Chili pepper	G22	Caman	*C. annuum*	Chili pepper
G11	Seroja	*C. annuum*	Cayenne	G23	Neno	*C. annuum*	Chili pepper
G12	Chenzo	*C. annuum*	Cayenne				

underwent gauging in mm using a digital caliper. The section of the chili fruit acquires assessment at the center of the fruit. Fruit hardness measurement followed previously reported for *Capsicum* spp., where firmness evaluation employed the penetration testing using a penetrometer, then expressed in Kgf (Bayogen *et al*., 2016).

Total soluble solids

For the measurement of total soluble solids (TSS), the methodology of Díaz-Pérez *et al*. (2006) was the basis. Chili juice extracts came from 15 g samples of 4–5 fruits. Pulverizing the fruits plus 10 ml of distilled water used a blender, with the homogenized samples filtered using filter paper. Taking the filter results, as much as 1–2 drops, proceeded their placement on the refractometer prism. The digital refractometer SNDWAY SW-593, as used, had ranges of measurement (0%–55% Brix), resolution (0.1% Brix), and accuracy (±0.02% Brix). The reading of the dissolved solids appeared on the scale indicated with units in percentage. The thickness of the fruit flesh was measured using digital calipers with a precision of two decimal places. The measurement results were presented in millimetres.

Color analysis

Color analysis progressed by using Colorimeter FRU WR10. The colorimeter measurements were in the form of variables: lightness (L), the red-green coordinate (a*), and the yellow-blue coordinate (b*). The three notations served to calculate hue, chroma, and whiteness index using the following equation (Kamal-eldin *et al*., 2020).

$$\text{Hue}\ (h^o) = \tan^{-1}\frac{b}{a}$$

$$\text{Chroma*}\ (C^*) = \sqrt{a^2 + b^2}$$

Data analysis

All the collected data underwent the analysis of variance (ANOVA). Differences among the various treatments and their separation took place by using the Tukey test at $p \leq 0.05$. The analysis used Minitab version 16 software (Minitab Inc., 2010).

RESULTS AND DISCUSSION

Physiological characters

The fruit quality in chili (*Capsicum* sp.) genotypes displayed significant differences depending on the genotypes and storage environments under ambient room conditions (Table 2). Each chili genotype responded differently to the environment, as indicated by the variations in physiological (weight loss, firmness, total soluble solids, and pericarp thickness) and color (lightness, red-green coordinate, yellow-blue coordinate, hue angle,

Table 2. Analysis of variance of the physiological and color traits in chili genotypes under uncontrolled environment.

Variables	Mean Squares					Coefficient of Variants
	Block	Genotype (A)	Shelf life (B)	A x B	Galat	(%)
Physiological characters						
Weight loss (%)	23.49	984.77**	24,297.98**	110.47**	17.67	16.50
Total soluble solids (%)	0.02	10.29**	8.03**	0.20**	0.03	10.03
Texture (Kgf)	0.00	0.01**	0.06**	0.01**	0.00	18.97
Pericarp thickness (mm)	0.01	1.53**	4.17**	0.07**	0.01	10.54
Color characters						
L*	1.92	1,498.11**	48.27**	9.29**	2.77	4.74
a*	5.16*	2,626.67**	10.55	18.74**	2.85	4.81
b*	9.94	2,283.43**	121.41**	72.27**	10.87	7.79
Hue°	2.94	1,770.52**	92.76**	9.36**	2.72	3.25
Chroma*	12.56	3,499.55**	72.61**	81.86**	11.19	5.99

and chroma value) characteristics (Figure 1). The principal component analysis showed the chili genotype Katokon emerged with the lowest physiological changes compared with the genotype Pesona (Figure 2). This revealed the genotype Katokon has a better level of adaptation to the uncontrolled existing environment than the other genotypes. Overall, four groups of the chili genotypes occurred in response to the environment (Figure 2). Most tested genotypes (16 genotypes) have at-par adaptation in dealing with the ambient uncontrolled environmental conditions (25 °C–27 °C; RH 60%–70%). The behavior of the genotype Pesona (G2) was similar to the cultivar Caman (G22), which has the same pattern of decline in the physiology slope.

The results further proved the weight loss of chili fruits during storage gained considerable influences from the genetic makeup of the genotypes (Figure 1). Variation occurs among the chili genotypes within the same species and among the species. The genotype Katokon was evident with the lowest water loss rate (approximately 18%), while the genotype Lemon Drop has the highest water loss (exceeding 70%), as illustrated in the weight-loss curve in Figure 1 (upper-left graph). Nearly half of the chili fruits were lost during this process and identified as a major concern. The water loss is due to a combination of factors, including the fruit's respiration activity and its physical properties, which differ because of genetic variation. The production and postharvest constraints of chili fruits in tropical regions resulted from the high temperature that causes rapid water loss in fruits. After chili harvest, water loss is the major problem in fruit handling and storage, affecting quality, shelf life, and market value (Gidado *et al*., 2024).

The shelf life of fresh chili fruits received adverse effects from high temperature, followed by continued respiration and transpiration processes after harvest (Lufu *et al.,* 2024). However, chili genotypes with heavier fruit weight and thicker exocarp have lower water loss (Elibox *et al.,* 2015). This pattern was also notable in our study. Genotypes with thicker pericarp, such as Katokon, showed lower water-loss rates, whereas those with very thin pericarp, such as Lemon Drop, exhibited the highest water loss (Figure 1). This is because thicker exocarp tissues provide a stronger barrier against moisture diffusion, while heavier fruits typically have a lower surface-area-to-volume ratio, which reduces transpiration. Different chili genotypes and species have varied rates of water loss during storage at room temperature. Large tomatoes had a longer shelf life and the lowest respiration and ethylene production than the smaller fruits (Islam *et al.,* 2019).

Total soluble solids (TSS) reflect the quality of fruits and their ripening as indicated by the minerals and sugars. The findings

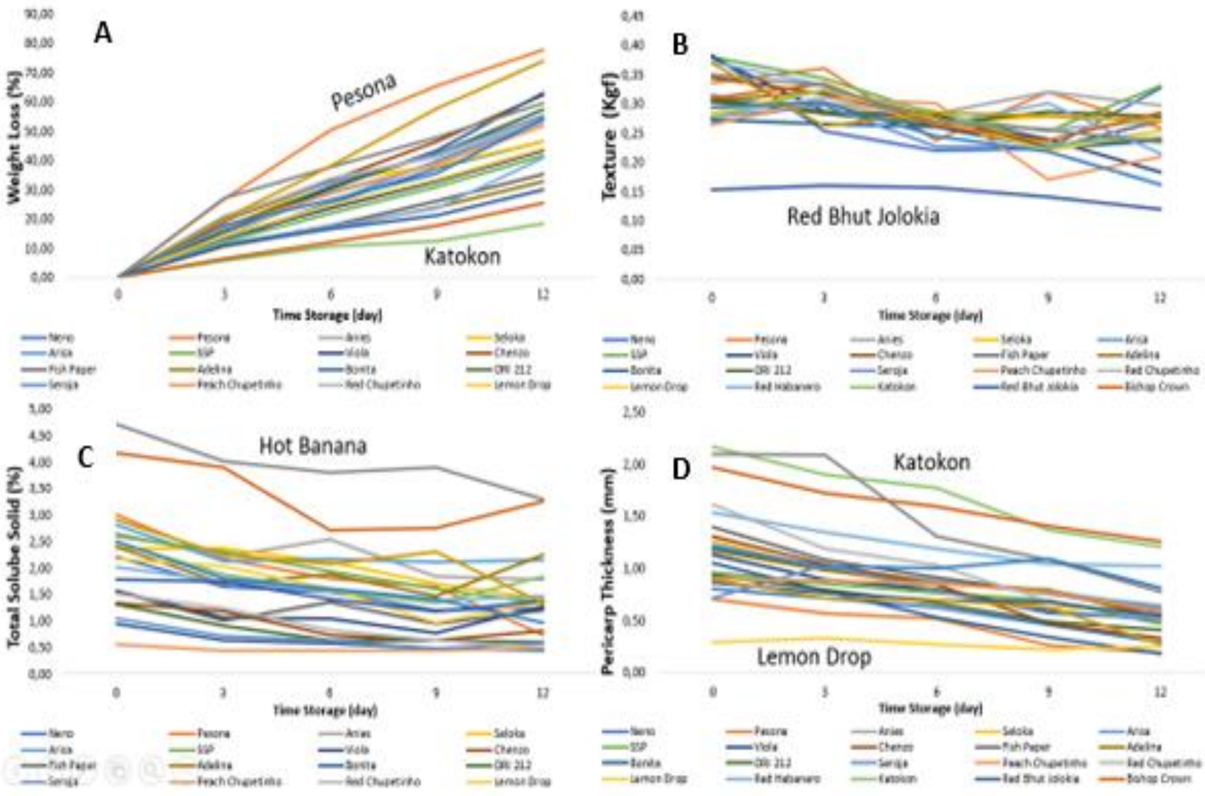

Figure 1. Trend of fruit physiological variations in chili genotypes under uncontrolled environment. Changes in chili pepper quality during storage, measured by A: percentage of fruit weight loss, B: fruit texture, C: percentage of total soluble solids, and D: fruit flesh thickness.

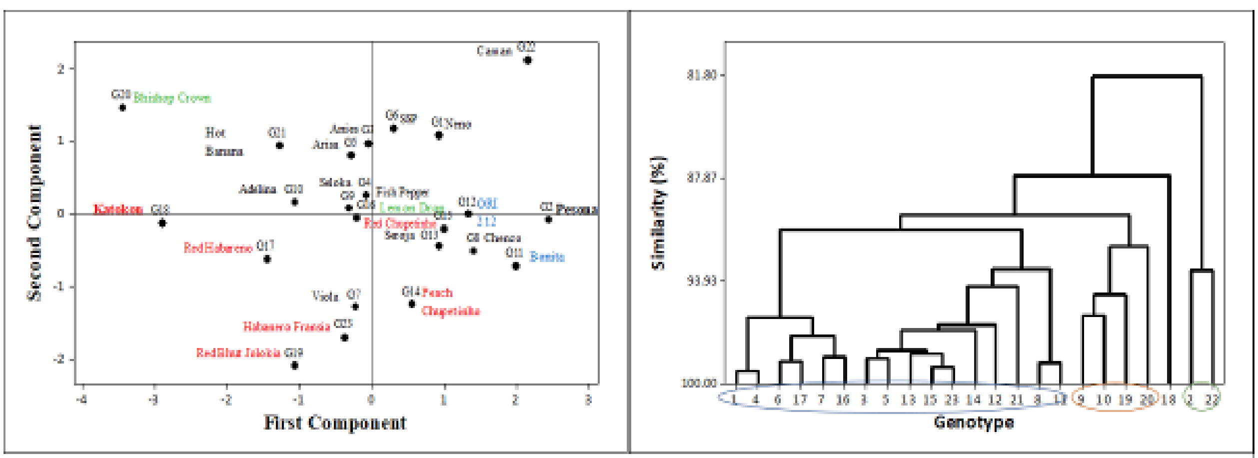

Figure 2. Principal component analysis and cluster analysis of the physiological traits in chili genotypes under uncontrolled environment.

showed total soluble solids decreased with storage period (0, 3, 6, 9, and 12 days). However, the magnitude of this decrease varied among the species and genotypes (Figure 1). Hot banana had the highest TSS content (decreasing from approximately 4.8% to 3.7%), while Peach Chupetinho had the lowest (reducing from around 1.5% to 1.0%). These results agreed with the findings of Al-Gaadi *et al*. (2024), who reported the total soluble solids in tomatoes declined during storage but increased in other fruits. Comparisons of TSS among the different chili genotypes can produce inconsistent results due to unusually high and low fruit acid levels. The total soluble solids and dry matter content of the cucumber at harvest can serve to indicate its shelf life and manage the value chain, especially to identify cultivars with a longer shelf life (Valverde-Miranda *et al.,* 2021). Total soluble phenols and capsaicin levels increased together at the temperature of 25 °C during storage (Miranda-Molina *et al.,* 2019).

The decrease in texture and firmness of chili fruits for the existing environment had the same pattern, except for the genotype Red Bhut Jolokia (Figure 1). Red Bhut Jolokia exhibited the highest firmness loss, decreasing by approximately 0.20–0.22 Kgf over 12 days. In contrast, Katokon showed the lowest decrease in firmness, with a reduction of only about 0.04 Kgf, indicating greater structural stability during storage. Fruit firmness depends on the genetic makeup of the genotypes and the storage methods. Decreased fruit firmness due to mass loss during room storage indicates senescence. Even a little loss of water can lead to undesirable variations in the texture of various fruits (Gidado *et al.,* 2024). Moisture loss caused the change in fruit firmness, as well as weight loss, which was the opposite course in tomatoes (Kibar and Sabir, 2018). During the storage period, a change in the texture of the fruit manifested due to an alteration in the composition of the cell wall, resulting in a decrease in cell turgor pressure and fruit firmness in chilies (Chitravathi *et al.,* 2015). The degree of maturity in the chili fruits was indicative of changes in fruit color, which affect the physio-morphological properties of the chili fruits. In this study, the Pesona genotype showed the highest weight loss but did not experience a notable decline in fruit firmness. This indicates firmness does not solely result from moisture loss but also from genotype-dependent factors, such as cell-wall structure, cuticle thickness, and pectin degradation rate. Thus, Pesona's ability to maintain firmness despite high weight loss reflects a structural resilience unique to this genotype.

The chili genotype Katokon has the thickest pericarp, with an initial thickness of approximately 2.3 mm, which decreased to about 1.4 mm after 12 days of storage. In contrast, the cultivar Lemon Drop has the thinnest, decreasing from roughly 1.2 mm at harvest to around 0.5 mm at the end of storage (Figure 1). Pulp volume degradation appeared to be associated with water loss that occurs during storage. It was also noticeable that the greater the water loss from the lenticels, the greater the decrease in cell wall turgidity and cell wall degradation, resulting in the cell's shrinkage and damage. Fruit firmness showed a strong association with structural parameters, except for roundness in pumpkin genotypes (Liu *et al.,* 2024). Specifically, the intercellular space rate and cell wall thickness showed a significant correlation with fruit firmness (Ma *et al.,* 2020). Pulp cell dimensions varied among the pumpkin cultivars, and they significantly increased during storage. The round, tightly packed, and compact cultivar of pumpkin proved to be resistant to storage conditions (Liu *et al.,* 2024).

From the analysis of variance of the physiological and color traits in chili genotypes under uncontrolled environments, physiological characteristics, viz., weight loss, TSS, texture, and pericarp thickness, achieved significant effects from genotype, shelf life, and their interaction (Table 2). These results indicate the performance of each genotype differs over time, and the physiological responses of the fruits during storage express a close relation to both their genetic makeup and the duration of storage. Similarly, color traits (L*, a*, b*, hue, and chroma*) attained remarkable influences from genotype, shelf life, and their interaction, suggesting a potential relationship between physiological changes and color variations in chili fruits during postharvest storage.

Color characters

Color is a quality factor affecting chili appearance, as determined by pigmentation. Fresh fruit's appearance is its most important attribute, influencing its shelf life. The quality of fresh chili fruits is dependent on appearance, firmness, flavor, and nutritional values (Stavang *et al.,* 2015). Variations in color and sensitivity to storage conditions, especially during the late storage period, were common in citrus fruits. Numerous factors impact the appearance, from wound effects to drying and microbial colonization. The higher transportation temperature and being transported over long distances showed the most substantial change in color of Ponkan oranges (Cao *et al.,* 2018) and tomatoes (Al-Dairi *et al.,* 2021).

In general, the presented research showed most chili genotypes had similar color change patterns under the uncontrolled environment (Figure 3). At the end of the observations, most genotypes produced duller and darker colors, except the genotype Lemon Drop, which appeared brighter. Out of the 23 tested chili genotypes, the genotype Red Bhut Jolokia showed the highest response to storage environment; the fruit color appeared redder and sharper with a clear yellowish tone (Figure 3). Color, which is crucial to consumer perceptions of food quality, is one of the vital attributes used in evaluating food products (Stavang *et al.,* 2015). Color significantly influences consumer behavior. It affects how they perceive other senses, as influenced by several factors, some of which are modified by genetic factors and pre- and post-harvest treatments.

Chili fruits contain naturally occurring pigments that enhance their color. Varying color stages at harvest impact the fruit and spice coloration but not pungency in chili (Krajayklang *et al.,* 2000). Tomato’s total color differences (L*, a*, and b* values) emerged to be statistically significant due to factors such as transportation, storage temperature, and duration (Al-Dairi *et al.,* 2021). During storage, the fruit peel changed from yellow-green to orange and orange-yellow, and the a* value remained consistent across the three storage modes during the entire period in *C. reticulata* (Cao *et al.,* 2018).

Overall, out of the 23 chili genotypes tested, three groups of genotypes succeeded in their formation with different responses to uncontrolled environmental conditions (Figure 4). Genotypes totaling 17 had similar responses from the species *C. annuum* and *C. frutescens*. However, the species *C. baccatum* and *C. chinense* resulted in two different groups (Figure 4). In chili genotypes, the loss of smooth glossy appearance, color change, and carotenoid accumulation showed varied responses during storage (Sharma *et al.,* 2024). The hue (h) decreased, suggesting a change in superficial color from reddish to dark red. Chroma increased slightly during storage, regardless of temperature. The hue and color index values occurred to have considerable influences from the investigated factors, including transportation distance, storage temperature, and storage duration (Al-Dairi *et al.,* 2021). Color variations in Shine Muscat fruit during low-humidity storage indicate a link between color and water loss (Watanabe *et al.,* 2018). The fruit’s weight loss significantly affects the fruit's mechanical and chromatic properties.

CONCLUSIONS

The chili (*Capsicum* sp.) quality was significantly dependent on the uncontrolled storage environment, as indicated by physiological and color characteristics, such as weight loss, firmness, TTS, and pericarp thickness. Likewise, their quality is reliant on color attributes, including lightness, hue angle, and chroma. Each chili genotype responds differently to the storage environment, with the most stable performance generally observed between 0 and 6 days, when physiological traits interacted more consistently across genotypes. This proves the physiological expression and chili fruit color

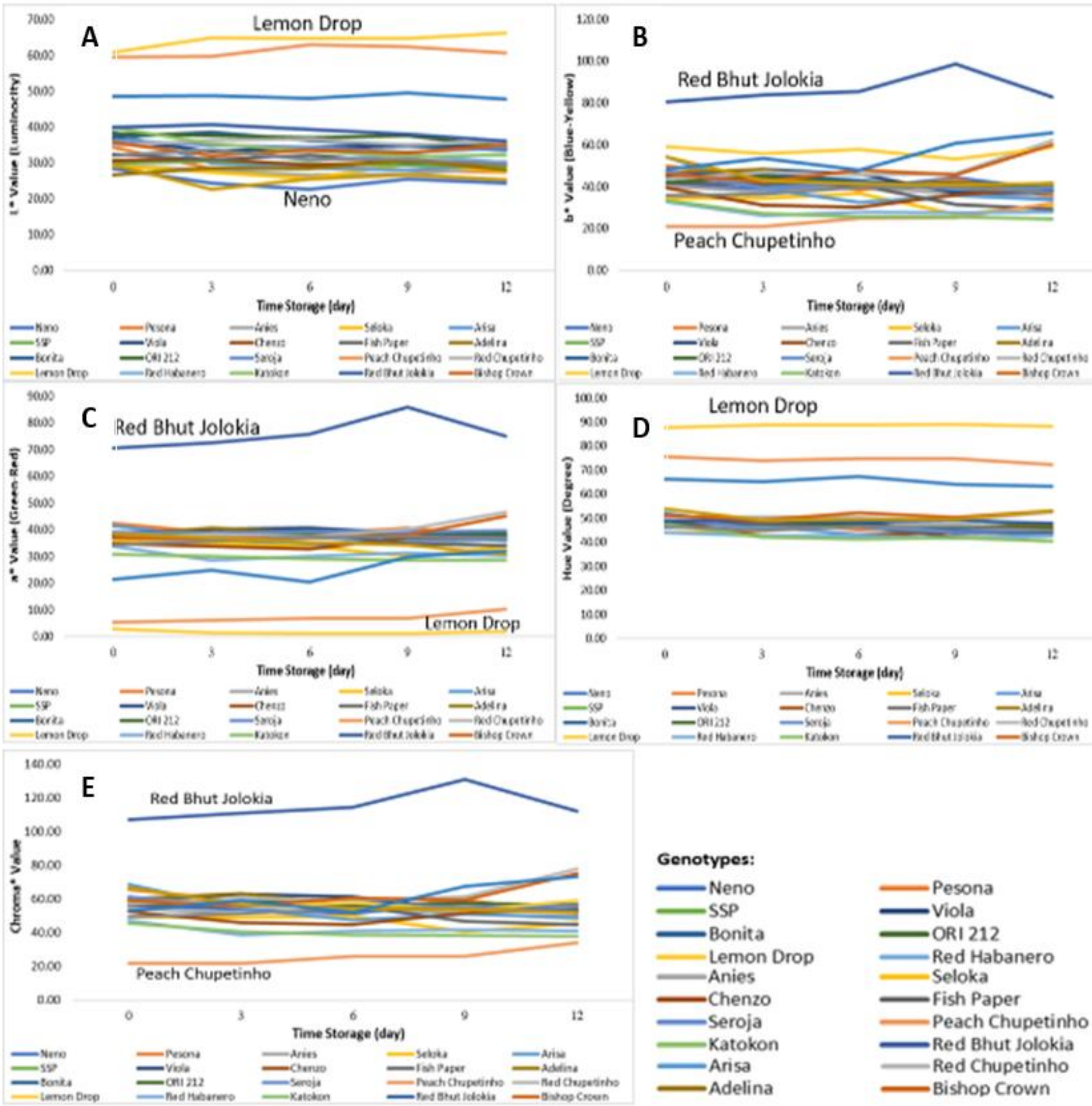

Figure 3. Trend of fruit color change in chili genotypes under uncontrolled environment. The color quality of chili peppers during storage measured by A: lightness (L*), B: blue-yellow, C: green-red, D: hue degree, and E: color intensity (chroma).

against the storage environment incurred influences from genetic factors, as seen in the clear differences in weight loss progression, firmness decline, and pericarp reduction among genotypes. The chili genotype Katokon (*C. chinense*) was an adaptable genotype to the uncontrolled environment, as indicated by low physiological variations (relatively stable weight loss, minor firmness reduction, and slower pericarp thinning), while the genotype Pesona (*C. annuum*) was the opposite. Both genotypes were suitable for use as cross parents for storage resistance studies. However, the intraspecific crossing will be a great challenge.

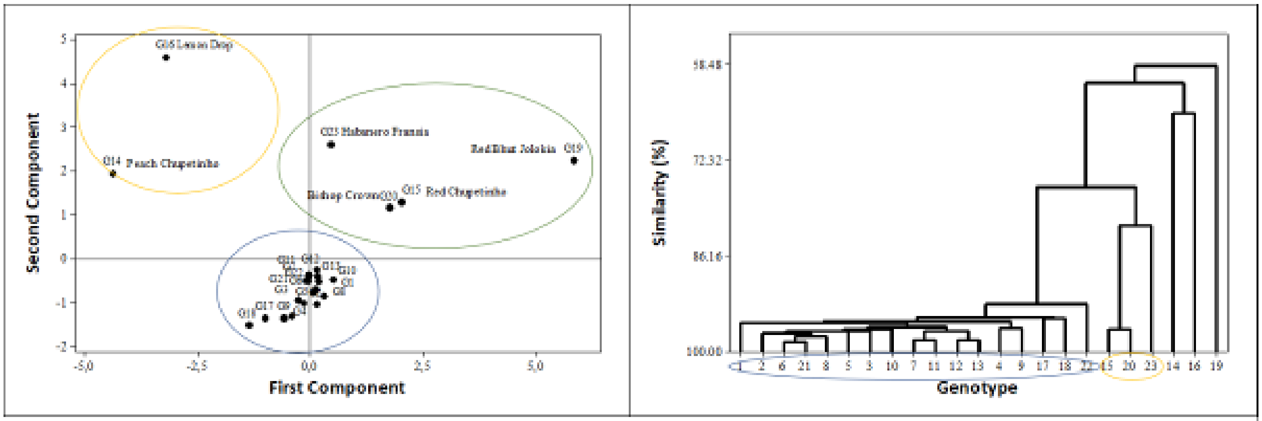

Figure 4. Principal component analysis (left) and cluster analysis (right) of the color quality in chili genotypes under uncontrolled environment.

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