

Quantitative taxonomy of flower color in *Gladiolus × gandavensis* cultivars

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ABSTRACT

Gladiolus × gandavensis is one of the world's four famous cut flowers. Flower color is an important basis for the classification of *Gladiolus × gandavensis*. To define the flower color of *Gladiolus × gandavensis* more scientifically, twenty-three *Gladiolus × gandavensis* horticultural cultivars were studied for flower color phenotypes to provide a preliminary phenotypic quantitative basis for the determination of the affinity of unknown cultivars in the species and provide a scientific basis for the definition of flower color in the classification process. Based on the results of the RHSCC colorimetric card and colorimeter, the 23 *Gladiolus × gandavensis* cultivars were classified into six flower color categories, i.e. white, pink, purple, greenish-yellow, orange, and red. Clustering analysis further verified the above classification results according to the flower color, indicating that the two have good correspondence. At the same time, the characteristics of the distribution of the parameters of the CIELab color system of *Gladiolus × gandavensis* were analyzed and preliminarily determined the distribution range of the phenotypic parameters of each color group, and the results filled a gap in the study of the floral phenotype of *Gladiolus × gandavensis*.

Keywords: *Gladiolus × gandavensis*, RHSCC method of designating colors, CIELab color system, flower color phenotype, cluster analysis

INTRODUCTION

Gladiolus × gandavensis is a perennial monocotyledonous geophyte flower of the genus *Gladiolus* in the family Iridaceae [Zhao 1985]. Their corms are oblate, with yellowish-brown or brown membranous outer skins. Basal leaves are sword-shaped, alternate, and arranged in two rows. Flowering stems are erect and drawn from the leaf tufts [Zhao 1985]. They have terminal spikes, flowers symmetrical on both sides and colorful, beautiful flowers making them one of

the world's four famous cut flowers [Yi et al. 2000]. *Gladiolus × gandavensis* is a hybrid species, probably formed by crossing *Gladiolus natalensis* and *Gladiolus oppositiflorus*. There are many cultivars of *Gladiolus × gandavensis*, and due to its complex genetic background, the classification of *Gladiolus × gandavensis* cultivars has been a difficult problem. According to the difference in blooming habit, it has been classified as a spring-flowering cultivar, which is planted in the fall

and blooms in the following spring, as well as a summer-flowering cultivar, which is planted in the spring and blooms in the summer and fall [Chen 1990].

Flower color is a qualitative and quantitative trait controlled by multiple genes, involved in plant pollination, plays a role in plant defense against biotic and abiotic stresses and other physiological functions, and is an important basis for the classification of plant cultivars [Zeng et al. 2021]. Within the same color family, differences in color shade are exhibited due to environmental factors such as cultivation conditions (light intensity, temperature, nutrient availability, growth season), pigment system, types of pigments (anthocyanins, carotenoids, etc.) and cell sap pH. Between different flower color families, the trait is relatively stable unless mutations or transposons occur [Liu et al. 1998]. Currently, studies on flower color in ornamental plants mainly focus on flower color phenotype, flower color glycoside composition, and the flower color formation mechanism [Ding et al. 2019]. In flower color phenotype studies, flower color description methods are usually adopted from RHSCC (Royal Horticultural Society Color Chart) and ISCC-NBS (Inter-Society Color Council-National Bureau of Standard, collated by the Domestic Color Research Society and the National Bureau of Standards) color name representations are dominant, while RHSCC is the most widely used type of colorimetric color scale for describing floral colors [Sakata et al. 1995, Hashimoto et al. 2000, Torskangerpoll and Andersen 2005]. The CIELab tabular color system, on the other hand, has been widely used for the quantitative determination of flower color in ornamental plants, and its scientific validity has been confirmed by several studies, such as *Rhododendron mucronulatum* [Li et al. 2008], *Chrysanthemum × morifolium* [Sun et al. 2010, Hong et al. 2012], *Phalaenopsis* type [Li et al. 2013], *Paeonia × suffruticosapeony* [Wu et al. 2016, Han et al. 2017], *Rosa chinensis* [Wang et al. 2017], *Malus* spp. [Jiang et al. 2017], *Rosa damascena* [Rasouli et al. 2018], *Paeonia lactiflora* [Wang et al. 2018], *Bougainvillea spectabilis* [Zeng et al. 2018], *Freesia refracta* [Ding et al. 2019], *Nelumbo* sp. [Wu et al. 2020, Liu et al. 2020], *Yulania denudata* [Du et al. 2021], *Dianthus caryophyllus* [Teng et al. 2022], *Oxalis obtusa* [Li et al. 2022] and *Viola cornuta* [Jang et al. 2023].

As an important fresh-cut flower, the flower color of *Gladiolus × gandavensis* is one of the most important indicators of its ornamental properties. The flower color of the existing *Gladiolus × gandavensis* horticultural cultivars is mainly concentrated in white, pink, red, purple, yellow series, etc., and the solid color cultivars are the mainstream. However, there are fewer studies on the flower color of *Gladiolus × gandavensis*. Wang et al. [2007] conducted a principal compositional analysis of 76 traits in 25 cultivars of *Gladiolus × gandavensis* and found that the contribution rate of flower color reached 73.93%. Liu et al. [2016] qualitatively described the flower color of *Gladiolus × gandavensis*. Years of hybridization have also brought more difficulties in classifying the flower color of *Gladiolus × gandavensis*, which has limited its promotion and related research. The objective of the current study was to provide a quantitative classification of the flower color phenotypes of *Gladiolus × gandavensis* as a reference for future research on the identification and classification of new cultivars to aid in the genetic breeding of novel flower colors.

MATERIAL AND METHODS

Plant material and cultivation conditions

Samples of 23 *Gladiolus × gandavensis* horticultural cultivars were used as experimental material (Table 1), all of which were imported summer-flowering cultivars from the Netherlands, including corms of ‘Adrenalin’, ‘Cartago’, ‘Flevo Shine’, ‘Flevo Snow’, ‘Indian Summer’ and ‘Natan’ purchased from Sichuan Bella Horticulture Co. (Chengdu, Sichuan, China), and corms of the remaining 17 cultivars were purchased from Zhejiang Hongan Horticulture Co. (Jiaxing, Zhejiang, China). *Gladiolus × gandavensis* corms were planted in April 2024 in the Experimental Garden of the College of Life Science and Biotechnology, Mianyang Teachers’ College. The base soil was yellow loam, loamy clay, with a planting density row spacing of 15 × 15 cm and a planting depth of 10–15 cm, with water and fertilizer applied per standard recommendations [Yi et al. 2000].

Flower color description and determination methods

During June–July 2024, when *Gladiolus × gandavensis* cultivars were in full bloom, six inflorescen-

Table 1. List of 23 *Gladiolus × gandavensis* cultivars used in the study at the College of Life Science and Biotechnology, Mianyang Teachers' College, China

| No. | Cultivar |
|-----|-------------------------|
| 1 | 'Adrenalin'* |
| 2 | 'Amsterdam' |
| 3 | 'Bra Val' |
| 4 | 'Cartago'* |
| 5 | 'Dador De Pan' |
| 6 | 'Essential' |
| 7 | 'Fairytale Pink' |
| 8 | 'Flevo Breezer' |
| 9 | 'Flevo Fusion' |
| 10 | 'Flevo Nautica' |
| 11 | 'Flevo Quote' |
| 12 | 'Flevo Shine'* |
| 13 | 'Flevo Snow'* |
| 14 | 'Green Star' |
| 15 | 'Indian Summer'* |
| 16 | 'Kio' |
| 17 | 'Mojito' |
| 18 | 'Natan'* |
| 19 | 'Orange Sun' |
| 20 | 'Prinses Margaret Rose' |
| 21 | 'Priscilla' |
| 22 | 'Sugar Babe' |
| 23 | 'Zamora' |

All cultivars were imported from the Netherlands: *purchased from Sichuan Bella Horticulture Co., China. Those without an asterisk were purchased from Zhejiang Hongan Horticulture Co., China

ces of each cultivar were selected, and three flowers that were in full bloom in each inflorescence were randomly selected. Since the middle and upper parts of the inner whorl petals of *Gladiolus × gandavensis* flowers are the main ornamental parts, and the main color expression of the petals is also determined by this part of the flower, the test was conducted only on the middle and upper parts of the inner whorl petals.

Colorimetry was performed using a visual inspection method and an RHSCC colorimetric card (6th edition, 920 colors) for the middle-upper center of the petal frontal surface in the inner whorl of each flower. At the same time, the lightness value (L^*), redness value (a^*), and yellowness value (b^*) of the middle-upper center of the adaxial surface of the petals of the inner whorl of each flower were measured sequen-

tially, using a colorimeter (Linshang LS173) with a fixed light source of D65, according to the CIELab colorimetric system.

Data analysis

For each cultivar, the color number with the most repetitions was selected as the color number of that cultivar for the floral description. Using Excel software, the average of the L^* , a^* , and b^* values of multiple measurements for each cultivar were calculated as the flower color. The chroma value (C^*) and hue angle value (h°) were calculated according to the formula: $C^* = (a^{*2} + b^{*2})^{1/2}$, and $h^\circ = \arctan(b^*/a^*)$. L^* , a^* , and b^* values were analyzed by squared Euclidean distance, intergroup linkage method of cluster analysis with IBM SPSS statistics 27.0 software. Correlation analy-

sis and graphing of L^* , a^* , b^* , and C^* values and different color groups were performed using Origin Pro 2020 software. The L^* value is a measure of the lightness and darkness of the flower color, and changes in the C^* value (indicates the degree of color) affect the L^* value (indicates the degree of lightness).

RESULTS

Flower color classification based on flower color description

Based on the results of the RHSCC colorimetric card, the measured floral phenotypic values of 23 *Gladiolus × gandavensis* cultivars were named and systematically categorized. A total of 13 color names were obtained (Table 2), which can be classified into six color groups (Fig. 1): I – white group, including ‘Amsterdam’, ‘Essential’ and ‘Flevo Snow’; II – pink group, including ‘Adrenalin’, ‘Flevo Fusion’, ‘Flevo Quote’, ‘Priscilla’ and ‘Sugar Babe’; III – purple group, including ‘Flevo Nautica’, ‘Mojito’ and ‘Zamora’; IV – greenish-yellow group, including ‘Bra Val’, ‘Flevo Breezer’, ‘Green Star’, ‘Kio’ and ‘Prinses Margaret Rose’; V – orange group, including ‘Cartago’, ‘Dador De Pan’ and ‘Orange Sun’; VI – red group, including ‘Fairytale Pink’, ‘Flevo Shine’, ‘Indian Summer’ and ‘Natan’.

Flower color classification based on flower color determination

According to the CIELab spatial color system, the colors of *Gladiolus × gandavensis* cultivars were widely distributed in the CIELab spatial color system, and the distribution of L^* , a^* , b^* , C^* and h° values ranged from 33.75 to 98.91, –4.65 to 61.95, –20.41 to 48.74, 3.35 to 67.68, and –1.39 to 1.52, respectively (Table 3). Among them, most of the a^* , b^* , and h° values were distributed in the range of positive values.

A cluster analysis of L^* , a^* , and b^* values of the flower color of 23 *Gladiolus × gandavensis* cultivars were classified into six color groups at the classification distance of five (Fig. 2): Class I – white, comprising three cultivars, Class II – pink, comprising five cultivars, Class III – purple, comprising three cultivars, Class IV – greenish-yellow, comprising five cultivars, Class V – orange, comprising three cultivars, Class VI – red, comprising four cultivars. The grouping results

of the cluster analysis basically coincided with the classification results based on the RHSCC colorimetric card (Table 4).

Evaluation of the CIELab color system based on the color group classified by the RHSCC color name representation method

The CIELab color system was used to evaluate the results of flower color classification of *Gladiolus × gandavensis* cultivars obtained by the RHSCC color name representation. By analyzing the CIELab color system parameters (i.e., L^* , a^* , and b^* values) of the six color groups of *Gladiolus × gandavensis*, it was found that the characteristics of each color group are obvious and can distinguish between the different color groups (Fig. 3), which are specifically expressed as follows. First, the L^* value of the white group was the largest, which was significantly higher than that of other color groups, and the L^* value of the red group was the smallest. There was an overlap between the L^* values of the purple and orange groups, but the a^* and b^* values of the orange group were much higher than those of the purple group, so the two could be clearly differentiated by the a^* and b^* values. The pink and greenish-yellow colors overlapped the L^* values, which could also be differentiated by the a^* and b^* values. Second, the a^* values of both the orange and red groups were larger and closer, but the b^* values were significantly different so that the two could be distinguished. The a^* value of the greenish-yellow group was the smallest and distributed over both the positive and negative range, while the a^* values of the remaining color groups were distributed in the positive range. Third, the b^* values of the greenish-yellow, white, pink, and orange groups were distributed in the positive range, and the greenish-yellow group was the largest. The b^* value of the purple group, which was distributed in the negative range, was the smallest, while the b^* value of the red group was distributed in both the positive and negative ranges. Although the b^* values of the white and pink groups overlapped, they could be differentiated by the a^* values. The results of the above analysis further showed that there is a good correspondence between the RHSCC color name representation and the CIELab color system, which can objectively differentiate different color groups. In addition, the classified color groups are reasonable and consistent with the phenotypic characteristics of *Gladiolus × gandavensis*.

Table 2. Flower color description (RHSCC numbers and their corresponding color description, and color groups) of petals of 23 tested *Gladiolus × gandavensis* cultivars

| No. | Cultivars | RHSCC No. | Color description | Color group |
|-----|-------------------------|------------|------------------------|-----------------|
| 1 | ‘Adrenalin’ | RHS 62C | light purplish-pink | pink |
| 2 | ‘Amsterdam’ | RHS NN155C | white | white |
| 3 | ‘Bra Val’ | RHS 8B | light greenish-yellow | greenish-yellow |
| 4 | ‘Cartago’ | RHS N30B | vivid reddish-orange | orange |
| 5 | ‘Dador De Pan’ | RHS 44B | vivid reddish-orange | orange |
| 6 | ‘Essential | RHS NN155C | white | white |
| 7 | ‘Fairytale Pink | RHS 58B | strong purplish-red | red |
| 8 | ‘Flevo Breezer’ | RHS 5D | light greenish-yellow | greenish-yellow |
| 9 | ‘Flevo Fusion’ | RHS 52D | strong pink | pink |
| 10 | ‘Flevo Nautica’ | RHS N82C | light purple | purple |
| 11 | ‘Flevo Quote’ | RHS 54D | moderate purplish-pink | pink |
| 12 | ‘Flevo Shine’ | RHS 61B | strong purplish-red | red |
| 13 | ‘Flevo Snow’ | RHS NN155D | white | white |
| 14 | ‘Green Star’ | RHS N144D | strong yellow-green | greenish-yellow |
| 15 | ‘Indian Summer’ | RHS 184D | moderate purplish-red | red |
| 16 | ‘Kio’ | RHS 154C | brilliant yellow-green | greenish-yellow |
| 17 | ‘Mojito’ | RHS N81C | brilliant purple | purple |
| 18 | ‘Natan | RHS 64A | moderate purplish-red | red |
| 19 | ‘Orange Sun’ | RHS 33B | vivid reddish-orange | orange |
| 20 | ‘Prinses Margaret Rose’ | RHS 7D | light greenish-yellow | greenish-yellow |
| 21 | ‘Priscilla’ | RHS N66C | deep purplish-pink | pink |
| 22 | ‘Sugar Babe’ | RHS 49A | strong pink | pink |
| 23 | ‘Zamora’ | RHS 75A | light purple | purple |



Fig. 1. Photographs showing the classification of 23 tested *Gladiolus* × *gandavensis* cultivars based on flower color description. I – ‘Amsterdam’, ‘Essential’, ‘Flevo Snow’; II – ‘Adrenalin’, ‘Flevo Fusion’, ‘Flevo Quote’, ‘Priscilla’, ‘Sugar Babe’; III – ‘Flevo Nautica’, ‘Mojito’, ‘Zamora’; IV – ‘Bra Val’, ‘Flevo Breezer’, ‘Green Star’, ‘Kio’, ‘Prinses Margaret Rose’; V – ‘Cartago’, ‘Dador De Pan’, ‘Orange Sun’; VI – ‘Fairytale Pink’, ‘Flevo Shine’, ‘Indian Summer’, ‘Natan’

Table 3. Determination of flower color on the middle and upper parts of the inner whorl petals of 23 tested *Gladiolus × gandavensis* cultivars

| No. | Cultivars | CIELab | | | | |
|-----|-------------------------|--------|-------|--------|-------|-----------|
| | | L^* | a^* | b^* | C^* | h° |
| 1 | ‘Adrenalin’ | 78.41 | 16.81 | 2.8 | 17.04 | 1.41 |
| 2 | ‘Amsterdam’ | 98.91 | 5.04 | 8.94 | 10.26 | 0.51 |
| 3 | ‘Bra Val’ | 86.62 | 1.93 | 41.17 | 38.69 | 0.05 |
| 4 | ‘Cartago’ | 54.58 | 55.72 | 38.42 | 67.68 | 0.97 |
| 5 | ‘Dador De Pan’ | 55.97 | 61.95 | 27.17 | 67.64 | 1.16 |
| 6 | ‘Essential’ | 84.07 | 3.06 | 4.75 | 5.65 | 0.57 |
| 7 | ‘Fairytale Pink’ | 50.90 | 58.03 | 3.93 | 58.16 | 1.5 |
| 8 | ‘Flevo Breezer’ | 97.75 | 3.44 | 46.8 | 46.92 | 0.07 |
| 9 | ‘Flevo Fusion’ | 75.72 | 31.71 | 7.64 | 32.62 | 1.33 |
| 10 | ‘Flevo Nautica’ | 66.98 | 14.84 | –14.14 | 20.5 | –0.81 |
| 11 | ‘Flevo Quote’ | 75.69 | 32.97 | 10.57 | 34.62 | 1.26 |
| 12 | ‘Flevo Shine’ | 33.75 | 48.92 | 2.62 | 48.99 | 1.52 |
| 13 | ‘Flevo Snow’ | 85.55 | 1.27 | 3.11 | 3.35 | 0.39 |
| 14 | ‘Green Star’ | 70.28 | –4.65 | 41.09 | 41.35 | –0.11 |
| 15 | ‘Indian Summer’ | 48.52 | 32.62 | 1.78 | 32.67 | 1.52 |
| 16 | ‘Kio’ | 76.52 | –1.8 | 37.37 | 37.41 | –0.05 |
| 17 | ‘Mojito’ | 58.41 | 14.42 | –20.41 | 24.99 | –0.62 |
| 18 | ‘Natan’ | 43.71 | 52.05 | –9.28 | 52.87 | –1.39 |
| 19 | ‘Orange Sun’ | 67.66 | 46.28 | 35.11 | 58.09 | 0.92 |
| 20 | ‘Prinses Margaret Rose’ | 80.56 | 0.25 | 48.74 | 48.74 | 0.01 |
| 21 | ‘Priscilla’ | 77.04 | 24.37 | 3.03 | 24.56 | 1.45 |
| 22 | ‘Sugar Babe’ | 86.56 | 33.79 | 13.97 | 36.57 | 1.18 |
| 23 | ‘Zamora’ | 68.75 | 15.83 | –9.95 | 18.7 | –1.01 |

L^* – lightness value; a^* – redness value; b^* – yellowness value; C^* – chroma value; h° – hue angle value; $C^* = (a^{*2} + b^{*2})^{1/2}$, $h^\circ = \arctan(b^*/a^*)$.

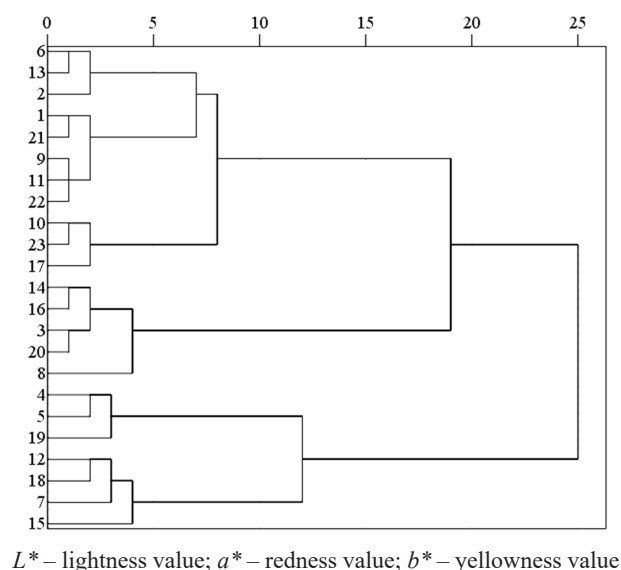


Fig. 2. Cluster analysis, based on the L^* , a^* , b^* values, of 23 tested *Gladiolus × gandavensis* cultivars. The numbering of the cultivars in Figure 2 is the same as in Table 1

Characterization of phenotypic parameters distribution

Distribution of the L^* , a^* , b^* values. The flower color phenotypic parameters of *Gladiolus × gandavensis* were widely distributed. On a two-dimensional quadrant map of a^* and b^* values (Fig. 4A), the flower colors of *Gladiolus × gandavensis* cultivars in this experiment were mainly distributed in quadrants I, II, and IV. The number of germplasm resources in quadrant I was the most distributed, with the distribution of the pink group, orange group, and white group. In addition to the distribution of the greenish-yellow and red groups in quadrant I, they were also distributed in quadrants II and IV. Quadrant IV was the distribution of the purple group. There were no cultivars distributed in quadrant III, i.e., there were no flower colors with negative a^* and b^* values, indicating that there was no blue-green color group. The three-dimensional distribution of L^* , a^* , and b^* (Fig. 4B) showed that the color group was roughly distributed in three-dimensional bands.

Relationship between the L^* and C^* values for the different color groups. The relationship between the L^* and C^* values of *Gladiolus × gandavensis* cultivars was different for different color groups. The 23

Gladiolus × gandavensis cultivars could be classified into three groups based on the distribution of the L^* and C^* values in two-dimensional coordinates (Fig. 5). White was the first group, pink, purple, and greenish-yellow were the second group, and orange and red were the third group. A linear regression fit test of the L^* and C^* values of the three groups showed that the L^* value increased with the C^* value, i.e., the lightness and chroma of the floral phenotype were positively correlated, and the correlation between the L^* and C^* values of the three groups was not significant. The correlation coefficient between the L^* and C^* values for the first taxon is 0.6621 (Fig. 6A), the correlation coefficient between the L^* and C^* values for the second taxon is 0.3040 (Fig. 6B), and the correlation coefficient between the L^* and C^* values for the third taxon is 0.0271 (Fig. 6C).

DISCUSSION

Cluster classification of flower color phenotype in *Gladiolus × gandavensis* cultivars

In the current study, it was found that the L^* , a^* , and b^* values measured by the colorimeter could more accurately describe the petal color of *Gladiolus × gandavensis* cultivars. The classification based on

Table 4. Distribution range of flower color parameters L^* , a^* , b^* , C^* , h° of each color group of 23 tested *Gladiolus* × *gandavensis* cultivars based on the cluster analysis

| No. | Color group | Sample number | Percentage (%) | CIELab | | | | |
|-----|-----------------|---------------|----------------|-------------|-------------|--------------|-------------|-------------|
| | | | | L^* | a^* | b^* | C^* | h° |
| 1 | white | 3 | 13.04 | 85.55~98.91 | 1.27~5.04 | 3.11~8.94 | 3.35~10.26 | 0.39~0.57 |
| 2 | pink | 5 | 21.74 | 75.69~85.56 | 16.81~33.79 | 2.80~13.97 | 17.04~36.57 | 1.18~1.45 |
| 3 | purple | 3 | 13.04 | 58.41~68.75 | 14.42~15.83 | -20.41~-9.95 | 18.70~24.99 | -1.01~-0.62 |
| 4 | greenish-yellow | 5 | 21.74 | 70.28~97.75 | -4.65~-3.44 | 37.37~48.74 | 37.41~48.74 | -0.11~0.07 |
| 5 | orange | 3 | 13.04 | 54.58~67.66 | 46.28~61.95 | 27.17~38.42 | 58.09~67.68 | 0.92~1.16 |
| 6 | red | 4 | 17.39 | 33.75~50.90 | 32.62~58.03 | -9.28~3.93 | 32.67~58.16 | -1.39~1.52 |

L^* – lightness value; a^* – redness value; b^* – yellowness value; C^* – chroma value; h° – hue angle value; $C^* = (a^{*2} + b^{*2})^{1/2}$; $h^\circ = \arctan(b^*/a^*)$.

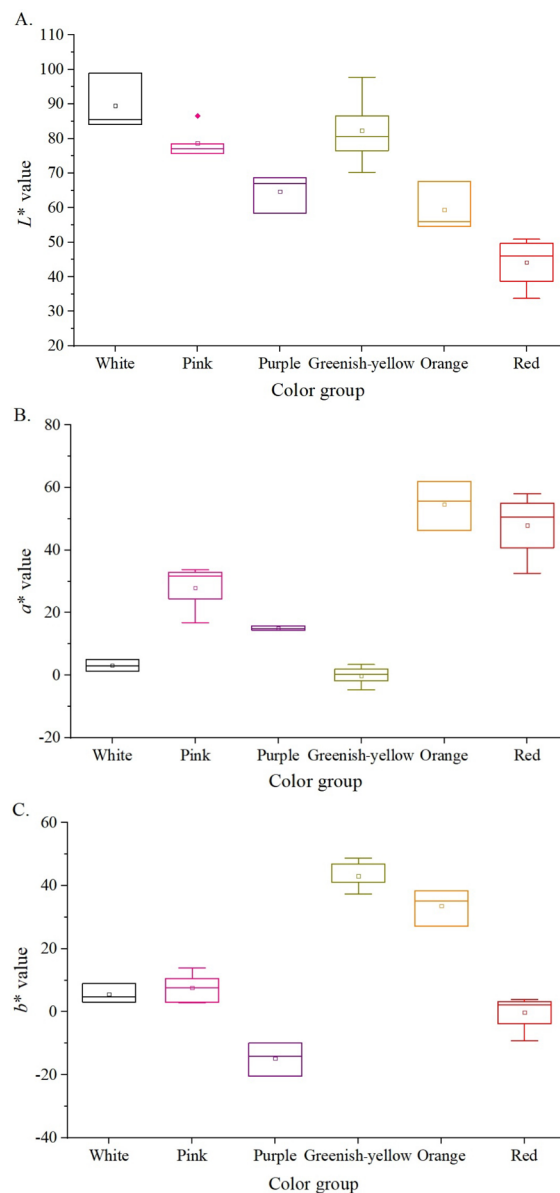


Fig. 3. Boxplot, based on the L^* , a^* , and b^* values, for each color group of 23 tested *Gladiolus × gandavensis* cultivars. A. Boxplot based on the L^* (lightness) values for each color group; B. Boxplot based on the a^* (redness) values for each color group; C. Boxplot based on the b^* (yellowness) values for each color group.

the description of the flower color of *Gladiolus × gandavensis* cultivars and the clustering results of the flower color measurements matched, and there was no phenomenon of individual clustering inaccuracy in the flower color classification studies such as those reported for *Freesia × hybrida* [Ding et al. 2019] and *Oxalis obtusa* [Li et al. 2022]. Previous researchers mostly

used cluster analysis to classify flower color phenotype and found that the problem of not being able to differentiate the light color lineage, such as Hong et al. [2012] on a cluster analysis of *Chrysanthemum × morifolium*, found that the light pink cultivars were dispersed into the white group. Cluster analysis by Liu et al. [2020] on the flower color of *Nelumbo* sp. found

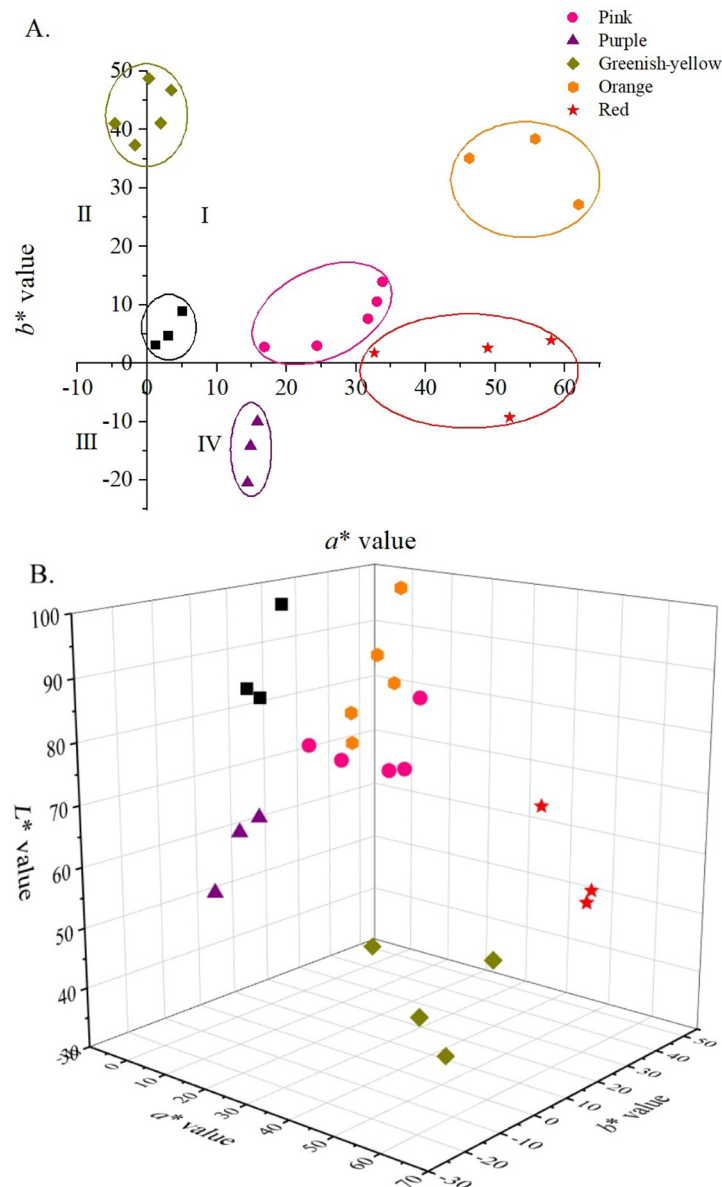
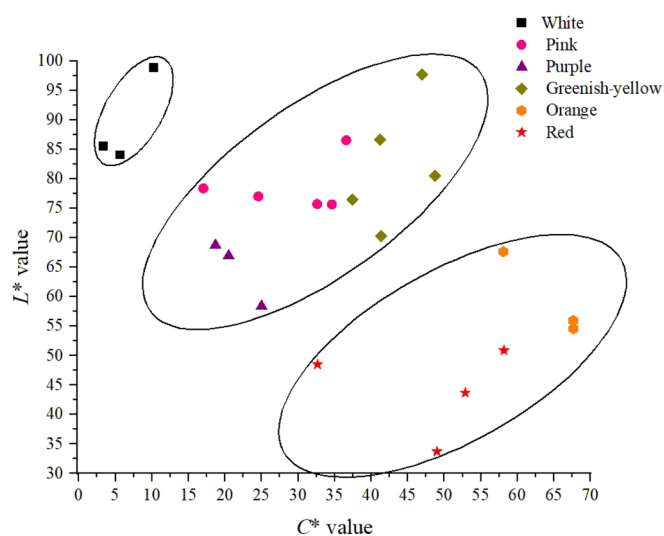


Fig. 4. Two-dimensional distribution map of the a^* and b^* values (A) and three-dimensional distribution map of the L^* , a^* , and b^* values (B) of each color group of 23 tested *Gladiolus × gandavensis* cultivars. L^* – lightness value, a^* – redness value, b^* – yellowness value

that it was not possible to differentiate the white group from the light color lineage, such as pale yellow and pink. Du et al. [2021] also found that it was not possible to distinguish the light color group on *Yulania denudata*, with white and light yellow crossing each other, but it was possible to cluster yellow-green into

a separate category. The white, pink, and greenish-yellow groups of *Gladiolus × gandavensis* cultivars in the current experiment could be clustered into a separate category, probably because cultivars have more typical white, pink, and greenish-yellow petals.



L^* – lightness value; C^* – chroma value

Fig. 5. Two-dimensional scatterplot of the L^* and C^* values of 23 tested *Gladiolus × gandavensis* cultivars

Distributional characteristics of flower color phenotype in *Gladiolus × gandavensis* cultivars

Gladiolus × gandavensis cultivars are rich in flower color and cover most color groups. On the two-dimensional quadrant map of a^* and b^* , they were mainly distributed in quadrants I, II, and IV, with no distribution in quadrant III, which is similar to some other ornamental plants, such as *Paeonia cathayana* [Han et al. 2010], *Chrysanthemum × morifolium* [Hong et al. 2012], *Rosa chinensis* [Wang et al. 2017], *Freesia refracta* [Ding et al. 2019], *Nelumbo* sp. [Liu et al. 2020], *Yulania denudate* [Du et al. 2021], and *Oxalis obtusa* [Li et al. 2022], in which blue-colored flowers are seldom seen. The main reason for the formation of blue flowers is the accumulation of blue-violet delphinidin glycosides in the petals. Many important ornamental plants in nature do not have blue flowers because they do not have the expression signal of the key enzyme gene for synthesizing delphinidin and do not have the core pigment that can show blue color [Li et al. 2021]. Therefore, in the breeding of *Gladiolus × gandavensis* for flower color improvement, it would be very meaningful to further study in depth the mechanism of flower color presentation, enrich the genetic resources of the synthesis pathway of anthocyanin glycosides in *Gladiolus × gandavensis*, and rebuild the pathway of frutillar in glycoside biosynthesis, to cultivate the blue *Gladiolus × gandavensis*.

Correlation of flower color phenotypic parameters in *Gladiolus × gandavensis* cultivars

In the quantitative classification of *Freesia refracta* [Ding et al. 2019] and *Nelumbo* sp. [Liu et al. 2020], the relationship between the L^* and C^* values was that the white and yellow groups were classified into one category, and the rest of the color groups were classified into another category. *Oxalis obtusa* [Li et al. 2022]. On the other hand, the yellow group classified into one category and the rest classified into another category. In the present experiment, in the two-dimensional scatter plot of L^* and C^* values, the white group was farther away from the greenish-yellow group, while the greenish-yellow group was closer to the pink and purple groups, and the orange group was closer to the red group. Therefore, the white group of *Gladiolus × gandavensis* cultivars was classified into one category alone, the greenish-yellow group was classified into one category with the pink and purple group, and the orange group was classified into one category with the red group.

The L^* and C^* values of flower color phenotype of *Gladiolus × gandavensis* cultivars in this experiment show a positive correlation, i.e., as the chroma increases, the lightness also increases. This is contrary to the findings that L^* and C^* values of flower color phenotype of *Consolida ajacis* [Hashimoto et al. 2000], *Freesia refracta* [Ding et al. 2019], and *Oxalis obtusa*

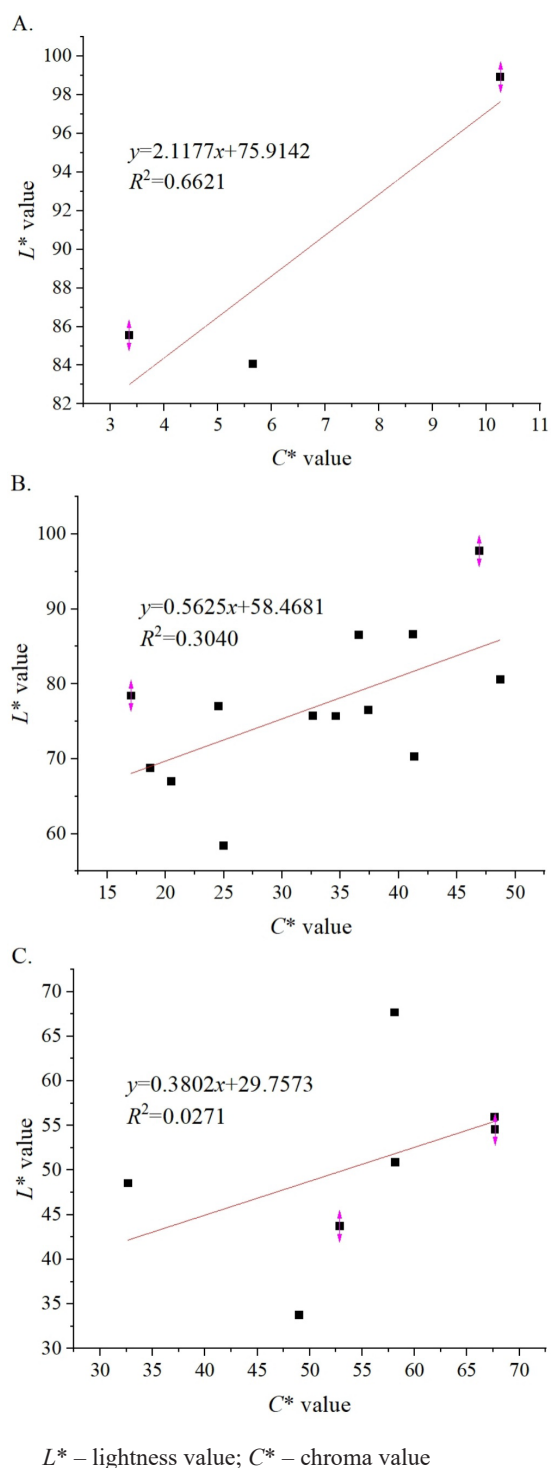


Fig. 6. . Linear regression analysis for the relationship between the L^* and C^* values of 23 tested *Gladiolus × gandavensis* cultivars. A. Relationship between the L^* and C^* values for the white group; B. Relationship between the L^* and C^* values for the greenish-yellow, pink, and purple groups. C. Relationship between the L^* and C^* values for the orange and red groups

[Li et al. 2022] were negatively correlated. Previous studies also found that total anthocyanin glycoside content, total carotenoid content, and total flavonoid content were negatively correlated with flower color lightness, while the magnitude of the colorimetric value was determined by the content of key pigments. Currently, there are fewer studies on the flower color of *Gladiolus × gandavensis*, and the reasons for the positive correlation between lightness and chroma in *Gladiolus × gandavensis* may need to be further determined through accurate pigment content testing.

Genetic diversity and taxonomic complexity of *Gladiolus × gandavensis* cultivars

Widely cultivated *Gladiolus × gandavensis* cultivars are made by interspecific, interspecies, intercultivar, species-hybrid, and intercultivar crosses of different species [Zhang 1982]. At present, there is no uniform method for their classification internationally, and most of them are classified according to their biological characteristics, reproductive period, flower shape, flower diameter, flower color, etc. Liu et al. [2016] used SRAP technology to construct fingerprints of *Gladiolus × gandavensis* and found that the classification of cultivars had no significant correlation with phenotypic traits and had certain genetic diversity at both the morphological and DNA molecular levels, which indicated that cultivars had a complex genetic background. The results of the cluster analysis of the flower color of *Gladiolus × gandavensis* cultivars in the current experiment provide a reference for the kinship relationship of *Gladiolus × gandavensis*. Due to the limited research resources, there are still many *Gladiolus × gandavensis* cultivars that have not been determined for flower color phenotype. In the future, the number of research cultivars can be increased to further optimize the quantitative classification of the flower color phenotype of *Gladiolus × gandavensis* and provide a basis for the study of the mechanism of flower color presentation.

CONCLUSIONS

This experiment used an RHSCC colorimetric card and colorimeter to describe and measure the flower colors of 23 *Gladiolus × gandavensis* cultivars, which can be roughly classified into six color groups, i.e.

white, pink, purple, greenish-yellow, orange, and red, through cluster analysis. Overall, there was a positive correlation between the lightness and the chroma of *Gladiolus × gandavensis* flower colors. The flower colors could be classified into three groups on the two-dimensional coordinate system of lightness and chroma values. The results of this study provide a theoretical basis for the selection and breeding of new cultivars and flower colors of *Gladiolus × gandavensis* and provide a scientific basis for the molecular breeding study of flower colors of other ornamental plants, as well as the determination and classification of flower colors.

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