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ARTICLE

Anthocyanin Profile and Color Diversity in Paeonia: From Phytochemical Analysis to Aesthetic Preferences

Abel Bala[1], Monica Hârţa[1,*], Doina Clapa[1], Floricuţa Ranga[2], Doina Angela Pui[3] and Mirela Irina Cordea[1,*]

[1]Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania
[2]Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania
[3]Al. Borza Botanical Garden, University of Babeş-Bolyai Cluj-Napoca, Cluj-Napoca, Romania
*Corresponding Authors: Monica Hârţa. Email: monica.harta@usamvcluj.ro; Mirela Irina Cordea. Email: mcordea@usamvcluj.ro

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ABSTRACT: This study presents an interdisciplinary approach to the chromatic diversity of the genus Paeonia, combining quantitative colorimetric analysis (CIELab and RHS systems) with biochemical profiling of anthocyanins across 14 genotypes. The results indicate a correlation between the biochemical composition of the petals and their visual appearance, suggesting that the intensity and distribution of certain pigments—mainly cyanidin and peonidin derivatives—determine the hue and saturation of the flowers. HPLC analysis revealed quantitative variation in pigmentation: dark-red genotypes showed high levels of anthocyanins (e.g., P. tenuifolia 'Flore-Plena'), whereas these compounds were not detected in yellow genotypes. In parallel, the evaluation of aesthetic preferences revealed a public bias toward pink shades and bicolor variants, highlighting the importance of shape and fragrance in the perception of ornamental quality. By correlating analytical data with visual attractiveness factors, the study provides relevant information for future selection strategies aimed at developing varieties that align phytochemical characteristics with the requirements of the ornamental market.

KEYWORDS: Peony; genotypes; anthocyanins; CIELab color; consumer preferences

1 Introduction

Ornamental plants have been domesticated and improved mainly for their aesthetic appeal, with flower color being one of the most important visual traits that influence public perception and cultural preferences [1]. In this context, flower color is a key indicator of ornamental quality and attractiveness, mainly determined by the type, distribution, and concentration of pigments within the petals [2]. It is also closely linked to cellular and biochemical factors that ultimately affect the petals' visual appearance [3]. Floral pigmentation primarily results from uneven accumulation of flavonoids, especially the balance between anthocyanins and anthoxanthins (flavones, flavonols, and chalcones), which directly influences the color variation observed in many ornamental species [4]. Among these compounds, anthocyanins are the primary pigments responsible for petal color, and variations in their composition and concentration lead to noticeable changes in hue and intensity [5].

Flower color is a complex phenotypic trait influenced by interactions among the spectrum of light reflected by the petals, the observer's visual system, and illumination conditions [6,7]. Additionally,

human color perception is affected by factors such as brightness, intensity, and saturation, which add a subjective element to color assessment [8]. These limitations are also encountered when using chromatic charts (e.g., RHS color charts), emphasizing the need for quantitative, standardized, and reproducible methods [9]. Therefore, using the CIELAB (L*, a*, b*) colorimetric system in flower color analysis can be a valuable tool [10,11]. The L* value indicates lightness, while a* and b* measure the chromatic components along the green–red and blue–yellow axes, respectively. This system allows comparisons of genotypes' flower-petal colors and their chromatic parameters [12]. However, the biochemical composition and pigment concentrations remain important factors in color discrimination among flowering ornamental plants [13]. Among ornamental plants, the peony is one of the most popular flowering species worldwide [14–18]. From a botanical point of view, the genus *Paeonia* (Paeoniaceae) is divided into three primary sections—section Moutan, sect. Onaepia, and sect. Paeonia—and includes 32 species and 26 subspecies worldwide [19]. It is highly valued for its ornamental qualities, which derive from floral features such as shape, color, blooming period, and fragrance [20]. Herbaceous and tree peonies come in a wide range of colors, reflecting significant phenotypic diversity [21,22]. Flower color is also a key factor in determining ornamental quality and is often used as a basis for peony classification. After a long period of artificial selection, the peony has developed nine common color categories among native species, including white, green, yellow, orange, red, pink, purple, blackish-red, and double- or multi-color [20,23].

In peony petals (*Paeonia* spp.), six key anthocyanins have been identified, including peonidin-3,5-di-O-glucoside (Pn3G5G), pelargonidin-3,5-di-O-glucoside (Pg3G5G), cyanidin-3,5-di-O-glucoside (Cy3G5G), peonidin-3-O-glucoside (Pn3G), cyanidin-3-O-glucoside, and pelargonidin-3-O-glucoside (Pg3G), all directly linked to flower pigmentation [24]. Overall, flower color is determined by several main pigment groups, including flavonoids, carotenoids, chlorophylls, and betalains. Anthocyanins typically produce red, blue, and violet shades. Carotenoids add yellow–orange hues, while flavonols and flavanones may produce white–yellow tones or alter the overall color appearance through interactions with anthocyanins [25].

To our knowledge, research combining color parameter analysis with biochemical profiling of petal extracts remains limited. There is also a lack of statistical data on the public's preferences for peony flower color and associated floral traits in Romania, where the peony holds significant symbolic meaning for human well-being. In this context, the current study aimed to compare 14 peony genotypes by evaluating petal color differences with colorimetric tools and analyzing the biochemical profiles of petal extracts. Additionally, the study aimed to investigate public preferences in Romania for peony flower color, fragrance, and shape, given the species' symbolic role as the national flower. This was accomplished using four Paeonia cultivars with different representative petal colors—bicolor, red, pink, and yellow—and variations in flower shape and fragrance. Respondents were also asked to explain their reasons for preferring specific flower colors.

2 Materials and Methods

2.1 Plant Material

Flowers from fourteen *Paeonia* accessions (Table 1) were collected in 2025 from "Alexandru Borza" Botanical Garden, Babeș-Bolyai University (UBB), Cluj-Napoca, Romania (46.762539° N, 23.588516° E; 46°45′45.1″ N, 23°35′18.7″ E). Due to constraints related to plant material, specifically the limited availability of flowering material at the time of analysis, eight petals were manually detached from fully bloomed flowers on a single representative plant per genotype, and the measurements were treated as technical

replicates. A subset of three petals/peony genotype was used for color evaluation, while the remaining plant material was immediately frozen and stored at −80°C until biochemical analysis.

Table 1: Characteristics of the *Paeonia* accessions' flowers used in this study.

Species/Cultivar	Sample Code	Flower Type	Flower Color*
Paeonia sp. 'Cora Louise'	P1	semi-double	White with purple center
Paeonia sp. 'Pastel Splendor'	P2	semi-double	Cream-pink with purple flares
Paeonia officinalis ssp. Villosa	P3	single	Deep pink
Paeonia peregrina	P4	single	Red
Paeonia suffruticosa	P5	double/semi-double	Pink to white, with purple basal patches
Paeonia tenuifolia 'Flore-Plena'	P6	double	Red
Paeonia lactiflora 'Sorbet'	P7	double	Pink, cream and white layered
Paeonia sp. 'Hillary'	P8	semi-double	Raspberry pink flowers fading to pale pink and cream
Paeonia sp. 'Pink Hawaiian Coral'	P9	semi-double	Coral-pink
Paeonia delavayi var. *Lutea*	P10	single	Yellow
Paeonia sp. 'Bartzella'	P11	large double	Yellow
Paeonia anomala	P12	single	Pink to magenta
Paeonia sp. 'Alexander Fleming'	P13	double	Pink
Paeonia sp. 'Callie's Memory'	P14	semi-double	Cream-yellow with purple center

Note: *Flower characteristics were recorded according to data provided by the American Peony Society (APS) [26].

2.2 Flower Color Assessment

Flower color was evaluated through visual inspection and digital image analysis. First, the flower colors of each sampled flower (three petals per genotype) were compared to the Royal Horticultural Society (RHS) Colour Chart to identify the RHS color codes and UCL (universal color language) names. Observations were made at full bloom under natural daylight around midday to reduce color distortion. Then, digital image analysis was conducted using Fiji (ImageJ 1.54p, National Institute of Health, USA) software [27,28] for quantitative color characterization of analyzed petals. High-resolution images were examined, and for each petal, three representative regions were selected: the proximal (P), median (M), and distal (D) areas of the petal surface. In each region, a square region of interest (ROI) measuring 15 × 15 pixels was defined on visually uniform sections of the petal, avoiding margins, veins, shadows, or reflections that could influence color measurements. The average values of the red (R), green (G), and blue (B) color channels were obtained using the "Color Histogram" feature in Fiji (ImageJ 1.54p). The RGB values were then converted into CIELAB coordinates using the ColorMine online tool (http://colormine.org), assuming the standard sRGB color space and D65 illumination conditions. To ensure consistency and reduce subjectivity, ROI selection followed a standardized protocol, with regions selected from comparable areas of each petal (proximal, median, distal), using an identical ROI size and avoiding structural features such as veins, edges, shadows, and reflective areas. All measurements were performed by the same operator under identical visualization conditions [29]. To facilitate visual comparison of flower colors across samples, color swatches were created using the corresponding hexRGB codes and integrated into the results table as pattern images. The color of each analyzed peony genotype was labeled using the RHS code, its corresponding hexRGB value, and the UCL name from the RHS color classification.

The mean values of the L*, a*, and b* for each analyzed part of the petal (P, M and D) were then used for cluster analysis to evaluate the relationships between genotypes based on their petal colors.

2.3 Anthocyanin Profiling by HPLC-DAD-ESI-MS

2.3.1 Anthocyanin Extraction

For anthocyanin extraction, 1 g of homogenized petal sample was mixed with 5 mL of methanol containing 1% (v/v) HCl (37%). The mixture was vortexed for 1 min (Heidolph Reax Top), sonicated for 15 min (Elmasonic E 15 H), and centrifuged at 10,000 rpm for 10 min at 40°C (Eppendorf AG 5804). The supernatant was collected, and the extraction procedure was repeated until the plant material became colorless. The combined extracts were concentrated to dryness under reduced pressure using a rotary evaporator (Heidolph Hei-Vap Expert) and stored at −20°C until HPLC analysis. Prior to injection, the dried extracts were reconstituted in 2 mL of methanol and filtered through a 0.45 μm nylon filter (Chromafil Xtra). An aliquot of 20 μL was injected into the HPLC system.

2.3.2 HPLC-DAD-ESI-MS Conditions

Chromatographic separation was performed using an Agilent 1200 HPLC system equipped with a quaternary pump, solvent degasser, autosampler, and diode-array detector (DAD), coupled to a single quadrupole mass spectrometer (Agilent 6110; Agilent Technologies, CA, USA). Separation was achieved on a Kinetex XB C18 column (4.6 × 150 mm, 5 μm; Phenomenex, USA). The mobile phases consisted of (A) water with 0.1% acetic acid and (B) acetonitrile with 0.1% acetic acid. The gradient program (expressed as %B) was as follows: 0 min, 5% B; 0–2 min, 5% B; 2–18 min, 5–40% B; 18–20 min, 40–90% B; 20–24 min, 90% B; 24–25 min, 90–5% B; and 25–30 min, 5% B. The column temperature was maintained at 25°C, and the flow rate was 0.5 mL/min. Spectral data were recorded in the 200–600 nm range, and chromatograms were monitored at 520 nm.

Mass spectrometric detection was performed in positive electrospray ionization (ESI+) mode, using full-scan acquisition under the following conditions: capillary voltage 3000 V, drying gas temperature 350°C, nitrogen flow rate 7 L/min, collision energy 100 eV, and m/z scan range 120–1200. Data acquisition and processing were conducted using Agilent ChemStation software (version B.02.01 SR2). Identification of anthocyanins was based on the combined use of chromatographic retention behavior, characteristic UV-Vis absorption maxima at 510–540 nm, the protonated molecular ion $[M]^+$ obtained by LC-ESI-MS, and comparison with authentic standards and previously published mass spectrometric data for Paeonia anthocyanins [24,30].

2.3.3 Chemical Standards and Calibration Curve

Acetonitrile (HPLC grade; Merck, Germany) and ultrapure water (Direct-Q UV; Millipore, USA) were used throughout the analysis. Cyanidin standard (>99% purity; Sigma, USA) was employed for calibration. Anthocyanin quantification was performed using a cyanidin calibration curve (R^2 = 0.9951).

2.4 Public Preference Survey

A public preference survey was conducted to assess Romanian consumers‘ perceptions and preferences regarding peony flower colors and flower color associated with other important characteristics such as fragrance and flower shape. The original high-resolution images of four representative peony genotypes (white-purple *Paeonia sp.* ‘Cora Louise’; pink-*Paeonia sp.* ‘Alexander Fleming’; red-*Paeonia peregrina*; and yellow-*Paeonia* sp. ‘Bartzella’) were included in the questionnaire and sent to the consumers for a preference survey. The questionnaire was specifically created for this study and distributed online from December 2025 to February 2026. Participation was voluntary and anonymous. The survey gathered sociodemographic

data, including gender, age group, and occupation. The respondents were asked to select their preference peony color: (bicolor ex. White-purple, pink, yellow, or red), fragrance and flower shape and to indicate their main reason for favoring a specific color (visual appeal, elegance, tradition, personal taste, or symbolic meaning). Additionally, fragrance and flower shape were included in this public preference survey. A total of 302 valid responses were collected and included in the subsequent statistical analysis.

2.5 Correlations between Data Sets

To investigate the relationships between pigment accumulation and flower color, Spearman rank correlation analyses were performed between total anthocyanin content and the CIELab parameters (L*, a*, and b*) measured in the proximal, median, and distal petal regions across all 14 peony genotypes. In addition, exploratory Spearman rank correlation analyses were conducted on the four flower color categories from the consumer survey to assess relationships between consumer preference and measured colorimetric and phytochemical traits. Consumer preference was expressed as the percentage of respondents selecting each flower color category. These preference percentages were correlated with the CIELab parameters (L*, a*, and b*) measured in the proximal, median, and distal petal regions, as well as with the total anthocyanin content of the corresponding representative genotypes.

2.6 Data Analysis

The mean values of the L, a, and b color parameters were used to create UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrograms for the proximal (P), median (M), and distal (D) areas of the petal in the analyzed peony genotypes. These dendrograms were generated using software (Paleontological Statistics (PAST) Version 4.11, Natural History Museum, University of Oslo, Norway) [31] and are based on the Euclidean similarity coefficient to explore the relationships between genotypes based on their petal colors.

Biochemical analyses were performed using three independent biological replicates per genotype, and results are presented as mean ± standard error (SE). Data were evaluated for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used as an exploratory approach because all groups had equal sample sizes and low within-group variability. Statistical significance was set at $p < 0.05$.

Survey responses were examined using descriptive statistics to summarize response frequencies and percentages. The relationships between categorical variables were tested with the Chi-square (χ^2) test of independence. When expected frequencies between data were low, Fisher's exact test was used. Statistical significance was set at $p < 0.05$.

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3 Results

3.1 Flower Color Assessment

Representative genotypes of the genus Paeonia were chosen to highlight the color diversity of the floral materials in this study (Fig. 1). As shown in Fig. 1, the selected genotypes included both botanical species and horticultural cultivars, demonstrating significant variation in flower color.

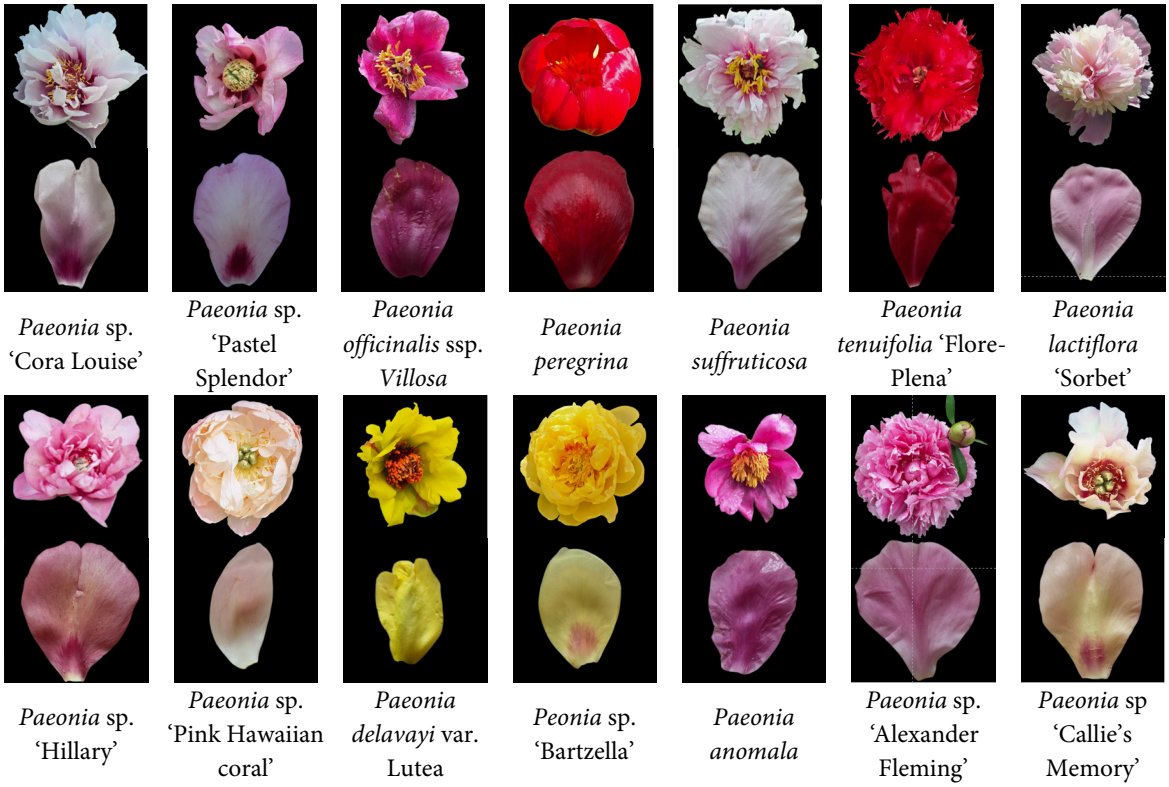


Figure 1: The analyzed peony genotypes illustrating the color diversity used in this study.

The analysis of color variations along the proximal (P), median (M), and distal (D) axes across 14 peony genotypes (P1-P14) shows how pigments are distributed within the petals, reflecting their genetic diversity. As shown in Table 2, the analysis of color reveals genotype-specific chromatic patterns. For example, in some genotypes (P1, P2), distinct contrasts between these regions are seen, while in other peony genotypes (P4, P6, P10 and P13), a more uniform petal color is dominant. Analyzing the data for the 14 genotypes reveals a remarkable diversity in how pigments are distributed on the petal surface. This variation makes the petal a visual indicator of the genetic complexity of peonies. Additionally, using a digital method like color pattern analysis can eliminate errors from subjective visual observation and allow for objective monitoring of color changes, making it more accurate than traditional RHS chart visual observation.

Table 2: Flower color characteristics of 14 analyzed peony genotypes.

Sample Code	Sample Name	RHS Code	hexRGB Code	UCL Name	Pattern Color Image
P1	*Paeonia* sp. 'Cora Louise'	187A (P) 186D (M) 202 (D)	#3c0d21 (P) #927d86 (M) #a9a99f (D)	Dark red (P) Moderate Purplish Pink (M) Light Greenish Grey (D)	 P M D
P2	*Paeonia* sp. 'Pastel Splendor'	187A (P) 186D (M) 84B (D)	#3a0314 (P) #8a818c (M) #8c7aa9 (D)	Dark red (P) Moderate Purplish Pink (M) Light Purple (D)	 P M D
P3	*Paeonia officinalis* ssp. Vilosa	187B (P) 185C (M) 67B (D)	#3f101e (P) #813259 (M) #923e5d (D)	Deep Purplish Red (P) Moderate Purplish Red (M) Vivid Purplish Red (D)	 P M D
P4	*Paeonia peregrina*	183D (P) 183B (M) 45A (D)	#683c37 (P) #570209 (M) #7c1020 (D)	Moderate Red (P) Dark Red (M) Vivid Red (D)	 P M D
P5	*Paeonia suffruticosa*	N77C (P) N187C 201C (D)	#7b5f76 (P) #9f9ca1 (M) #abacac (D)	Greyish Reddish Purple (P) Purplish Grey (M) Reddish Grey (D)	 P M D
P6	*Paeonia tenuifolia* 'Flore-Plena'	187A (P) 183B (M) 45A (D)	#47020c (P) #4f010a (M) #900d23 (D)	Dark Red (P) Dark Red (M) Vivid Red (D)	 P M D
P7	*Paeonia. lactiflora* 'Sorbet'	62D (P) 70B (M) 62B (D)	#bbafb4 (P) #987089 (M) #ccb0c5 (D)	Pale Purplish Pink (P) Strong Reddish Purple (M) Moderate Purple Pink (D)	 P M D
P8	*Paeonia* sp. 'Hillary'	67A (P) 177D (M) 59D (D)	#a6435e (P) #ac7b6c (M) #a05e6e (D)	Strong Purplish Red (P) Greyish Reddish Orange (M) Strong Purplish Red (D)	 P M D

Table 2: *Cont.*

Sample Code	Sample Name	RHS Code	hexRGB Code	UCL Name	Pattern Color Image
P9	*Paeonia* sp. 'Pink Hawaiian Coral'	202D (P) 177C (M) 68D (D)	#bbc3b6 (P) #9a705c (M) #a28783 (D)	Light Greenish Grey (P) Greyish Reddish Orange (M) Light Purplish Red (D)	 P M D
P10	*Paeonia delavayi* var. lutea	152C (P) 151B (M) 12B (D)	#725c03 (P) #a3a138 (M) #cabc36 (D)	Dark Greenish Yellow (P) Strong Greenish Yellow (M) Briliant Yellow (D)	 P M D
P11	*Paeonia* sp. 'Bartzella'	177D (P) 150C (M) 149D (D)	#b9735f (P) #b1a956 (M) #bab87d (D)	Grayish Reddish Orange (P) Brilliant Yellowish Green (M) Pale Yellowish Green (D)	 P M D
P12	*Paeonia anomala*	77C (P) 70A (M) N74B (D)	#b380ab (P) #6d2b51 (M) #aa6390 (D)	Light Purple (P) Moderate Purplish Red (M) Strong Reddish Purple (D)	 P M D
P13	*Paeonia* sp. 'Alexander Fleming'	71B (P) 71A (M) 63C (D)	#825163 (P) #813556 (M) #a98196 (D)	Strong Purplish Red (P) Deep Purplish Red (M) Strong Purplish Red (D)	 P M D
P14	*Paeonia* sp. 'Callie's Memory'	182C (P) 145C (M) 156C (D)	#8b434b (P) #b5ab74 (M) #b5ab74 (D)	Dark Pink (P) Light Yellow (M) Yellowish Gray (D)	 P M D

Regarding the variation in color name (UCL name), hybrid genotypes like P1 (‘Cora Louise’) and P2 (‘Pastel Splendor’) show notable variation: they display strong dark red in the proximal (P) area and gradually shift to neutral or light colors, such as light greenish grey or light purple, toward the distal (D) petal area. This pattern indicates that anthocyanins are concentrated at the base of the petals, forming a visible basal blotch. Conversely, in the genotype coded P10 (*P. delavayi* var. *Lutea*), the saturation gradient is reversed: the P zone appears dark yellow (Dark Greenish Yellow) and becomes more vibrant (Brilliant Yellow) toward the distal (D) area. In the case of P12 (*P. anomala*) and P13 (‘Alexander Fleming’), although these genotypes are part of the purple/red group, there is a noticeable tone-to-tone color shift from light to deep or strong red, creating a velvety, three-dimensional visual effect. In contrast, a complementary color transition was observed in P9 (‘Pink Hawaiian Coral’), with a light greenish-grey at the base of the petal (P), then shifting to coral/orange-grey hues in the middle and upper areas (M, D) of the petals. In terms of chromatic stability, P4 (*P. peregrina*) and P6 (*P. tenuifolia*) show the highest consistency. Although the UCL names for the petals differ slightly—moderate red for P4 and vivid red for P6—both peony genotypes are included in the red color spectrum along their entire petals. This indicates a uniform distribution of the pigments that give the petals their red color. P14 (‘Callie’s Memory’) displays a complex pattern in color distribution across the proximal, median, and distal regions of the petals, beginning with dark pink (P), then light yellow (M), and ending with yellowish gray (D). This pattern indicates the simultaneous presence of anthocyanins (pink) and carotenoids (yellow). In the case of P11 (‘Bartzella’), a well-known yellow peony, its proximal zone features a grayish reddish orange, which is the visual hallmark of Itoh (intersectional) hybrids. In Table 3, the mean values of CIELab parameters recorded from 3 petals per genotype are presented and used for the cluster analysis of the analyzed peony genotypes.

Table 3: Values of color space parameters (L*, a*, b*) in 14 analyzed peony genotypes.

Sample Code	L*			a*			b*		
	Proximal (P)	Median (M)	Distal (D)	Proximal (P)	Median (M)	Distal (D)	Proximal (P)	Median (M)	Distal (D)
P1	17.27	47.90	70.80	33.83	15.67	0.47	−0.02	−0.97	2.48
P2	21.27	55.62	50.87	20.63	5.57	18.80	−4.39	−4.39	−24.33
P3	19.92	26.38	39.66	30.16	39.79	38.82	−2.20	−0.52	−3.50
P4	33.85	17.51	26.05	32.70	38.35	44.58	13.05	23.00	22.99
P5	28.20	59.78	72.58	23.66	6.60	−0.07	−6.06	−3.38	−1.18
P6	10.54	18.38	31.30	29.45	39.48	50.11	12.01	22.64	21.88
P7	51.70	56.69	72.29	10.72	21.22	14.77	−0.01	−7.75	−6.16
P8	30.57	61.17	50.87	36.69	−1.93	25.14	6.08	19.99	2.62
P9	70.10	62.18	71.58	−2.14	8.41	5.28	10.92	8.82	−1.08
P10	37.98	59.03	72.13	0.02	0.88	−2.40	44.84	57.16	65.63
P11	49.87	66.37	75.50	25.63	−8.10	−8.80	17.63	42.37	25.17
P12	39.96	53.98	45.28	25.74	20.25	37.38	−3.63	7.55	−9.38
P13	45.12	47.07	60.25	17.65	27.97	14.18	−1.12	−7.39	−3.55
P14	39.96	68.09	68.50	25.09	−5.45	3.79	13.11	26.64	12.21

The dendrogram that grouping the 14 peony genotypes based on the similarity of their color values (L, a, b) across all three petal areas (P, M, D) is shown in Fig. 2.

Regarding the relationships among peony genotypes based on their chromatic parameters, two main clusters (A and B) are observed in the dendrogram, with P10 (*Paeonia delavayi* var. *Lutea*) considered an outlier due to its highest b* values across all analyzed petal areas (44.84, 57.16, and 65.63), even though most varieties have near-zero or negative b* values. P10 appears yellow across the entire petal surface. Cluster A

(with light/pastel color) included the majority of the peony genotypes. As can be seen in Fig. 2, subcluster 1 grouped genotypes with medium values of luminosity (L*) and moderate variation of color in P, M and D areas of petals (P5, P1, P7, P13, P8, P12, P2). These genotypes are classified together because they exhibit high brightness values (L* > 60) and low color saturation. Subcluster 2 comprises genotypes (P9, P11, and P14) that exhibit high luminosity values in the M and D regions of the petals (62.18, 71.58; 66.37, 75.50; 68.09, 68.50). Notably, P9 (*Paeonia* sp. 'Pink Hawaiian Coral') recorded a negative a* value (−2.14) at its base (P), which imparts a subtle green-cream hue that slightly differentiates it from P11 (*Peonia* sp. 'Bartzella') and P14 (*Paeonia* sp. 'Callie's Memory') genotypes.

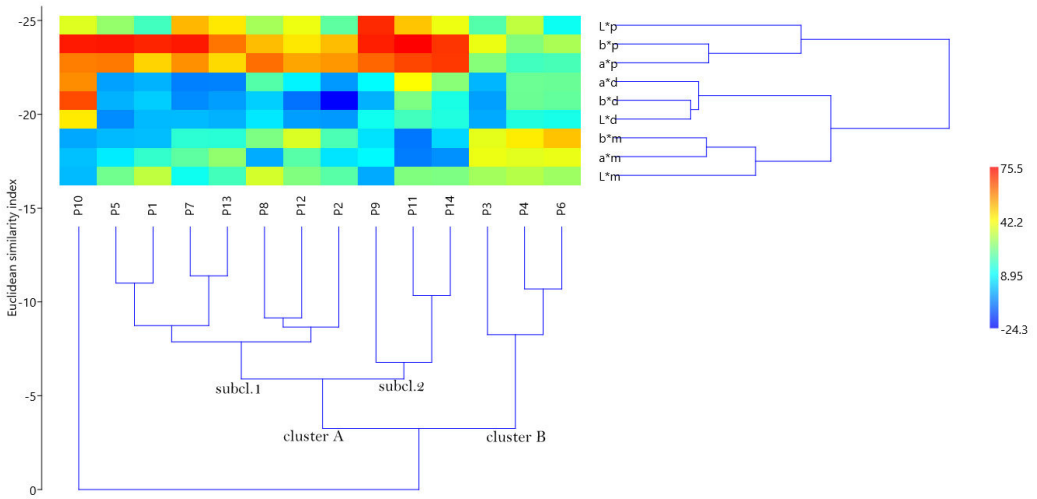


Figure 2: Two-way UPGMA dendrogram based on the Euclidean similarity index showing the cluster analysis of 14 peony genotypes; main clusters (A; B); subclusters (subcl 1 and subcl 2); P1-P14 coded genotypes; L*, a*, b*-color space parameters; p-proximal, m-median, and d-distal areas of petals.

As shown in Fig. 2, main cluster B of the UPGMA dendrogram included the saturated red-colored peony genotypes (P3, P4, P6). In cluster B, P4 and P6 show the highest a* values for the D region of the petal (44.58 and 50.11, respectively) and b* values for M/D (both above 20). These parameters suggest a very intense warm red at the petal's edge. Although the P3 genotype has an a* value over 30 (indicating red), it has negative b* values, producing a purplish/cool red hue, which explains why P3 stands alone in the dendrogram alongside P4 and P6. These results suggest that peony genotypes in Cluster B are useful for generating vibrant colors, characterized by consistent hues (with minimal brightness variation) and peak saturation of the red pigment.

3.2 Anthocyanin Profile of Paeonia Petals by HPLC-DAD-ESI-MS

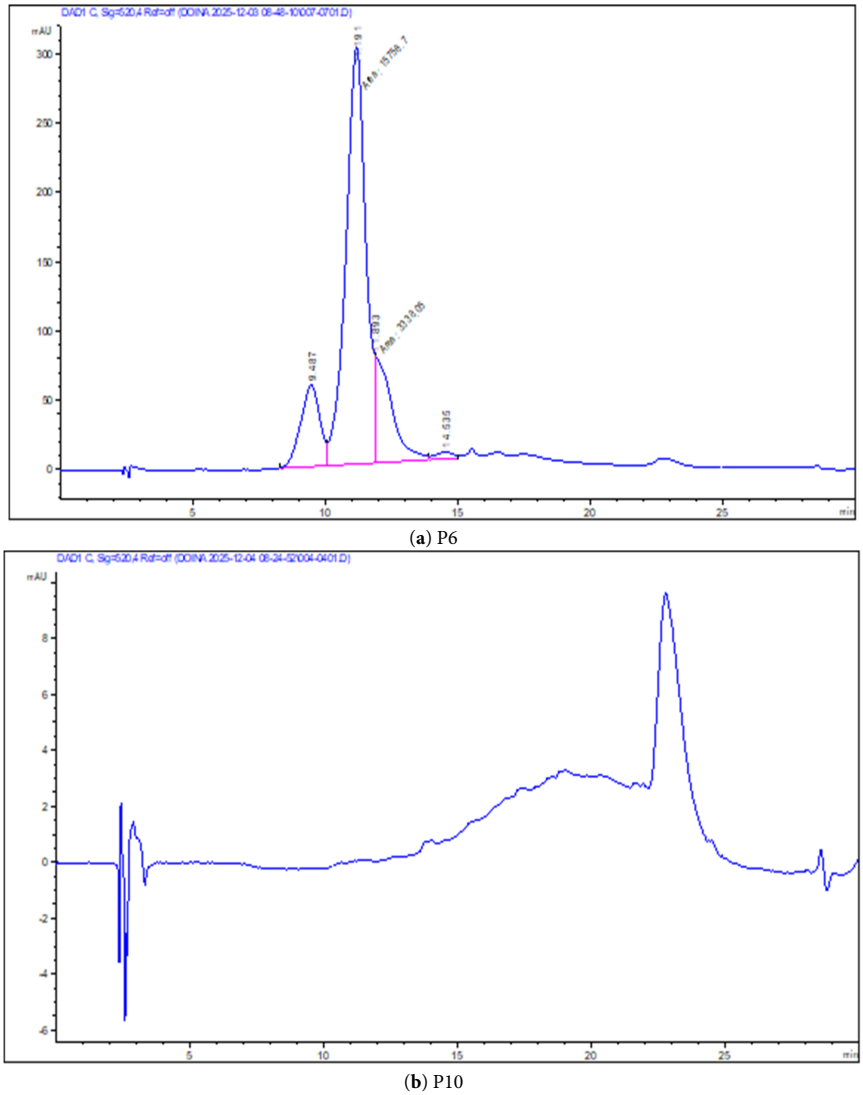
The anthocyanin composition of the analyzed *Paeonia* petal extracts was examined using HPLC-DAD-ESI-MS. The analysis identified several major anthocyanin compounds that contribute to the petals' color and appearance pigmentation.

As shown in Table 4, five anthocyanins were detected and identified based on their retention times and molecular ions $[M+H]^+$. These included cyanidin-diglucoside, peonidin-diglucoside, cyanidin-glucoside, peonidin-glucoside, and pelargonidin-diglucoside. The detected compounds belong to three main classes of anthocyanidins, which are commonly associated with red, pink, and purple pigmentation in ornamental flowers.

Representative chromatograms obtained at 520 nm are shown in Fig. 3, illustrating the separation of the identified anthocyanins in the analyzed petal extracts. The chromatographic profiles revealed noticeable differences in the relative abundance of detected compounds among the samples. After qualitatively identifying the anthocyanins, their quantitative content in the petal samples was measured.

Table 4: Retention times (RT) and $[M+H]^+$ values of the anthocyanins identified in *Paeonia* petal samples.

Peak No.	RT (min)	$[M+H]^+$ (m/z)	Anthocyanin
1	9.42	611	Cyanidin-diglucoside
2	10.49	625	Peonidin-diglucoside
3	11.23	449	Cyanidin-glucoside
4	12.13	463	Peonidin-glucoside
5	13.17	595	Pelargonidin-diglucoside



(**a**) P6

(**b**) P10

Figure 3: Representative HPLC-DAD chromatograms (520 nm) of *Paeonia* petal extracts in the 8–15 min retention time range: (**a**) P6 (highest total anthocyanins) and (**b**) P10 (no detectable anthocyanins). Peaks 1–5 correspond to the identified anthocyanins listed in Table 4.

3.3 Anthocyanin Content in Paeonia Petal Samples

The quantitative analysis of anthocyanins showed significant variation among the *Paeonia* petal samples examined. The concentrations of individual anthocyanins and the total anthocyanin content are listed in Table 5 as mean values ± standard deviation (n = 3).

Table 5: Anthocyanin content in *Paeonia* petal samples (mean ± SE, n = 3).

Sample	Cyanidin-Diglucoside μg/g FW	Peonidin-Diglucoside μg/g FW	Cyanidin-Glucoside μg/g FW	Peonidin-Glucoside μg/g FW	Pelargonidin-Diglucoside μg/g FW)	Total Anthocyanins μg/g FW
P1	9.21 ± 0.03^{fg}	161.15 ± 0.25^{b}	4.36 ± 0.03^{jk}	4.34 ± 0.03^{g}	1.91 ± 0.02^{k}	180.96 ± 0.34^{c}
P2	3.90 ± 0.03^{i}	13.42 ± 0.07^{h}	5.43 ± 0.03^{ij}	4.80 ± 0.03^{g}	2.66 ± 0.02^{j}	30.21 ± 0.18^{ij}
P3	15.89 ± 0.12^{d}	29.30 ± 0.23^{e}	16.66 ± 0.14^{cd}	19.74 ± 0.16^{c}	13.42 ± 0.12^{b}	95.01 ± 0.76^{e}
P4	152.95 ± 1.30^{b}	264.49 ± 2.18^{a}	92.76 ± 0.75^{b}	88.90 ± 0.81^{b}	11.55 ± 0.11^{d}	610.64 ± 5.13^{b}
P5	3.50 ± 0.03^{i}	6.48 ± 0.04^{k}	3.33 ± 0.03^{k}	3.35 ± 0.02^{h}	3.31 ± 0.02^{i}	19.97 ± 0.16^{j}
P6	338.09 ± 2.34^{a}	262.38 ± 1.75^{a}	1530.57 ± 9.73^{a}	110.83 ± 0.87^{a}	51.41 ± 0.41^{a}	2293.28 ± 15.03^{a}
P7	11.43 ± 0.09^{e}	36.22 ± 0.29^{d}	14.53 ± 0.12^{de}	0.00 ± 0.00^{i}	12.20 ± 0.12^{c}	74.38 ± 0.58^{f}
P8	23.48 ± 0.23^{c}	67.68 ± 0.67^{c}	21.57 ± 0.21^{c}	11.75 ± 0.12^{d}	13.27 ± 0.13^{b}	137.75 ± 1.36^{d}
P9	10.60 ± 0.10^{ef}	9.40 ± 0.09^{ij}	9.00 ± 0.09^{f}	8.78 ± 0.09^{e}	8.95 ± 0.09^{f}	46.72 ± 0.46^{gh}
P10	0.00 ± 0.00^{j}	0.00 ± 0.00^{l}	0.00 ± 0.00^{l}	0.00 ± 0.00^{i}	0.00 ± 0.00^{l}	0.00 ± 0.00^{k}
P11	6.20 ± 0.06^{h}	8.27 ± 0.08^{jk}	6.05 ± 0.06^{hi}	6.67 ± 0.07^{f}	0.00 ± 0.00^{l}	27.19 ± 0.27^{ij}
P12	6.82 ± 0.07^{h}	17.29 ± 0.17^{g}	9.98 ± 0.10^{f}	8.19 ± 0.08^{e}	6.98 ± 0.07^{h}	49.27 ± 0.48^{g}
P13	9.58 ± 0.09^{ef}	24.75 ± 0.24^{f}	11.75 ± 0.12^{ef}	0.00 ± 0.00^{i}	9.54 ± 0.09^{e}	55.62 ± 0.55^{g}
P14	7.34 ± 0.07^{gh}	12.46 ± 0.12^{hi}	7.69 ± 0.08^{gh}	0.00 ± 0.00^{i}	7.73 ± 0.08^{g}	35.21 ± 0.35^{hi}

Note: Different letters within the same column indicate significant differences among samples (one-way ANOVA followed by Tukey's HSD post-hoc test, $p < 0.05$).

Differences were found in the accumulation of cyanidin-, peonidin-, and pelargonidin-derived compounds across the analyzed samples, reflecting differences in pigmentation intensity. Some genotypes exhibited higher levels of certain anthocyanins, while others had a more even distribution of the detected compounds, as shown in Table 5.

3.4 Public Preferences for the Color, Fragrance and Shape of Peony Flowers

Besides the biochemical analysis of petal pigments, this study also assessed public perception of peony flower colors and other important traits such as fragrance and flower shape. A total of 302 valid responses were collected through an online questionnaire and included in the statistical analysis. The sociodemographic characteristics of the respondents are summarized in Table 6.

Table 6: Sociodemographic characteristics and survey responses of participants (N = 302).

Sociodemographic Characteristic	Category	n	%
Gender			
	Male	88	29.1
	Female	214	70.9
Age			
Group 1	≤25 years	167	55.3
Group 2	≥26 years	135	44.7

Most participants were female (70.9%), while male respondents accounted for 29.1% of the sample. The largest groups of respondents were in the group 1 ≤ 25 age (55.3%), with group 2 being less represented

(44.7%), as shown in Table 6. Participants reported a variety of occupations, including education and research, healthcare, public administration, private sector work, and freelance or independent professions (data not shown). Respondents' preferences for flower color, along with other traits such as fragrance and flower shape, were further analyzed by sociodemographic group.

The results of respondents' preferences by gender and age group for the color and the most valued characteristic of peony flowers—such as color, fragrance, or shape—are shown in Table 7.

As shown in Table 7, male and female respondents do not differ significantly in their preferences for flower colors. Female respondents mainly preferred pink (107) and bicolor (66), while male respondents favored the same colors: pink (30) and bicolor (29), with a small difference between the recorded values for pink and bicolor peony genotypes. Regarding the peony's yellow color, it can be noted that this color was the least preferred, regardless of whether respondents were female or male (15 vs. 9), compared to the other flower colors analyzed. Notably, red ranked only third among respondents' preferred colors, regardless of gender.

Table 7: Distribution of flower color preferences (%) and most valued peony characteristics by gender and age group (n = 302).

Distribution of Flower Color Preferences by Gender					
Gender %	Bicolor	Pink	Yellow	Red	*p*-Value
Male	33	34.1	10.2	22.7	0.030
Female	30.8	50	7	12.2	
Distribution of the Most Valued Characteristic of Peony Flowers by Gender					
Gender %	Color	Fragrance	Shape Flower		*p*-Value
Male	47.7	22.7	29.5		<0.001
Female	20.1	33.2	46.7		
Distribution of Flower Color Preferences by Age Group					
Age %	Bicolor	Pink	Yellow	Red	*p*-Value
Group 1	34.7	45.5	7.2	12.6	0.351
Group 2	27.4	45.2	8.9	18.5	
Distribution of the Most Valued Characteristic of Peony Flowers by Age Group					
Age %	Color	Fragrance	Shape Flower		*p*-Value
Group 1	25.1	26.3	48.5		0.029
Group 2	31.9	34.8	33.3		

Note: The relationships between categorical variables were tested with the Chi-square (χ^2) test of independence. Statistical significance was set at $p < 0.05$.

Consumer preferences differed significantly between male and female respondents for both preferred flower color ($p = 0.030$) and the floral characteristic considered most important (color, fragrance, or flower shape; $p < 0.001$) (Table 7). Female respondents showed a stronger preference for pink flowers, whereas male respondents more often selected red flowers and placed greater emphasis on color itself. In contrast, women more often identified flower shape as the most important ornamental trait. No significant age-related differences were observed in flower color preference ($p = 0.351$). However, the relative importance assigned to color, fragrance, and flower shape differed significantly between age groups ($p = 0.029$), with younger respondents placing greater emphasis on flower shape. Regarding the reasons for flower color preferences, Fig. 4 displays the reported motivations, which serve as the foundation for subsequent analyses of consumer preferences. A statistically significant relationship was identified between preferred flower color and the reason given for selecting that color (Fisher's exact test, $p < 0.001$).

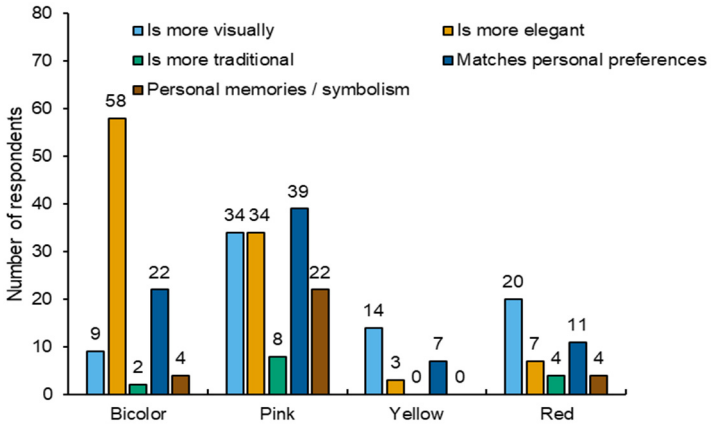


Figure 4: Reasons for respondents' preferences for flower colors behind their choices (n = 302). The relationships between categorical variables were tested with the Fisher's exact test, ($p < 0.001$).

As shown in Fig. 4, bicolor flowers were mainly linked to perceived elegance, while pink flowers were mostly selected for their aesthetic appeal and personal taste. In contrast, red and yellow flowers were primarily chosen for their visual impact rather than for elegance or symbolic meaning.

3.5 Correlations between Data Sets

Spearman rank correlation analysis across all 14 peony genotypes revealed several significant associations between total anthocyanin content and CIELab parameters (Supplementary Table S1). Total anthocyanin concentration was significantly negatively correlated with L* values in the median ($\rho = -0.666$, $p = 0.009$) and distal ($\rho = -0.682$, $p = 0.007$) petal regions, indicating that higher pigment accumulation was associated with darker petals. Significant positive correlations were observed between total anthocyanin content and a* values in the proximal ($\rho = 0.657$, $p = 0.011$), median ($\rho = 0.681$, $p = 0.010$), and distal ($\rho = 0.669$, $p = 0.010$) regions, confirming that increased anthocyanin accumulation was associated with stronger red coloration. No significant correlations were found between total anthocyanin content and b* values.

Exploratory Spearman rank correlations between consumer preference and flower traits are presented in Supplementary Table S2. None of the correlations were statistically significant ($p > 0.05$), reflecting the limited sample size (n = 4). Nevertheless, consumer preference showed consistent negative associations with b* values across all petal regions ($\rho = -1.0$), indicating lower preference for flowers with stronger yellow coloration. Moderate positive associations were observed with a* values in the median and distal regions ($\rho = 0.4$), whereas total anthocyanin content showed no meaningful relationship with consumer preference ($\rho = -0.105$).

4 Discussion

Flower color is an important and complex trait that enhances the ornamental appeal of flowering plants [32]. In this context, the results of the present study emphasize that combining colorimetric analyses (RHS and CIELab) with anthocyanin profiling is essential for establishing the link between visual flower coloration and the biochemical composition of pigments in *Paeonia* petals. In this study, five main anthocyanins—cyanidin-diglucoside, peonidin-diglucoside, cyanidin-glucoside, peonidin-glucoside, and pelargonidin-diglucoside were identified—each known for its contribution to red, pink, and purple hues in ornamental plants [33]. The analysis of anthocyanin composition in the petals of the analyzed peony

samples revealed notable differences in both quality and quantity among genotypes. The total anthocyanin content ranged from 0.00 μg g^{-1} FW (P10) to 2293.28 μg g^{-1} FW (P6), showing considerable variation in pigment accumulation. This variation supports earlier research that associates anthocyanin levels with flower color intensity and hue [5]. The sample coded P6 had the highest anthocyanin concentration, more than tripling that of P4, mainly due to cyanidin-glucoside (1530.57 μg g^{-1} FW), along with cyanidin- and peonidin-diglucosides. The dominance of cyanidin derivatives is typical of red and dark-colored peony cultivars [34]. Sample P4 also showed high levels (610.64 μg g^{-1} FW), primarily consisting of peonidin-diglucoside and cyanidin-diglucoside, similar to profiles observed in *Paeonia lactiflora* and related species [24]. Thus, the genotypes (P3, P4, P6) previously grouped in cluster B based on CIELab parameters (Fig. 2, Section 3) exhibited the highest total anthocyanin contents, confirming the direct relationship between pigment accumulation and color intensity. In particular, P6 (*Paeonia tenuifolia* 'Flore-Plena'), characterized by a uniform and highly saturated red coloration across all petal zones (P–M–D), showed the highest total anthocyanin content (2293.28 μg/g FW). This was mainly due to the extremely high levels of cyanidin-glucoside (1530.57 ± 9.73 μg/g FW) and cyanidin-diglucoside (338.09 ± 2.34 μg/g FW), compounds known to produce intense red coloration [35]. The high a* values recorded for this genotype further support the strong contribution of these anthocyanins to red chromatic expression. Similarly, P4 (*Paeonia peregrina*) displayed elevated concentrations of peonidin- and cyanidin-derived compounds, consistent with its classification as a vivid red genotype. Although it total anthocyanin content (610.64 μg/g FW) was significantly lower than that of P6, the relatively high pigment levels explain its stable and intense coloration across the petal surface. These findings align with previous studies indicating that both cyanidin and peonidin derivatives are key determinants of red pigmentation in peony flowers [4,36]. In contrast, genotypes included in cluster A (e.g., P1, P2, P5, P7, P9, P11, P14), characterized by higher luminosity (L*) and lower saturation, showed considerably lower total anthocyanin contents. For instance, P2 ('Pastel Splendor') and P5 (*Paeonia suffruticosa*) exhibited very low concentrations of all identified anthocyanins, which corresponds to their pale and pastel-like coloration. These results suggest that reduced anthocyanin biosynthesis or accumulation leads to diminished chromatic intensity, allowing lighter tones to dominate [37]. The variation in anthocyanin profiles reflects genetic differences among genotypes and differences in how the biosynthetic pathway is regulated, involving structural genes and transcription factor complexes [38].

Moreover, genotypes displaying pronounced color gradients across petal regions (e.g., P1 and P14) showed moderate anthocyanin levels, primarily composed of peonidin derivatives. The accumulation of anthocyanins in the proximal petal region (basal blotch) and their reduction toward the distal region is consistent with the observed color transitions and reflects spatial regulation of pigment biosynthesis [39]. Intermediate genotypes such as P8 and P12 showed balanced distributions of cyanidin- and peonidin-derived anthocyanins, corresponding to moderate color intensity and mixed tonal colors. The presence of pelargonidin derivatives, although in lower concentrations, may also contribute to subtle variations toward warmer hues [33,36,40]. A distinct case is represented by P10 (*Paeonia delavayi* var. *Lutea*), in which no anthocyanins were detected. This result is consistent with its chromatic profile, characterized by high b* values and a uniform yellow coloration. The absence of detectable anthocyanins in yellow-flowered genotypes suggests that coloration may be associated with other pigment classes, such as carotenoids, flavonols and chalcones [22]. The results of the present study provide initial insights into the phytochemicals present in the petals of the analyzed genotypes. Although technical replicates are a limitation and future studies with multiple biological replicates are required, the results suggest that variation in flower color among Paeonia genotypes is closely linked to both the concentration and composition of anthocyanins.

High anthocyanin levels are associated with intense red coloration and high a* values, whereas reduced pigment content results in lighter, pastel shades. Additionally, spatial variation in pigment accumulation across petal regions contributes to complex color patterns [20,41]. Besides the biochemical analysis of petal pigments, this study also assessed Romanian public perception of peony flower colors and other important traits (scent and flower shape). The peony (*Paeonia* spp.), known locally as "bujor", was officially designated Romania's national flower by Law no. 285/2022, owing to its botanical importance and cultural symbolism [42]. Several wild species naturally grow in Romania, including *Paeonia peregrina*, *P. tenuifolia*, and *P. officinalis* subsp. *banatica*, many of which thrive in protected habitats and are associated with oak forest ecosystems [43]. Beyond its ecological value, the *Paeonia* genus is highly valued for its ornamental appeal and the diversity of its flower shapes and colors, with over 100–130 taxa identified in Romania [43]. The results of this study on public color preferences partly align with previous research showing sex-based differences in color perception—women generally prefer pink and reddish hues, while men favor more saturated colors [44,45]. In this study, preferences for bicolor peonies are similar across genders, with about 30–33% favoring them, and yellow is the least popular among both groups (7.0–10.2%). Such preferences might be connected to evolutionary and social factors, including responses to red–pink tones linked to fruit detection and social signals [44]. In ornamental horticulture, flower color significantly influences aesthetic appeal and consumer choice, with pink and red often being the most attractive [46,47]. The strong preference for pink among females probably reflects cultural influences and emotional significance [45]. The preference for pink stems from both emotional and perceptual reasons, as it is often associated with warmth and positive feelings, shaped by experiences described in ecological valence theory [48–50]. Conversely, red and yellow are prominent colors associated with arousal, energy, and attention, which explains their choice being mainly based on visual impact rather than symbolic meaning [50–52]. The appreciation of bicolored peony genotypes indicates they are less gender-specific, while the low preference for yellow matches earlier research on their limited aesthetic appeal [46]. These insights highlight the importance of considering socio-demographic factors, such as gender, when breeding and marketing ornamental plants like *Paeonia*. Overall, flower color preferences are shaped by perceptual, evolutionary, and cultural factors, with gender being a key influence.

Regarding the distribution of the most preferred floral traits by gender, male respondents displayed a relatively balanced interest in color, fragrance, and flower shape. In contrast, females favored shape and fragrance more than color, indicating that gender shapes not only color preferences but also the emphasis placed on ornamental features.

The stronger female preference for flower shape aligns with research identifying morphology as a key element in aesthetic perception, often surpassing the importance of color [46]. This may reflect heightened sensitivity to structural qualities such as symmetry and form. Additionally, females' higher valuation of fragrance suggests a multisensory approach, as olfactory cues are closely linked to emotional responses and memory [53], enhancing overall ornamental appeal.

Conversely, males tend to evaluate traits more holistically or generally, a trend seen in consumer research within ornamental horticulture [47]. These differences in trait prioritization may also stem from perceptual processing, where structural fluency influences aesthetic judgments [54]. Practically, these findings highlight the importance of considering gender-specific preferences in *Paeonia* breeding programs. Cultivars targeting female consumers might focus on consistent flower shape and fragrance, while a balanced trait approach could appeal to a wider market.

The preferred floral traits (flower shape and fragrance) are similarly distributed across different age groups. Fisher's exact test (p = 0.101) confirms there is no significant link between age and the main

trait valued. This suggests that aesthetic evaluation of key ornamental features remains fairly consistent throughout adulthood, aligning with studies indicating that preferences for natural elements are largely universal [55,56].

The consistent emphasis on color, fragrance, and flower shape supports the idea that multisensory perception depends on stable cognitive and emotional processes [53,57].

In contrast, a significant relation between flower color and the reason for choosing it was assessed in this study. This indicates that respondents associate different symbolic and aesthetic meanings with various colors. Pastel colored flowers are primarily associated with elegance, pink with visual appeal and personal preference, while red and yellow are mainly chosen for their strong visual impact. The association of light-colored flowers with elegance aligns with its cultural ties to purity, simplicity, and refinement, as well as its lower perceptual intensity compared to more saturated colors [51,58,59].

Exploratory correlations based on the four flower color categories in the consumer survey indicated that consumer preference was more closely associated with perceived color attributes than with pigment concentration itself. In particular, preference showed a consistent negative association with b* values across all petal regions, suggesting lower appreciation for flowers with stronger yellow coloration. Moderate positive associations with a* values in the median and distal petal regions further indicate a preference for flowers with more pronounced red and pink hues. By contrast, total anthocyanin content showed no meaningful relationship with preference, emphasizing that consumer choices are driven primarily by visual perception of flower color rather than by pigment concentration alone.

Survey responses also highlighted the importance of flower shape and fragrance, demonstrating that ornamental appreciation depends on multiple visual and sensory attributes. Taken together, these findings support a sequential relationship in which anthocyanin accumulation influences flower color, flower color contributes to consumer preference, and flower shape and fragrance further modulate the overall ornamental value of peony cultivars.

From a practical standpoint, breeding and marketing strategies should consider not only color variety, but also the meanings associated with different hues, enabling targeted development of cultivars suited for specific aesthetic and functional needs. These results highlight the role of color as a multifaceted trait in ornamental horticulture, influencing consumer preferences through visual, emotional, and cultural factors [60].

5 Conclusions

Flower color diversity in Paeonia is strongly influenced by anthocyanin concentration and composition, particularly cyanidin and peonidin derivatives. Higher anthocyanin accumulation was associated with darker petals and more intense red coloration. Survey participants showed a clear preference for pink and bicolor flowers, whereas yellow flowers were the least preferred. Together, these results highlight pigment composition, objective color measurements, and consumer preferences, providing practical guidance for breeding and selecting ornamental peony cultivars.

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Hârţa and Doina Clapa; Supervision: Monica Hârţa and Doina Clapa; Validation: Mirela Irina Cordea; Visualization: Floricuţa Ranga and Doina Angela Pui; Writing—original draft: Abel Bala and Doina Clapa; Writing—review and editing: Abel Bala and Monica Hârţa. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The data supporting the findings of this study are available from the first author, Abel Bala, upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Supplementary Materials: The supplementary material is available online at https://www.techscience.com/doi/10.32604/phyton.2026.083046/s1, Table S1: Spearman rank correlation coefficients among total anthocyanin content and CIELab parameters measured in proximal, median, and distal petal regions across 14 peony genotypes; Table S2: Correlation matrix including consumer preference, regional CIELab color parameters, and total anthocyanin content for the four peony flower color categories included in the consumer survey.

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