



## Article

# Morphological Trait Variations and Flower Color Differences in Wild *Crocus* Species

Nataša Krstić<sup>1</sup>, Goran Jaćimović<sup>1</sup> , Branka Ljevnaić-Mašić<sup>1</sup> , Sofija Petrović<sup>1</sup>, Željana Prijić<sup>2,\*</sup> , Đorđe Krstić<sup>1</sup> and Borislav Banjac<sup>1</sup>

<sup>1</sup> Faculty of Agriculture, University of Novi Sad, Dositej Obradovića Square 8, 21000 Novi Sad, Serbia; natasa.sovilj@gmail.com (N.K.); goran.jacimovic@polj.uns.ac.rs (G.J.); brana@polj.uns.ac.rs (B.L.-M.); sofija.petrovic@polj.uns.ac.rs (S.P.); djordje.krstic@polj.uns.ac.rs (Đ.K.); borislav.banjac@polj.edu.rs (B.B.)  
<sup>2</sup> Institute for Medicinal Plants Research “Dr Josif Pančić” Belgrade, Tadeuša Košćuška 1, 11000 Belgrade, Serbia  
\* Correspondence: zprijic@mocbilja.rs; Tel.: +381-(0)64-81-88-748

**Abstract:** This study investigates the phenotypic diversity of spring-flowering *Crocus* species native to Serbia by analyzing their morphological traits and flower color variations. Detailed phenotypic characterization was performed on seven species: *C. alexandri*, *C. chrysanthus*, *C. heuffelianus*, *C. randjeloviciorum*, *C. rujanensis*, *C. variegatus*, and *C. weldenii*. The study examines how morphological parameters of tested species diverged from average values during a hot and dry winter, resulting in smaller plants, earlier and shorter flowering periods, and reduced flower size, regardless of species. Hierarchical clustering based on morphological traits grouped the species into three clusters: the first cluster included *C. alexandri*, *C. chrysanthus*, *C. rujanensis*, and *C. weldenii*, characterized by the highest number of leaves and the lowest number of flowers; the second consisted of *C. randjeloviciorum*, distinguished by shorter plant height and the greatest number of flowers; and the third cluster comprised *C. heuffelianus* and *C. variegatus*, notable for the tallest plants, widest corms and leaves, and longest perigon segments. Grouping based on petal color components (L\*, a\*, b\*, chroma, and hue) formed five distinct clusters, demonstrating differences in flower color. These findings are valuable for taxonomic classification, conservation measures, and breeding programs, which ultimately support the conservation of biodiversity and the promotion of ornamental plant cultivation. Flower color in particular has proven to be a reliable species indicator, as it remains constant despite the year and/or habitat. The use of a colorimeter can speed up the identification of species in the field and provides researchers and conservationists with a practical tool.

**Keywords:** *Crocus alexandri*; *Crocus chrysanthus*; *Crocus heuffelianus*; *Crocus randjeloviciorum*; *Crocus rujanensis*; *Crocus variegatus*; *Crocus weldenii*; clustering; PCA; spring flowering



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## 1. Introduction

The genus *Crocus* L. belongs to the family Iridaceae Juss., which includes irises, saffron, and gladioli [1,2]. *Crocus* L. species are perennial herbaceous plants, most of which bloom in early spring, while a smaller number bloom in autumn [3]. Crocuses, like other geophytes, survive unfavorable conditions with seeds and underground organs called corms, from which the stem develops each year. They have narrow, linear leaves, while the flowers are mostly single, actinomorphic, and most often white, yellow, or purple [4]. The perigon is funnel-shaped with a long, narrow tube. The flower of *Crocus* species typically exhibits intricate details, with six petals arranged in two circular rows.

The flowering time of *Crocus* species varies, with autumn forms blooming late in the season and spring forms blooming early. This flowering biology is conditioned by the natural habitats of these species. Vegetation of crocuses starts before or after the growing season of meadows and pastures, during which the grass cover is at rest and non-competitive, allowing crocuses to complete their vegetation period and form new

corms [5–7]. Spring-blooming *Crocus* species are primarily used for decoration but also have potential medicinal applications [8,9]. These plants begin to flower with the first sunny days of spring. If there is a sudden cold period, the first flowers may be damaged, but with the warm periods that follow, a new flowering can emerge. This flowering pattern has also been observed in plants during the beginning and retreat of the Ice Age, which also confirms that crocuses are from the mentioned era [3].

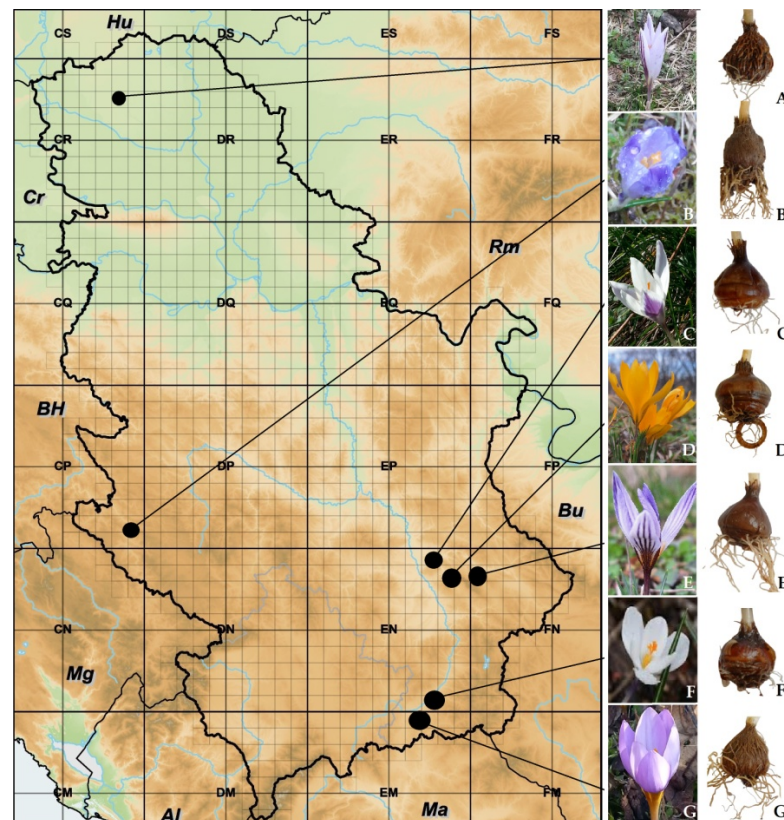
The genus *Crocus* L. is distributed from the western Mediterranean to Central Asia in the east and from Poland in the north, to North Africa in the south [10]. Asia Minor and Southeast Europe, particularly the Balkan Peninsula, are recognized as the most significant centers of diversity for this genus [3,11–18]. According to some authors, this genus includes about 180 species [14,18,19], while others state the existence of 237 species [3]. In Serbia, according to some literature, there are 17 recognized species [11], which belong to the sections *Crocus* sp. Mathew (series *Verni* Mathew, *Scardici* Mathew, *Crocus* Mathew) and section *Nudiscapus* Mathew (series *Retikulati* Mathew, series *Biflori* Mathew), or more according to the literature [20,21].

Molecular and morphological studies [22] have led to a taxonomic change, revealing that *Crocus adamii* Gay, previously described in 1990, as occurring in Serbia [3] is actually *Crocus. randjeloviciorum* Kernd., Pasche, Harpke, and Raca [23]. This species is more closely related phylogenetically to *C. alexandri* and *C. chrysanthus*, rather than to *C. adamii*, whose distribution ranges from the Anatolian Diagonal to Iran and the Caucasian mountains [23,24]. The *Crocus* series *Vernus* is now classified into eleven distinct species, three of which are found in Serbia [17,25]. Due to current taxonomic doubts and unresolved phylogenetic relationships within the *C. vernus* complex, *C. heuffelianus* is currently recognized as a distinct taxon within this group [26]. However, Mathew [27] classified *C. heuffelianus* as a synonym of *C. vernus*. For a long time, the Balkan endemic species *Crocus pallidus* has been listed as a synonym of *C. weldenii* [11]. Recent research in Bulgaria, however, has demonstrated a clear distinction between *C. pallidus* and the closely related *C. weldenii* Hoppe and Fumrohr [28]. *C. reticulatus* is not a distinct species with extensive variability and distribution, but rather a species aggregate. Within this aggregate, two species are identified: *C. danubensis* and *C. variegatus* [29]. Although big morphological differences are not so obvious between *C. danubensis* and *C. variegatus* [30], distinctions are evident in several characteristics: flower color (white to soft lilac—*C. danubensis*, lilac—*C. variegatus*), throat color (white to pale yellow—*C. danubensis*, yellow—*C. variegatus*), filament length (5–6.5 mm—*C. danubensis*, 2.5–7 mm—4), anther length (6–9.7 mm—*C. danubensis*, 6–13—*C. variegatus*, etc.) [31]; additionally, the leaf cross sections of *C. danubensis* are larger in size than *C. variegatus* [29]. Among the species in the *Crocus* genus, *Crocus sativus* has been the most studied due to its significance as the world's most expensive spice [32].

Despite several scientific studies [3,7–9,33], the species of the genus *Crocus* L. in Serbia are not sufficiently researched, particularly regarding the impact of warm winters on morphological traits. To address this gap, a study was conducted to determine the morphometric properties and differences in flower colors among seven *Crocus* species native to Serbia (*C. alexandri* Nicic ex Velen, *C. chrysanthus* Herb., *C. heuffelianus* Herb., *C. weldenii* Hoppe et Fürnr. 1840, *C. randjeloviciorum* Kernd., Pasche, Harpke and Raca, *C. rujanensis* Randjel and D.A. Hill [34], and *C. variegatus* Hoppe and Hornsch). This research provides a detailed analysis of the variations among and within *Crocus* species, facilitating their classification and identification while exploring possibilities for cultivation. The present study aimed to investigate morphological traits and flower colors to determine the variability of different *Crocus* species in Serbia, to improve species identification in the field. Additionally, the study examined the impact of a hot and dry winter on the tested parameters across different species, while also assessing their suitability for conservation efforts and potential commercial propagation.

## 2. Materials and Methods

One of the primary objectives of the present study was to examine the basic morphological traits of seven different *Crocus* species native to Serbia (Table 1, Figure 1), *C. alexandri*, *C. chrysanthus*, *C. heuffelianus*, *C. randjeloviciorum*, *C. rujanensis*, *C. variegatus*, and *C. weldenii*, with the goal of determining the diversity and variability among them. Sampling of the tested material was conducted in the full flowering phase of the species. For that reason, sampling for most of the tested species was conducted in February 2018, while *C. heuffelianus* was sampled in April 2018. The selection of the tested species was influenced by several factors. Endemic species such as *C. alexandri*, *C. rujanensis*, and *C. randjeloviciorum* were prioritized. Additionally, the choice was influenced by their vertical distribution: selected species were found in lowland areas (*C. variegatus*), hilly regions (*C. alexandri*, *C. chrysanthus*, *C. weldenii*, *C. rujanensis*, and *C. randjeloviciorum*), and mountainous regions (*C. heuffelianus*). Flower color was also a crucial factor, with both multicolored species (*C. alexandri*, *C. rujanensis*, *C. randjeloviciorum*, *C. variegatus*) and single-colored species (*C. weldenii*, *C. chrysanthus*, *C. heuffelianus*) being considered. To ensure accurate color determination, single-color flowers in white (*C. weldenii*), yellow (*C. chrysanthus*), and purple (*C. heuffelianus*) were used as reference species.



**Figure 1.** Map of Serbia with the distribution of tested *Crocus* species with pictures of flowers and corms: (A) *C. variegatus*; (B) *C. heuffelianus*; (C) *C. alexandri*; (D) *C. chrysanthus*; (E) *C. randjeloviciorum*; (F) *C. weldenii*; (G) *C. rujanensis*.

To assess the diversity of the selected *Crocus* species, the following ten morphological traits were determined during the flowering period: plant height (PH), corm width (CW), number of leaves (NL), leaf width (LW), number of flowers (NF), perigon length (PL), length of the outer perigon segment (LOP), length of the inner perigon segment (LIP), length of the stigma lobes (LSL), and length of the anthers (LA).

In addition to morphological characteristics, to determine the diversity of selected species, the color of the flower, i.e., the color of the outer and inner petals of the perianth on both sides, was also determined (Figure 1).

**Table 1.** *Crocus* species from different regions of Serbia and their locations.

Species	Collection Site	Longitude	Latitude	Altitude (m a.s.l.)	Voucher No (BUNS *)
<i>C. alexandri</i>	Seličevica—Čelopek	N 43°12'27.78"	E 21°51'17.26"	365	2-1470
<i>C. chrysanthus</i>	Suva mountain—Kunovica	N 43°18'22.92"	E 22°05'29.6"	578	2-1471
<i>C. heuffelianus</i>	Zlatar mountain	N 43°27'3.78"	E 19°49'31.8"	1183	2-1476
<i>C. randjeloviciorum</i>	Suva mountain—Kunovica	N 43°18'21.00"	E 22°05'32.00"	568	2-1473
<i>C. rujanensis</i>	Rujan mountain—Čukarka	N 42°16'19.44"	E 21°42'20.16"	431	2-1475
<i>C. variegatus</i>	Tomislavci	N 45°49'32.64"	E 19°33'39.18"	96	2-1474
<i>C. weldenii</i>	Rujan mountain—Samoljica	N 42°24'8.94"	E 21°43'50.64"	434	2-1472

\* BUNS Herbarium collection. Legator: MSc. Nataša Krstić; Identifier Prof. Dr Goran Anačkov.

### 2.1. Sample Collection and Measurements

To determine the morphological characteristics of the plants, the plant material used in the study consisted of ten randomly chosen plants for each of the seven selected Serbian *Crocus* species with diverse colors (sample size:  $n = 70$ ). The measurements of morphological traits were performed using a Digital Sliding Caliper (RND 555-00167, RND Digital Sliding Caliper Stainless Steel 150 mm, Elfa Distrelec A/S, Aarhus, Denmark).

Considering that flower color is primarily determined by genetic predisposition as well as by the climatic conditions of the location, the color was evaluated based on the plant material collected from their natural habitat. To determine the differences in flower color, i.e., for color coordinate analyses, three completely matured flowers of each *Crocus* species (in five replicates, totaling 15 flowers per species) were harvested at the full bloom stage. Determination of petals color was performed using a Konica Minolta CR-400 Chroma Meter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) equipped with a light projection tube. The light source used for the measurement was type C, corresponding to daylight.

### 2.2. Determination of Flower Color of the *Crocus* sp. (Colorimetric Analysis)

To determine the diversity of selected *Crocus* species, the color of the flower, i.e., the color of the outer (O-) and inner (I-) petals of the perianth on both sides (outer (-o) and inner (-i)), was also determined. In this way, to determine the flower colors using principal component analysis (PCA) and cluster analysis (CA), a total of 28 observations were obtained (7 *Crocus* sp., with petals/side defined as O-o, O-i, I-o, and I-i).

Color components ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue) of each crocus plant petal are presented in the CIE  $L^*a^*b^*$  (CIELab) color space, which is based on a three-dimensional (spherical) colored space. The CIELab model is based on the opponent color system with linear measures of lightness ( $L^*$ ) and two color dimensions (color coordinates):  $a^*$  and  $b^*$ , which represent the spectrum from green ( $-a^*$ ) to red ( $+a^*$ ); and blue ( $-b^*$ ) to yellow ( $+b^*$ ), respectively [35–37]. Lightness ( $L^*$ ) and color components  $a^*$  and  $b^*$  can approximate the human-perceived color attributes: lightness (L), hue (H), and chroma (C). The  $L^*$  coordinate indicates the darkness or lightness of a color and ranges from black (0) to white (100). Hue is an angular value (hue angle from  $0^\circ$  to  $360^\circ$ ) representing the dominant wavelength. Chroma is a linear measure that represents the purity of a color (saturation, or vividness). As chromaticity increases, the color becomes more intense; as it decreases, the color becomes duller [38]. Both chroma and hue are derived from  $a^*$  and  $b^*$  coordinates using the following equations [37,38].

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

$$\text{Hue angle, H} = \tan^{-1} (b^*/a^*) \text{ (in degrees, } ^\circ \text{)}$$



The conversions represented in the CIELAB (L\*a\*b\*) were accurately translated into the RGB (R—red; G—green; B—blue) format consistent with human perception “<https://www.e-paint.co.uk/convert-lab.asp>” (accessed on 20 July 2024).

### 2.3. Statistical Analysis

The analysis of variance (ANOVA) procedure for the morphological characterization of *Crocus* species was performed using one-way ANOVA without blocking, i.e., without replications, on a sample consisting of ten randomly chosen plants for each of the seven selected *Crocus* sp. For the colorimetric determination of flower color (specifically petals), the analysis of variance was performed according to a completely randomized design with five replications. Before analysis, all datasets were tested according to the basic assumptions of ANOVA at a 95% confidence interval. When testing all analyzed parameters for normality of distribution, the Shapiro–Wilk test confirmed that all datasets were normally distributed. The assumption of constant variance was checked using a plot of residuals vs. fitted values. The ANOVA procedure was performed considering *Crocus* species as independent variables (fixed-effect factors) and the analyzed parameters as dependent variables, with a 95% confidence interval. The statistical significance of the differences among the means of the analyzed parameters (morphometric traits and parameters of color components of crocuses petals) was determined using Fisher’s least significant difference (LSD) test at the 5% probability level. Correlation analysis among the analyzed parameters was calculated using Pearson’s correlation at the significance level  $\alpha = 0.05$ . To determine the relationships between the various parameters and to identify the set of parameters that explain the maximum variability among the *Crocus* species, the multivariate data analysis method PCA (principal component analysis) was used. Grouping of *Crocus* sp. based on morphological traits and flower color was carried out using cluster analysis (hierarchical clustering), employing the complete linkage agglomeration method. Statistical analyses were performed using the Statistica software package, version 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA) and GenStat for Windows 12th Ed. (VSN International, Hemel Hempstead, UK).

## 3. Results

### 3.1. Distribution

*Crocus* species differ both in their vertical and horizontal distribution [3]. In terms of vertical distribution, *C. variegatus* is found in plain regions, with habitat in the remnants of steppes. Species *C. alexandri*, *C. chrysanthus*, *C. weldenii*, *C. rujanensis*, and *C. randjeloviciorum* are distributed in hilly areas at the edges of deciduous forests, while *C. heuffelianus* is found mainly in mountainous regions at the edges of coniferous forests. Horizontally, *C. alexandri*, *C. rujanensis* and *C. randjeloviciorum* have a very limited distribution range, which categorizes them as endemic species [3,22].

### 3.2. Morphological Traits of *Crocus* Species

Significant differences were observed among the different *Crocus* sp. regarding their morphological properties (Table 2).

All the studied traits, except for the number of leaves (NL;  $p = 0.439$ ), significantly differed ( $p < 0.001$ ) among the species studied. The high  $R^2$  values for some traits (such as PH—plant height, PL—perigon length, LSL—length of the stigma lobes, and LA—length of the anthers) also suggest a strong differentiation among species for these characteristics. Coefficients of variation (CV) showed varying degrees of variability within each characteristic. The highest CVs were recorded for the number of flowers (NF) (36.0%) and leaf width (LW) (35.0%), while the lowest CVs were observed for the length of the inner perigon segment (LIP) (11.9%) and length of the outer perigon segment (LOP) (12.0%).

PH varied significantly among species ( $p < 0.001$ ), (Table 2). On average, the tallest plants were found in the species *C. variegatus*, while the shortest were in *C. weldenii*, which is 38.14% shorter than the highest value.

**Table 2.** Morphological traits of seven *Crocus* species native to Serbia.

Species *	PH (mm)	CW (mm)	NL	LW (mm)	NF	PL (mm)	LOP (mm)	LIP (mm)	LSL (mm)	LA (mm)
<i>C. alexandri</i>	98.9 ± 6.8 <sup>cd</sup>	10.2 ± 1.2 <sup>cd</sup>	3.3 ± 0.5 <sup>b</sup>	0.8 ± 0.2 <sup>c</sup>	1.0 ± 0.0	44.1 ± 9.8 <sup>bc</sup>	25.2 ± 1.5 <sup>e</sup>	22.8 ± 1.6 <sup>c</sup>	7.9 ± 0.7 <sup>a</sup>	9.3 ± 0.7 <sup>e</sup>
<i>C. chrysanthus</i>	92.9 ± 5.4 <sup>e</sup>	12.4 ± 2.0 <sup>a</sup>	4.1 ± 1.0 <sup>a</sup>	1.4 ± 0.1 <sup>b</sup>	1.3 ± 0.5	31.9 ± 6.3 <sup>d</sup>	25.6 ± 2.1 <sup>e</sup>	22.8 ± 2.0 <sup>c</sup>	4.7 ± 0.4 <sup>d</sup>	10.1 ± 0.5 <sup>de</sup>
<i>C. heuffelianus</i>	120.7 ± 6.8 <sup>b</sup>	11.2 ± 0.7 <sup>bc</sup>	2.4 ± 0.5 <sup>c</sup>	1.8 ± 0.2 <sup>a</sup>	1.2 ± 0.4	46.9 ± 2.9 <sup>b</sup>	32.1 ± 2.8 <sup>a</sup>	29.6 ± 1.6 <sup>a</sup>	3.9 ± 0.4 <sup>e</sup>	21.7 ± 2.2 <sup>a</sup>
<i>C. randjeloviciorum</i>	94.6 ± 5.6 <sup>de</sup>	9.5 ± 1.8 <sup>de</sup>	3.6 ± 0.7 <sup>ab</sup>	0.8 ± 0.3 <sup>c</sup>	1.3 ± 0.7	16.6 ± 1.9 <sup>e</sup>	26.4 ± 3.2 <sup>de</sup>	25.3 ± 2.8 <sup>b</sup>	5.5 ± 0.2 <sup>bc</sup>	10.4 ± 0.3 <sup>d</sup>
<i>C. rujanensis</i>	100.1 ± 1.0 <sup>c</sup>	10.6 ± 0.8 <sup>bc</sup>	4.1 ± 1.1 <sup>a</sup>	1.4 ± 0.2 <sup>b</sup>	1.3 ± 0.5	43.7 ± 4.6 <sup>bc</sup>	29.1 ± 1.6 <sup>bc</sup>	26.2 ± 1.6 <sup>b</sup>	2.7 ± 0.3 <sup>f</sup>	11.6 ± 1.0 <sup>c</sup>
<i>C. variegatus</i>	140.4 ± 7.5 <sup>a</sup>	11.7 ± 0.6 <sup>ab</sup>	3.9 ± 0.9 <sup>ab</sup>	1.3 ± 0.1 <sup>b</sup>	1.2 ± 0.4	56.3 ± 5.0 <sup>a</sup>	30.3 ± 1.4 <sup>ab</sup>	26.2 ± 2.3 <sup>b</sup>	5.3 ± 0.3 <sup>c</sup>	12.8 ± 1.4 <sup>b</sup>
<i>C. weldenii</i>	86.6 ± 5.6 <sup>f</sup>	8.6 ± 0.9 <sup>e</sup>	3.6 ± 0.5 <sup>ab</sup>	0.9 ± 0.2 <sup>c</sup>	1.0 ± 0.0	41.0 ± 3.3 <sup>c</sup>	28.0 ± 3.6 <sup>cd</sup>	24.9 ± 3.1 <sup>b</sup>	5.8 ± 0.3 <sup>b</sup>	12.0 ± 1.5 <sup>bc</sup>
Average ± SD	104.9 ± 18.6	10.6 ± 1.7	3.6 ± 0.9	1.2 ± 0.4	1.2 ± 0.4	40.1 ± 12.9	28.1 ± 3.4	25.4 ± 3.0	5.1 ± 1.6	12.6 ± 4.1
CV (%)	17.7	16.0	25.9	35.0	36.0	32.2	12.0	11.9	30.7	32.5
ANOVA R <sup>2</sup>	0.903	0.511	0.357	0.793	0.086	0.839	0.507	0.517	0.935	0.914
F (n = 70)	97.990	10.986	5.842	40.168	0.991	54.542	10.784	11.218	151.689	111.134
p	<0.001	<0.001	<0.001	<0.001	0.439	<0.001	<0.001	<0.001	<0.001	<0.001

PH—plant height, CW—corm width, NL—number of leaves, LW—leaf width, NF—number of flowers, PL—perigon length, LOP—length of the outer perigon segment, LIP—length of the inner perigon segment, LSL—length of the stigma lobes, LA—length of the anthers, *n*—sample size (*n* = 70), R<sup>2</sup>—coefficient of determination, F—variance ratio (F-test), *p*—probability value corresponding to a variance ratio. \* Mean value ± standard deviation (SD). Within each column, means followed by different letters are significantly different at 5% level of significance according to Fisher's LSD test (*p* ≤ 0.05).

The largest CW was observed in *C. chrysanthus*, which was 30.6% larger compared to the smallest one found in *C. weldenii*. In our study, *C. chrysanthus* and *C. rujanensis* showed the highest NL, in contrast to *C. heuffelianus*, which had an NL value that was more than 41% lower in comparison. Although the differences in NL among species were statistically significant (*p* < 0.001), the coefficient of determination was relatively low (R<sup>2</sup> = 0.357).

The LW varied significantly among the analyzed species (*p* < 0.001). *C. heuffelianus* had the widest leaves, measuring 42.9% wider than those of *C. randjeloviciorum* and *C. alexandri*, which had the narrowest leaves. In contrast, no statistically significant difference was found in the NF among species (*p* = 0.439), with values ranging from 1.0 to 1.3. The difference in PL between the longest, observed in *C. variegatus*, and the shortest, in *C. randjeloviciorum*, was 70%. Comparing the longest LOP in *C. heuffelianus* to the shortest in *C. alexandri* revealed a difference of 21.5%. Similarly, *C. heuffelianus* had the longest LIP, while the shortest were recorded in *C. alexandri* and *C. chrysanthus*, showing a 23% difference. The LSL were found in *C. alexandri*, whereas *C. rujanensis* had the shortest, with a 65.8% difference. *C. heuffelianus* exhibited the LA, while the anthers of *C. alexandri* were more than twice as short. *C. variegatus* had the longest PL, while *C. randjeloviciorum* revealed the shortest, measuring only 70% of that length. The longest LOP was recorded in *C. heuffelianus*, whereas *C. alexandri* had the shortest, at just 21.5% of the maximum. Similarly, *C. heuffelianus* also had the longest LIP, while the shortest segments were observed in *C. alexandri* and *C. chrysanthus*, both at 23% of the longest measurement. The longest LSL were found in *C. alexandri*, with *C. rujanensis* having the shortest, which was 65.8% of the maximum length. Lastly, *C. heuffelianus* had the longest anther length (LA), while the shortest anthers in *C. alexandri* were less than half that length.

Parameters LSL and LA also showed highly significant differences (*p* < 0.001) among *Crocus* species, with R<sup>2</sup> values of 0.935 and 0.914, respectively.

The correlation analysis revealed several significant relationships among the morphological traits (Table 3).

PH showed moderate to strong positive correlations with CW, LW, PL, LOP, LIP, and LA. This suggests that taller plants tend to have wider corms, broader leaves, longer perigon segments, and longer anthers, indicating an overall larger plant size. CW was positively correlated with LW and NF, suggesting that plants with wider corms tend to have wider leaves and more flowers. However, CW did not show significant correlations with other traits. The NL had a weak positive correlation with NF but negatively correlated with LIP and LA, indicating that a plant with more leaves tends to have shorter inner perigon segments and anthers. LW exhibited several significant correlations: a positive relationship with PL, LOP, LIP, and LA, and a strong negative correlation with LSL. This suggests that plants with wider leaves tend to have longer perigons, outer and inner perigon segments, and anthers, but shorter stigma lobes.

**Table 3.** Pearson's correlation coefficients (r) between morphological traits of *Crocus* sp. \*.

Variables	CW	NL	LW	NF	PL	LOP	LIP	LSL	LA
PH	<b>0.35</b>	−0.14	<b>0.44</b>	0.02	<b>0.56</b>	<b>0.48</b>	<b>0.36</b>	−0.13	<b>0.47</b>
CW	1	0.16	<b>0.35</b>	<b>0.34</b>	0.23	0.07	0.00	−0.21	0.12
NL	-	1	−0.16	<b>0.28</b>	−0.12	−0.23	− <b>0.28</b>	−0.07	− <b>0.44</b>
LW	-	-	1	0.16	<b>0.35</b>	<b>0.51</b>	<b>0.50</b>	− <b>0.65</b>	<b>0.66</b>
NF	-	-	-	1	−0.10	−0.02	−0.02	−0.16	0.01
PL	-	-	-	-	1	<b>0.44</b>	<b>0.31</b>	−0.04	<b>0.35</b>
LOP	-	-	-	-	-	1	<b>0.85</b>	− <b>0.43</b>	<b>0.59</b>
LIP	-	-	-	-	-	-	1	− <b>0.43</b>	<b>0.64</b>
LSL	-	-	-	-	-	-	-	1	− <b>0.41</b>

\* Bolded values in italics are different from 0 with a significance level  $\alpha = 0.05$ . Sample size:  $n = 70$  (7 *Crocus* sp.  $\times$  10 randomly chosen plants). (PH—plant height, CW—corm width, NL—number of leaves, LW—leaf width, NF—number of flowers, PL—perigon length, LOP—length of the outer perigon segment, LIP—length of the inner perigon segment, LSL—length of the stigma lobes, LA—Length of the anthers) of seven *Crocus* species.

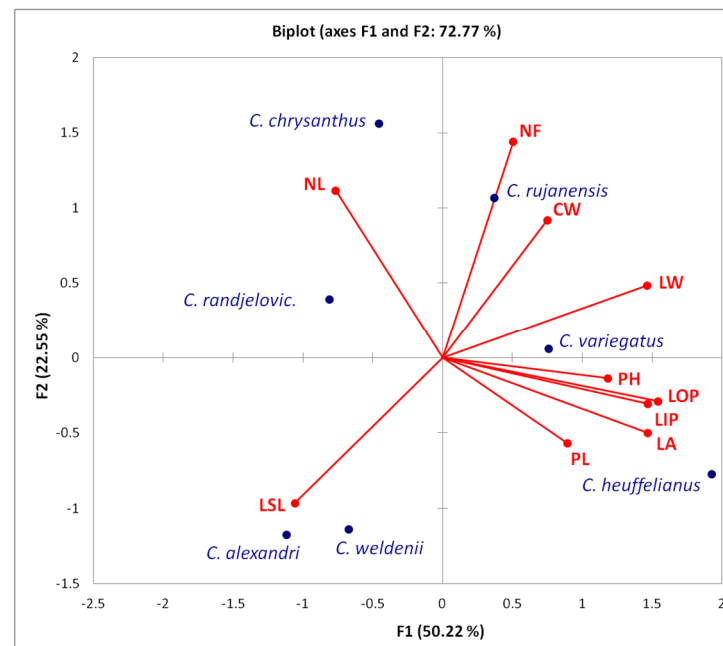
NF did not show statistically significant correlations with any of the other morphological traits. PL was positively correlated with LOP, LIP, and LA, indicating that plants with longer perigons tend to have longer outer and inner perigon segments and anthers. Length of the outer perigon segment (LOP) showed a very strong positive correlation with LIP and a significant positive correlation with LA, as well as a negative correlation with LSL, which indicates that plants with longer outer perigon segments also tend to have longer inner perigon segments and anthers but shorter stigma lobes. Length of the inner perigon segment (LIP) had a significant positive correlation with LA and a negative correlation with LSL, suggesting that longer inner perigon segments are associated with longer anthers but shorter stigma lobes. Finally, the LSL had a negative correlation with LA, indicating that plants with longer stigma lobes tend to have shorter anthers.

The PCA results indicated that the first three principal components (PCA dimensions F1, F2, and F3), each with eigenvalues greater than 1, together accounted for 87.33% of the total variability in the morphological traits of *Crocus* species (Table S1). Specifically, the first PCA dimension (F1) explained 50.22%, F2 explained 22.55%, and F3 explained 14.56% of the total variability. This suggests that these three dimensions effectively captured the majority of the variability in the dataset, simplifying the complexity of the morphological data while retaining its most significant patterns.

The partial contribution of each variable to the individual PCA dimensions was calculated (Table S1), and the results showed that variables PH, LW, LOP, LIP, LSL, and LA contributed a total of 83.77% to the variability in the F1 PCA dimension, with the maximum contribution from LOP (17.54%), followed by LIP (15.90%), LA (15.89%), and LW (15.84%). NF, CW, NL, and PL contributed less significantly (1.89–5.86%; i.e., a total of 16.22%) to the variability in F1. This indicates that F1 primarily captures variations related to floral and foliar dimensions of the *Crocus* species. The partial contribution of the observations (*Crocus* species) showed that significant contributions to the variability in the first PCA dimension (F1; 80.34%) were made by the species *C. randjeloviciorum* (9.34%), *C. alexandri* (17.87%), and *C. heuffelianus* (53.13%), with the greatest contribution from *C. heuffelianus*. The second PCA dimension (F2) was predominantly influenced by variables CW (13.90%), NL (20.39%), and NF (34.09%), which together contributed 68.38% to the total variability. Meanwhile, variables PH, LW, PL, LOP, LIP, LSL, and LA contributed 31.62% to the total variability in F2, having previously shown significant variability in F1. This suggests that F2 captures variations related to reproductive traits and plant size, complementing the floral and foliar emphasis seen in F1. Observing the partial contribution of the observations, it was noted that the species *C. chrysanthus* (34.80%), *C. weldenii* (18.51%), and *C. rujanensis* (16.32%) made the greatest contributions to the variability in F2 dimension (69.63% in total), with the highest contribution from *C. chrysanthus*. In the third PCA dimension (F3), the main contributors were PL (31.23%) and *C. variegatus* (41.96%), which together accounted for 73.19% of the total variability. This dimension highlights the importance of perigon length

and the specific contribution of the species *C. variegatus*, which were less emphasized in the first two dimensions.

The PCA analysis of *Crocus* species, as depicted in Figure 2 (biplot of principal components 1 and 2; i.e., PCA dimensions F1 and F2), provides a comprehensive overview of the relationships between various morphological traits and the differentiation of species based on these traits. In the PCA biplot (a combination of the loading plot of variables and the score plot of genotypes), the vector for each variable is drawn as a line from the origin to help in identifying the relationship between the traits. The correlation between any two traits is approximated by the cosine of the angle between these vectors [39].



**Figure 2.** Biplot of principal components 1 and 2 (PCA dimensions F1 and F2) for the morphological traits (PH—plant height, CW—corm width, NL—number of leaves, LW—leaf width, NF—number of flowers, PL—perigon length, LOP—length of the outer perigon segment, LIP—length of the inner perigon segment, LSL—length of the stigma lobes, LA—length of the anthers) of seven *Crocus* species.

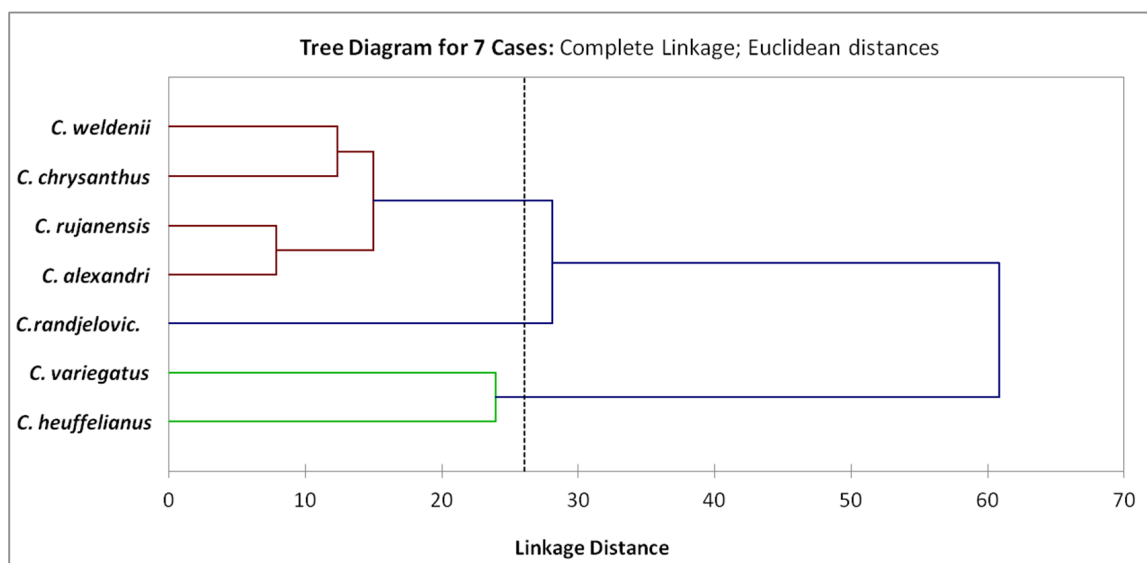
In PCA dimension F1, the relationships between traits are evident from the angles between the red vectors of the variables shown in the biplot (Figure 2). The traits LOP, LIP, LA, LW, and PH were positively correlated, as indicated by the narrow angles between their vectors. Conversely, LSL exhibited a negative association with these traits, which is evident from the obtuse angle between its vector and the vectors of the positively related traits, corroborating the results obtained by the Pearson's correlation (Table 3). In the second PCA dimension (F2), the narrow angles between NF, NL, and CW indicate positive links among these traits in F2, emphasizing their importance in species differentiation in this dimension. Analyzing the contributions of individual *Crocus* species, *C. heuffelianus* was the dominant contributor in dimension F1, characterized by traits such as longer perigon segments, wider leaves, longer anthers, and taller plants, but with shorter stigma lobes. *C. alexandri* and *C. randjeloviciorum* also significantly contributed to F1, with *C. alexandri* showing high values for LSL (length of the stigma lobes) and *C. randjeloviciorum* having high values for NL (number of leaves). In PCA dimension F2, the species *C. chrysanthus* and *C. rujanensis* were characterized by a greater number of flowers, leaves, and larger corm width, which were positively associated with each other, while *C. weldenii* (along with *C. alexandri*) stood out with longer stigma lobes.

The PCA analysis reveals that the first three principal components captured a substantial portion of the variability in the morphological traits of *Crocus* species. PCA dimension F1, which accounted for more than half of the total variability, was heavily influenced by



LOP, LIP, LA, and LW. Dimension F2 was mainly influenced by NF, NL, and CW, while F3 was largely influenced by PL. Different *Crocus* species contributed differently to the variability in each principal component. For example, *C. heuffelianus* was the major contributor to F1, *C. chrysanthus* to F2, and *C. variegatus* to F3. Overall, this analysis provides a clear understanding of how different morphological traits and species contribute to the overall variability within the *Crocus* genus [40].

Grouping of *Crocus* species based on their morphological traits was carried out using hierarchical clustering employing the complete linkage agglomeration method. The cluster analysis showed that the species were initially divided into two clusters (Figure 3); one with two species: *C. variegatus* and *C. heuffelianus*, and the other with the remaining five genotypes. All seven species were further divided into three clusters. The first cluster consisted of *C. alexandri*, *C. chrysanthus*, *C. weldenii*, and *C. rujanensis*, characterized by the highest number of leaves (NL) and the lowest number of flowers (NF) and length of the inner perigon segment (LIP). The second cluster included only *C. randjelovicorum*, distinguished by having the shortest plant (PH), smaller values of corm width (CW), leaf width (LW), perigon length (PL), shorter length of the outer perigon segment (LOP) and anthers (LA), but with the greatest number of flowers (NF) and the longest stigma lobes (LSL). The third cluster comprised *C. heuffelianus* and *C. variegatus*, notable for the tallest plant height (PH), widest corms (CW) and leaves (LW), longest perigon length (PL), outer and inner perigon segments (LOP, LIP), and anthers (LA), while having the lowest number of leaves (NL) and the shortest stigma lobes (LSL), which largely corroborated the results obtained from the PCA.



**Figure 3.** Hierarchical clustering of *Crocus* species based on morphological traits. The clusters are color-coded as follows: brown (Cluster 1), blue (Cluster 2), and green (Cluster 3).

### 3.3. Analysis of Flower Color Components in *Crocus* Species

The flower color varied among the tested species. The analysis of color components in the petals of various *Crocus* species, based on the CIELab color space parameters (lightness ( $L^*$ ), color coordinates ( $a^*$  and  $b^*$ ), chroma, and hue), revealed significant differences ( $p < 0.0001$ ) among species and between different parts (outer and inner petals/side) of the petals (Table 4). The highest lightness ( $L^*$ ) values observed in *C. weldenii* and *C. alexandri*, indicating light petals, while *C. heuffelianus* exhibited the lowest values, indicating darker petals. The color coordinate  $a^*$ , which represents the red/green component, also showed significant variation, with *C. heuffelianus* displaying the highest positive values, indicating a strong red component, whereas *C. weldenii* and *C. alexandri* had negative values, suggesting a slight green component. *C. chrysanthus* had the highest  $b^*$  coordinate, reflecting

a strong yellow component, while *C. heuffelianus*, *C. rujanensis*, and *C. variegatus* exhibited negative values, indicating a blue component. Chroma values, representing color saturation, differed significantly, with *C. chrysanthus* and *C. heuffelianus* showing the highest saturation, while *C. weldenii* and *C. variegatus* displayed lower saturation, indicating less vivid colors. Hue values, which denote the dominant wavelength of color, showed significant variation as well ( $p < 0.0001$  and  $R^2 = 0.999$ ), with *C. heuffelianus* and *C. randjelovitorium* exhibiting the highest hue angle, indicating a shift towards the purple spectrum, and *C. chrysanthus* the lowest, indicating a shift towards yellow.

**Table 4.** Color components (L\*, a\*, b\*, chroma, and hue) measured in the outer and inner petals of *Crocus* sp. with diverse flower colors.

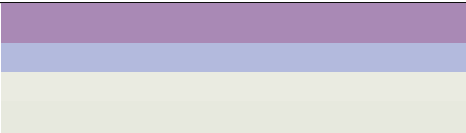



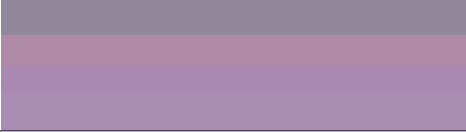
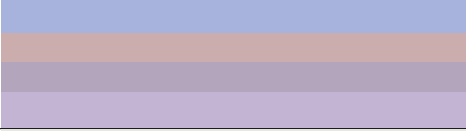
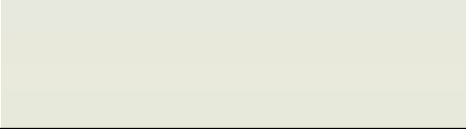
Species_Petal Side <sup>1,2</sup>	L*	a*	b*	Chroma	Hue (°)	
<i>C. alexandri</i> _O-o	61.23 ± 7.01 <sup>g</sup>	18.51 ± 2.48 <sup>e</sup>	−18.41 ± 1.59 <sup>k</sup>	26.18 ± 2.18 <sup>f</sup>	315.0 ± 4.41 <sup>cd</sup>	
<i>C. alexandri</i> _O-i	75.84 ± 3.03 <sup>d</sup>	2.89 ± 0.48 <sup>no</sup>	−1.52 ± 0.17 <sup>f</sup>	3.28 ± 0.40 <sup>op</sup>	331.7 ± 5.44 <sup>a</sup>	
<i>C. alexandri</i> _I-o	92.73 ± 1.65 <sup>a</sup>	−1.75 ± 0.28 <sup>P</sup>	4.70 ± 0.67 <sup>e</sup>	5.01 ± 0.69 <sup>no</sup>	290.5 ± 2.47 <sup>h</sup>	
<i>C. alexandri</i> _I-i	91.86 ± 2.58 <sup>a</sup>	−1.95 ± 0.32 <sup>P</sup>	4.97 ± 0.60 <sup>e</sup>	5.35 ± 0.65 <sup>m–o</sup>	291.4 ± 2.02 <sup>h</sup>	
<i>C. chrysanthus</i> _O-o	70.78 ± 3.09 <sup>ef</sup>	5.59 ± 0.67 <sup>kl</sup>	68.35 ± 4.22 <sup>b</sup>	68.59 ± 4.18 <sup>b</sup>	85.3 ± 0.74 <sup>k</sup>	
<i>C. chrysanthus</i> _O-i	70.18 ± 3.19 <sup>ef</sup>	4.45 ± 0.71 <sup>lm</sup>	66.90 ± 2.43 <sup>bc</sup>	67.05 ± 2.44 <sup>bc</sup>	86.2 ± 0.57 <sup>k</sup>	
<i>C. chrysanthus</i> _I-o	71.50 ± 5.23 <sup>ef</sup>	3.31 ± 0.45 <sup>mn</sup>	65.91 ± 4.52 <sup>c</sup>	66.00 ± 4.52 <sup>c</sup>	87.1 ± 0.39 <sup>k</sup>	
<i>C. chrysanthus</i> _I-i	68.97 ± 5.27 <sup>f</sup>	5.69 ± 0.69 <sup>kl</sup>	71.59 ± 4.16 <sup>a</sup>	71.82 ± 4.15 <sup>a</sup>	85.4 ± 0.61 <sup>k</sup>	
<i>C. heuffelianus</i> _O-o	50.90 ± 5.57 <sup>i</sup>	31.46 ± 4.35 <sup>a</sup>	−27.46 ± 3.23 <sup>m</sup>	41.77 ± 5.36 <sup>d</sup>	318.8 ± 1.09 <sup>b</sup>	
<i>C. heuffelianus</i> _O-i	52.05 ± 4.19 <sup>i</sup>	30.21 ± 1.78 <sup>b</sup>	−27.22 ± 1.53 <sup>m</sup>	40.67 ± 2.28 <sup>d</sup>	318.0 ± 0.76 <sup>b</sup>	
<i>C. heuffelianus</i> _I-o	62.89 ± 5.04 <sup>g</sup>	23.80 ± 3.88 <sup>c</sup>	−23.63 ± 3.73 <sup>l</sup>	33.54 ± 5.37 <sup>e</sup>	315.2 ± 0.55 <sup>d</sup>	
<i>C. heuffelianus</i> _I-i	64.08 ± 3.66 <sup>g</sup>	22.43 ± 2.21 <sup>d</sup>	−22.39 ± 2.38 <sup>l</sup>	31.69 ± 3.23 <sup>e</sup>	315.1 ± 0.54 <sup>d</sup>	
<i>C. randjelovicianorum</i> _O-o	83.87 ± 2.79 <sup>b</sup>	1.99 ± 0.25 <sup>P</sup>	−2.04 ± 0.20 <sup>f</sup>	2.85 ± 0.25 <sup>P</sup>	314.3 ± 3.88 <sup>d</sup>	
<i>C. randjelovicianorum</i> _O-i	75.73 ± 4.80 <sup>d</sup>	9.29 ± 1.75 <sup>hi</sup>	−13.01 ± 2.22 <sup>h</sup>	16.05 ± 2.41 <sup>i</sup>	305.5 ± 5.23 <sup>g</sup>	
<i>C. randjelovicianorum</i> _I-o	71.94 ± 4.11 <sup>ef</sup>	10.31 ± 1.34 <sup>gh</sup>	−13.10 ± 1.51 <sup>h</sup>	16.71 ± 1.67 <sup>i</sup>	308.2 ± 3.99 <sup>ef</sup>	
<i>C. randjelovicianorum</i> _I-i	79.70 ± 3.12 <sup>c</sup>	6.01 ± 0.75 <sup>k</sup>	−8.63 ± 1.35 <sup>g</sup>	10.54 ± 1.33 <sup>k</sup>	305.1 ± 4.19 <sup>g</sup>	
<i>C. rujanensis</i> _O-o	57.77 ± 5.29 <sup>h</sup>	7.48 ± 0.93 <sup>j</sup>	−9.26 ± 1.59 <sup>g</sup>	11.92 ± 1.69 <sup>jk</sup>	309.1 ± 3.35 <sup>e</sup>	
<i>C. rujanensis</i> _O-i	61.77 ± 8.40 <sup>g</sup>	17.90 ± 1.66 <sup>e</sup>	−16.48 ± 1.78 <sup>j</sup>	24.36 ± 2.10 <sup>fg</sup>	317.4 ± 2.98 <sup>bc</sup>	
<i>C. rujanensis</i> _I-o	61.06 ± 6.56 <sup>g</sup>	17.96 ± 2.36 <sup>e</sup>	−18.16 ± 2.18 <sup>k</sup>	25.56 ± 3.08 <sup>f</sup>	314.7 ± 2.17 <sup>d</sup>	
<i>C. rujanensis</i> _I-i	62.31 ± 5.30 <sup>g</sup>	15.98 ± 1.79 <sup>f</sup>	−15.90 ± 1.46 <sup>j</sup>	22.57 ± 1.96 <sup>g</sup>	315.1 ± 3.09 <sup>d</sup>	
<i>C. variegatus</i> _O-o	73.13 ± 6.87 <sup>de</sup>	2.88 ± 0.45 <sup>no</sup>	3.87 ± 0.62 <sup>e</sup>	4.84 ± 0.66 <sup>op</sup>	53.16 ± 4.57 <sup>l</sup>	
<i>C. variegatus</i> _O-i	73.36 ± 3.77 <sup>de</sup>	11.19 ± 2.40 <sup>g</sup>	−15.09 ± 3.28 <sup>ij</sup>	18.81 ± 3.95 <sup>h</sup>	306.6 ± 2.96 <sup>fg</sup>	
<i>C. variegatus</i> _I-o	69.44 ± 4.97 <sup>f</sup>	8.57 ± 1.73 <sup>ij</sup>	−10.26 ± 1.57 <sup>g</sup>	13.39 <sup>j</sup> ± 2.18	309.6 ± 3.71 <sup>e</sup>	
<i>C. variegatus</i> _I-i	75.45 ± 7.16 <sup>d</sup>	10.47 ± 1.59 <sup>gh</sup>	−14.05 ± 2.34 <sup>hi</sup>	17.53 ± 2.76 <sup>hi</sup>	306.8 ± 2.04 <sup>fg</sup>	
<i>C. weldenii</i> _O-o	92.01 ± 0.82 <sup>a</sup>	−1.99 ± 0.42 <sup>P</sup>	7.30 ± 1.51 <sup>d</sup>	7.58 ± 1.51 <sup>l</sup>	285.5 ± 2.89 <sup>j</sup>	
<i>C. weldenii</i> _O-i	91.97 ± 1.37 <sup>a</sup>	−2.25 ± 0.44 <sup>P</sup>	6.99 ± 1.39 <sup>d</sup>	7.35 ± 1.38 <sup>lm</sup>	288.1 ± 3.61 <sup>i</sup>	
<i>C. weldenii</i> _I-o	92.19 ± 0.95 <sup>a</sup>	−2.16 ± 0.53 <sup>P</sup>	6.61 ± 1.50 <sup>d</sup>	6.96 ± 1.56 <sup>l–n</sup>	288.1 ± 2.39 <sup>i</sup>	
<i>C. weldenii</i> _I-i	91.95 ± 1.20 <sup>a</sup>	−2.46 ± 0.49 <sup>P</sup>	7.59 ± 1.46 <sup>d</sup>	7.98 ± 1.51 <sup>l</sup>	288.1 ± 2.24 <sup>i</sup>	
ANOVA	R <sup>2</sup>	0.885	0.972	0.994	0.984	0.999
(n = 420)	F	111.241	504.453	2502.855	919.081	13,327.590
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup> Seven *Crocus* sp., with petal side defined as O-o, O-i, I-o, and I-i (O-outer and I-inner petals of the perianth; on outer (-o) and inner (-i) side). <sup>2</sup> Mean value ± standard deviation (SD). Within each column, means followed by different letters are significantly different at 5% level of significance according to Fisher's LSD test ( $p \leq 0.05$ ). L\*—Lightness, a\* and b\*—color coordinates, chroma—saturation (vividness), hue—an angular value (°) representing the dominant wavelength.

Examining individual species (Tables 4 and 5), *C. randjelovitorium* displayed high lightness and a low chroma value in its outer-outer petals, indicating white-purple petals with dark purple patterns, while its inner-inner petals were characterized as light purple. *C. alexandri* showed a notable difference between its outer-outer and inner-inner petals, with the former being dark purple with white borders and the latter being white. *C. chrysanthus*

exhibited dull deep yellow petals with higher chroma in its inner-inner petals compared to the outer-outer petals. *C. weldenii* and *C. rujanensis* both had variations in lightness and color components between their outer-outer and inner-inner petals. *C. weldenii* was characterized by very light, white petals, while *C. rujanensis* displayed dull purple petals, lighter in the inner-inner petals. *C. heuffelianus* had the darkest petals among the species studied, with strong red and blue components, described as purple for outer-outer petals and lighter purple for inner-inner petals. *C. variegatus* showed white-blue outer-outer petals with dark brown-purple patterns, while its inner-inner petals were light white-purple.

**Table 5.** Color of the petals of *Crocus* species: outer and inner parts of the perigon based on visual description and the conversion of the recorded colors from the LAB system to the RGB system.

Species_Petal Side <sup>1</sup>	Color of the Petals (Visual Description)	Color RGB <sup>2</sup>
<i>C. alexandri</i> _O-o	Dark purple with white borders	
<i>C. alexandri</i> _O-i	Purple base with white blue top	
<i>C. alexandri</i> _I-o	White	
<i>C. alexandri</i> _I-i	White	
<i>C. chrysanthus</i> _O-o	Dull deep yellow	
<i>C. chrysanthus</i> _O-i	Dull deep yellow	
<i>C. chrysanthus</i> _I-o	Dull deep yellow, lighter than O-o and O-i petals	
<i>C. chrysanthus</i> _I-i	Dull deep yellow, darker than the other petals	
<i>C. heuffelianus</i> _O-o	Purple	
<i>C. heuffelianus</i> _O-i	Purple, lighter than O-o	
<i>C. heuffelianus</i> _I-o	Purple	
<i>C. heuffelianus</i> _I-i	Dull purple, lighter than I-o	
<i>C. randjeloviciorum</i> _O-o	White-purple base with dark purple stripes	
<i>C. randjeloviciorum</i> _O-i	L. purple with dark purple pat., darker than the O-o	
<i>C. randjeloviciorum</i> _I-o	Light purple, similar color to O-i petals	
<i>C. randjeloviciorum</i> _I-i	Light purple	
<i>C. rujanensis</i> _O-o	Dull purple	
<i>C. rujanensis</i> _O-i	Dull purple, lighter than O-o	
<i>C. rujanensis</i> _I-o	Dull purple, darker than O-i	
<i>C. rujanensis</i> _I-i	Dull purple, lighter than I-o	
<i>C. variegatus</i> _O-o	White-blue with dark brown-purple stripes	
<i>C. variegatus</i> _O-i	White-purple with light brown patterns	
<i>C. variegatus</i> _I-o	White purple	
<i>C. variegatus</i> _I-i	Light white-purple	
<i>C. weldenii</i> _O-o	White	
<i>C. weldenii</i> _O-i	White	
<i>C. weldenii</i> _I-o	White	
<i>C. weldenii</i> _I-i	White	

<sup>1</sup> Seven *Crocus* sp., with petal side defined as O-o, O-i, I-o, and I-i (O—outer and I—inner petals of the perianth; on outer (-o) and inner (-i) side). <sup>2</sup> RGB (R—red, G—green, B—blue) color values.

The analysis of correlations between the color components of *Crocus* petals (Table 6) revealed significant relationships among various parameters (at a significance level of  $\alpha = 0.05$ ). Lightness ( $L^*$ ) exhibited a strong negative correlation ( $r = -0.87$ ) with the coordinate  $a^*$ , indicating that lighter petals had a considerably lower red component. Lightness also showed a moderate negative correlation with chroma ( $r = -0.49$ ), implying that lighter petals had lower color saturation. The red/green coordinate ( $a^*$ ) had a moderate negative correlation ( $r = -0.52$ ) with the yellow/blue coordinate ( $b^*$ ), showing that an increase in the red component was accompanied by a decrease in the yellow component. The correlation between  $a^*$  and chroma, as well as  $a^*$  and hue, was weak (statistically insignificant) and positive ( $r = 0.33$  and  $0.32$ , respectively). Coordinate ( $b^*$ ) demonstrated a strong positive

correlation with chroma ( $r = 0.63$ ), meaning that petals with a higher yellow component had higher color saturation, while a very strong negative correlation with hue ( $r = -0.86$ ) indicated that an increase in the yellow component was accompanied by a decrease in hue, suggesting a shift towards the yellow spectrum. Finally, chroma and hue had a significant negative correlation ( $r = -0.63$ ), showing that petals with higher color saturation had a lower hue, indicating a shift towards more vivid and distinct colors.

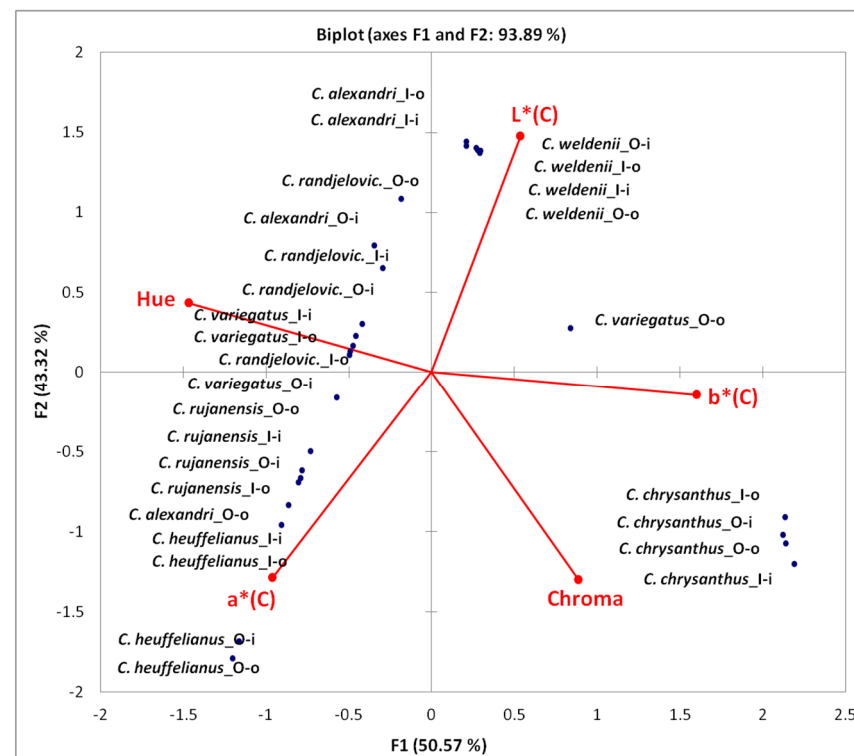
**Table 6.** Pearson's correlation coefficients ( $r$ ) between color components of *Crocus* species petals \*.

Variables	a* (C)	b* (C)	Chroma	Hue
L* (C)	<b><i>-0.87</i></b>	0.24	<b><i>-0.49</i></b>	-0.02
a* (C)	1	<b><i>-0.52</i></b>	0.33	0.32
b* (C)	-	1	<b><i>0.63</i></b>	<b><i>-0.86</i></b>
Chroma	-	-	1	<b><i>-0.63</i></b>

L\*—lightness, a\* and b\*—color coordinates, chroma—saturation (vividness), hue—an angular value (°) representing the dominant wavelength. \* Bolded values in italics are different from 0 with a significance level  $\alpha = 0.05$ .

The principal component analysis of color components in *Crocus* species petals revealed significant insights into the variability and contributions of different color attributes (Table S2 and Figure 4). The PCA identified three principal components (F1, F2, and F3), which together explain 98.15% of the variance in the initial dataset. The first principal component (F1) accounted for 50.57% of the total variance, and was predominantly influenced by the b\* coordinate (38.16%) and hue (32.10%), indicating that F1 mainly represented the contrast between the yellow/blue color spectrum and hue. The second principal component (F2) explained 43.32% of the variance, and was characterized by the contributions of the lightness (L\*) (38.00%), chroma (29.47%), and a\* coordinate (28.87%). This suggests that F2 captured the variations related to lightness, color saturation of the petals, and the red/green spectrum. The third principal component (F3) accounted for a minor proportion of the variance (4.27%). Given the low eigenvalue and variance explained, F3 appears to have had a negligible impact on the overall color variation in *Crocus* species petals.

Analyzing the contributions of individual *Crocus* species to the principal components revealed distinct patterns, highlighting the variability in petal coloration among the species (Table S2 and Figure 4). *C. chrysanthus* showed the highest contributions to the first PCA dimension (F1), with values ranging from 16.06% to 17.18% across its outer and inner petals, primarily through its b\* coordinate and hue. The high contributions of *C. chrysanthus* indicated that this species displays notable differences in these color attributes compared to others, emphasizing its distinct petal coloration. *C. heuffelianus* contributed significantly to dimension F2, particularly in its outer-outer petals (11.43%) and outer-inner petals (10.14%), highlighting its vivid petal coloration. *C. alexandri* showed moderate contributions to both F1 and F2, with its inner-outer petals contributing 7.43%, and inner-inner petals contributing 7.16% to the F2 dimension, reflecting lighter petal coloration and moderate impact on color variation. *C. weldenii* had notable contributions to F2, with values of 6.72% to 7.00% across its various petal parts, indicating its lighter petal coloration. *C. variegatus* exhibited a remarkable influence on F3, especially in its outer-outer petals, contributing 82.85%, underscoring its unique color characteristics in the yellow/blue spectrum. *C. rujanensis* displayed moderate contributions to F1, with values such as 2.20% for the outer-inner petals and 2.24% for the inner-outer petals, reflecting its intermediate positions in the color attribute spectrum. *C. randjeloviciorum* contributed less prominently compared to other species, with values such as 4.19% for the outer-outer petals to F2, indicating its moderate influence on color variability.



**Figure 4.** Biplot of PCA dimensions F1 and F2 for color components ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue) of *Crocus* species petals.

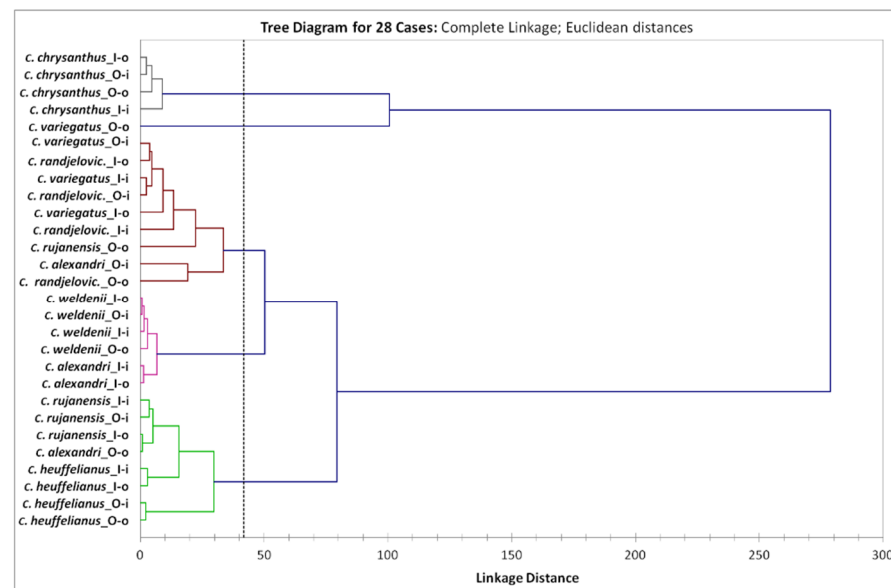
These patterns of contributions across different *Crocus* species and their respective petal parts emphasize the diversity in petal coloration within the genus. The PCA analysis effectively captured these variations, providing insights into the specific color attributes that distinguish each species and highlighting the complex interplay of color components in *Crocus* species petals.

A visual representation of the relationships between the color components of *Crocus* species petals and the contributions of individual *Crocus* species to the principal components (as explained in Table S2) is graphically depicted using a correlation biplot (Figure 4).

Hierarchical clustering of *Crocus* species based on their petal color components reveals clear groupings that help understand the relationships and similarities among these species (Figure 5). Using the complete linkage agglomeration method, the cluster analysis divided the species based on the color of their petals and their outer and inner sides into five clusters.

The first cluster consisted of all petal sides (O-o, O-i, I-o, and I-i) of *C. chrysanthus*, characterized by high values of the  $b^*$  coordinate (68.19) and chroma (68.36), indicating a bright yellow color component, and a hue value of 86.01, suggesting a yellowish hue. Cluster 2 included only *C. variegatus* (O-o) with low values of  $a^*$  (2.88),  $b^*$  (3.87), and the lowest values of chroma (4.84) and hue (53.16), indicating a less intense, greenish-yellow color component. The third cluster comprised *C. randjelovicorum* (all petal sides), *C. alexandri* (O-i), *C. rujanensis* (O-o), and *C. variegatus* (O-i, I-o, and I-i), characterized by a bluish component (negative  $b^*$  value of  $-9.66$ ) and a high hue value (310.77) indicating a bluish hue. Cluster 4 included *C. alexandri* (I-o, I-i), and *C. weldenii* (all petal sides), with the highest  $L^*$  value (92.12) indicating light colors, the lowest and negative  $a^*$  value ( $-2.09$ ) suggesting a greenish component, and a moderate hue (288.60) indicating a bluish-green hue. Cluster 5 consisted of *C. alexandri* (O-o), *C. rujanensis* (O-i, I-o, I-i), and all petal sides of *C. heuffelianus*, characterized by the lowest lightness ( $L^*$ ) value (59.53) and  $b^*$  coordinate ( $-21.21$ ), but the highest  $a^*$  coordinate (22.28) and hue (316.15), and high chroma (30.79), indicating an intense, dark purplish-blue color component.





**Figure 5.** Hierarchical clustering of *Crocus* species based on color components of petals. Seven *Crocus* sp., with petal side defined as O-o, O-i, I-o, and I-i (O—outer and I—inner petals of the perianth; on outer (-o) and inner (-i) side). The clusters are color-coded as follows: gray (Cluster 1), blue (Cluster 2), pink (Cluster 3), and green (Cluster 4).

#### 4. Discussion

Understanding the natural distribution of species is crucial for successful cultivation and growth, as well as for selecting species for further breeding. Although cultivation is generally effective, high-altitude species often do not flower regularly at the lower altitudes where collections are maintained, resulting in slow deterioration over time [41].

Based on plant height, the tested species *C. alexandri*, *C. chrysanthus*, *C. weldenii*, and *C. randjeloviciorum* were slightly below the average reported in previous studies [3,28]. On the other hand, *C. variegatus*, *C. rujanensis*, and *C. heuffelianus* were consistent with previous results in Serbia [3], but within a lower range. These observed deviations can be attributed to the influence of habitat conditions and warmer winter temperatures during the study year [42]. This is consistent with previous research, which indicates that the height of *C. vernus* plants decreases under higher winter temperature conditions [43].

Analysis of temperature data from 1888 to 2006 at 15 stations in Serbia indicates a significant increase in minimum winter temperatures with occurrence of extremely warm and dry winters on average once in ten years [44]. However, in the past decade, these extremes have been recorded three times (2014, 2018, and 2024) [42]. Consistent with the mentioned climate scenario, similar deviations in plant morphology of *Crocus* species may become more frequent in the future. According to previous studies [45], such temperature shifts result in plant species' migration to higher altitudes where they could not previously survive and/or shifting northwards from their current habitats in the Northern Hemisphere [46]. This presents a challenge for species like *Crocus* sp., which have limited seed dispersal capabilities, and decreasing habitats.

In this research, the tested species with height below the average of previous studies [3] also had fewer leaves than those reported in the literature [3]. This finding is consistent with previous research [41,42,47], which indicates that higher winter temperatures contribute to a reduction of plant growth and decreased biomass, with smaller underground organs as a result. The NL of tested species *C. weldenii* was in the range of previous studies [3,28]. However, *C. heuffelianus* was in the range of a previous study conducted in Serbia [3], but not with the research of Maw [32]. The obtained results indicate significant differences in morphological characteristics among *Crocus* species in Serbia. These differences will be valuable for taxonomic and ecological studies, as well as for conservation strate-

gies. Moreover, the observed variability in morphological traits underscores the diversity among *Crocus* sp. in their native habitats. This diversity is crucial for understanding the adaptability and ecological significance of these species in various environments [48].

The influence of temperature is also noted in time and duration of the flowering period. The flowering period of the tested spring species varies: *C. heuffelianus* blooms in April or May, which is later than other tested species that flower in February [3,49]. In spring, the flowers began to open at temperatures between 10 °C and 12 °C, with the opening rate increasing at higher temperatures [50]. Higher growth temperature (17 °C compared to 10 °C) promoted anthesis but shortened the flowering period, reduced flower longevity, and decreased flower size [49], which is consistent with our findings. The number of flowers (NF) observed in our investigation is consistent with previous research conducted in Serbia [3] for tested species, as well as with findings for *C. reticulatus* in Greece [41]. However, in Turkey, *C. chrysanthus* has been reported to produce only one flower per plant [51], while in Serbia, this species typically produces 1–3 flowers per plant [3].

Findings from our research have several practical applications. In taxonomy, hierarchical clustering helps classify and differentiate *Crocus* species, providing deeper insights into their morphological diversity [52]. In conservation, identifying clusters with unique traits can help prioritize species for conservation efforts, especially those with distinct or rare characteristics. For breeding programs, understanding these morphological clusters can assist breeders in selecting parent species for developing new varieties with desired traits [53]. The hierarchical clustering analysis effectively categorized *Crocus* species into distinct groups based on their morphological traits. This method provides valuable insights into the relationships and differences among species, applicable in taxonomy, conservation, and breeding programs [54,55]. The distinct clusters highlight the diversity within the genus and underscore the importance of considering multiple traits for a comprehensive understanding of species differentiation, providing valuable information for further botanical, ecological, and horticultural studies [31,38]. The differentiation of species based on petal color components enhances the classification and understanding of *Crocus* sp. diversity. The obtained parameters can be used for quick field identification of species, as well.

Considering that certain species are endemic and limited to small population sizes, future research directions should prioritize the domestication potential, optimized propagation techniques, and the establishment of ex situ conservation collections (gene banks). Developing efficient methods for cultivation and multiplication will support the preservation of genetic diversity and enhance population resilience in *Crocus* species. Sustainable cultivation practices and habitat restoration are essential to ensure these valuable plants' survival and continued utility for future generations.

Identifying species with unique color traits can help prioritize them for conservation efforts, especially those with rare or distinct characteristics [49]. However, the problem arises due to the very short flowering period of the genus *Crocus* (flowers live 3–4 days) [56].

For breeding programs, understanding the color components assists breeders in selecting parent species to develop new varieties with specific desired traits, such as unique or more vibrant petal colors. The clustering patterns observed align with the PCA results, offering a comprehensive understanding of species differentiation based on petal color traits [57,58]. This analysis highlights significant similarities and differences in petal coloration, underlining the importance of these color components in distinguishing *Crocus* species. In a study involving 106 *Crocus* species [59], researchers investigated whether the style length of *Crocus* flowers is influenced by petal color. Notably, this trend does not apply to *C. randjeloviciorum*, where all three size ratios (longer, shorter, or equal style to stamen) are equally represented [59], which is in agreement with our research as well. The hierarchical clustering analysis based on petal color components provides a detailed visualization of the relationships and differentiation among *Crocus* species' petal coloration, flower fragrance [60], and population density [61], factors that are also significant for pollination [62]. As different species use different strategies to attract insects,

the relationship between the styles and the height of the stamens, which is often linked to the color of the flower, also influences the pollination process [60].

The detailed analysis of color components using CIELab parameters effectively categorizes *Crocus* species, providing a robust framework for further research and practical applications in taxonomy, conservation, and breeding programs [56]. This comprehensive understanding of petal color diversity within the genus underscores the importance of considering multiple color traits for a thorough understanding of species differentiation [38]. The remarkable diversity of flower types and colors in *Crocus* species is determined by the synthesis of various pigments [38,57,63–65]. Previous studies have shown that anthocyanins and carotenoids are the key pigments responsible for the color spectrum, ranging from purple or brownish striped patterns to uniformly lilac, violet, or blue crocus flowers [63]. Anthocyanins, in particular, contribute to color variation. The color variation in the tested species, *C. alexandri*, *C. chrysanthus*, and *C. heuffelianus*, is influenced by the presence of anthocyanins A5 and A6. Additionally, *C. alexandri* and *C. heuffelianus* also contain A3 and A4 anthocyanins, adding to all the mentioned pigments. Beyond these, *C. heuffelianus* possesses A8 and A9 anthocyanins as well [63]. *C. chrysanthus* shows considerable variability in flower colors that does not correlate with its distribution, habitat, or chromosome number, making the study of its phenotypic variation difficult [27,66]. The analysis of correlations between the color components of *Crocus* species petals provides insights into the interrelationships of different color components in *Crocus* species petals, revealing significant associations that can be useful for understanding and predicting petal coloration patterns in this genus [50].

## 5. Conclusions

The phenotypic characterization of seven spring-flowering *Crocus* species native to Serbia has offered significant insights into the relationships among various morphological traits. Our research has shown that while the year significantly influences the morphological characteristics of individual species, the overall ratio among species according to these traits remains mostly consistent. The phenotypic profiles established by this study enhance our understanding of *Crocus* species and their morphological characteristics. The research highlights the negative impact of high temperatures and low winter precipitation, which result in smaller plants, earlier and shorter flowering periods, and smaller flower sizes across species. These findings are crucial for taxonomic classification, conservation efforts, and breeding programs, ultimately supporting biodiversity preservation and horticultural development.

Although DNA analysis is the most reliable method for identifying *Crocus* species, it is essential to have rapid identification indicators in the field based on the shoot of the plant. In the tested species, in addition to flowering time, flower color can also serve as a useful species indicator, as it remains mainly consistent despite variations in the year or habitat.

The study's results facilitate rapid field identification of *Crocus* species, allowing researchers and conservationists to quickly distinguish species based on flower color, thereby enabling more efficient monitoring and conservation efforts that aid in biodiversity assessments and the targeted preservation of endangered or endemic species in their natural habitats.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae10111214/s1>, Table S1: Principal component analysis: eigenvalues, eigenvectors, and contributions of variables and observations for the first three components (PCA dimensions F1 to F3) in *Crocus* species. Table S2: principal component analysis: eigenvalues and contributions of color components of *Crocus* species petals for the first three dimensions (F1 to F3).

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