



Article

The Composition of Anthocyanins and Carotenoids Influenced the Flower Color Heredity in Asiatic Hybrid Lilies

Jiewen Li ¹, Jiawei Chen ¹, Qian Zhang ^{1,2}, Pengcheng Yu ¹, Yanping Zhou ^{1,3} and Guixia Jia ^{1,*}

¹ Beijing Key Laboratory of Ornamental Plants Germplasm Innovation & Molecular Breeding, National Engineering Research Center for Floriculture, Beijing Laboratory of Urban and Rural Ecological Environment, Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants of Education Ministry, School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China

² College of Forestry, Shanxi Agricultural University, Jinzhong 030801, China

³ College of Agriculture and Forestry, Longdong University, Qingyang 745000, China

* Correspondence: gxjia@bjfu.edu.cn

Abstract: Flower color, including color hues and spot patterns, is an extremely important trait in Asiatic hybrid lilies. Investigating flower color variation across different cross combinations may help us select breeding parents efficiently. Thus, three hybridizations with different compositions of anthocyanins and carotenoids were performed, and 65, 289 and 125 offspring were obtained, respectively. For the parents and progenies, flower color hues were quantified by the CIELAB system, and the total number of raised spots on tepals was counted. Then, a cluster analysis and a statistical analysis were used to explore the inheritance patterns of flower color. The results showed that an orange flower color with high levels of carotenoids was highly heritable and that the progenies were less segregated, showing orange, orange-red, and yellow flowers. Parents showing red and purple-red flowers with differing levels of carotenoids and anthocyanins produced offspring with extensive segregation, including pink-white, pink, orange-pink, orange-red, yellow, and orange flowers. Meanwhile, different contents of anthocyanins and carotenoids resulted in variable proportions in color groups. Additionally, for F1 generation, the number of raised spots was continuously separated, demonstrating distinct quantitative genetic characteristics. For parents with few or intermediate spots, the hybrid plants showed both prominent negative and positive heterosis. For parents with many spots that covered almost the full tepals, the hybrid seedlings all exhibited raised spots.

Keywords: Asiatic hybrid lilies; flower color; raised spots; CIELAB color system; anthocyanins; carotenoids



Citation: Li, J.; Chen, J.; Zhang, Q.; Yu, P.; Zhou, Y.; Jia, G. The Composition of Anthocyanins and Carotenoids Influenced the Flower Color Heredity in Asiatic Hybrid Lilies. *Horticulturae* **2022**, *8*, 1206. <https://doi.org/10.3390/horticulturae8121206>

Academic Editors: Margherita Beruto, Emmy Dhooghe and Bruce Dunn

Received: 21 November 2022

Accepted: 10 December 2022

Published: 16 December 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Lilium is a major commercial floricultural crop around the world, due to its large, showy, fragrant, and multicolored flowers. The genus, which includes 110–115 species, is classified into eight sections, including *Sinomartagon*, *Martagon*, *Pseudolirium*, and others [1–3]. Interspecific and intersection hybridizations are the principal methods used for lily breeding [4–6]. Currently, more than 10,000 cultivars are registered with the Royal Horticulture Society, which are classified into different established hybrid groups, such as Asiatic, Oriental, and Longiflorum hybrids [7].

Asiatic hybrid lilies refer to the large hybrid population in the *Lilium* genus, which is derived from interspecific crosses of the *Sinomartagon* section [4]. These lilies are popular and are widely cultivated for landscaping due to their extensive variation in flower color [8], which is one of the most important characteristics determining the commercial value of lilies. Anthocyanins [9] and carotenoids [10–12] are the main pigments accumulated in their flowers. Generally, anthocyanins are responsible for pink colors, whereas carotenoids are primarily responsible for orange and yellow hues, and a combination of the two is responsible for red hues [9,10,13–15].

Some lily breeders have focused on the heredity of flower color hues for Asiatic hybrid cultivars to improve the aesthetics of their flowers. It was found that in orange \times yellow and orange \times orange crosses, the seedlings primarily bore orange flowers, and a few of them showed yellow flowers. For example, hybrids with orange-red as their dominant flower color were produced when the orange-red-flowered cultivars ('Compass', 'Gran Paradiso' and 'Alisa') were selected as parents [16]. The yellow-flowered Asiatic hybrid cultivar 'Dreamland' was crossed with the orange-flowered wild species *L. lancifolium* to produce F1 plants with flowers showing varying degrees of orange and a few individuals with yellow flowers [17]. Similarly, 'Kotnali', an orange-flowered cultivar, and 'Tiny Bee', a yellow-flowered cultivar, were crossed to produce hybrid progeny, which separated into plants with orange and yellow flowers [18]. Furthermore, the offspring from the following crosses, white \times salmon-orange, orange-red \times pink, and pink \times orange, displayed parental codominance for the color hues. In the cross between 'Olga' (apricot-white) and 'Arabeska' (salmon-orange), the progenies showed flowers with intermediate hues of rosy-pink, apricot, and salmon-orange [16]. Progenies of the cross between the pink-flowered Asiatic hybrid cultivar 'Renoir' and the orange-flowered wild species *L. davidii* var. *willmottiae* presented flowers with different degrees of pink, orange-pink, and yellow [19]. However, due to the lack of hybrid populations and quantitative descriptions, the separation regularity of flower color controlled by different compositions and contents of anthocyanin and carotenoid remains unclear.

Currently, the quantification of color phenotypes is an important and objective method for defining flower color. The CIELAB (International Commission on Illumination $L^*a^*b^*$) scale has been widely utilized in quantifying flower and fruit color phenotypes. The CIELAB color system is a color space that characterizes certain colors into geometric coordinates by employing the coordinate axes L^* , a^* , and b^* , which represent lightness, redness (red to green), and yellowness (yellow to blue), respectively [20,21]. As this color system can measure the target color rapidly and precisely using colorimetry, it is frequently used in the determination and classification of flower color hues in ornamental plants, such as *Magnolia biondii*, *Alstroemeria pallida*, *Narcissus*, *Rhododendron*, *Hemerocallis*, *Chrysanthemum*, *Zantedeschia hybrida*, *Gerbera hybrida*, rose, and *Lilium* [22–31].

In addition to color hues, spot patterns, including raised spots (also known as papillae), splatter-type spots, and brush marks, are also important factors affecting flower color [8,32–34]. Among them, raised spots are a unique pigmentation morphology in lilies [35]. The interior surfaces of tepals are raised to develop bumps where parenchymal and epidermal cell numbers increase and anthocyanin pigments accumulate [36]. Several studies have focused on the heredity of raised spots. In the Asiatic hybrid lily crosses with spotted cultivars ('Montreux', 'Renoir', and 'Orlito') \times a non-spotted cultivar ('Connecticut King'), F1 plants with and without spots segregated at a 1:1 ratio, which indicated that the presence or absence of raised spots was regulated by a single locus [32,35]. Moreover, progenies of the cross between the 'White Fox' (no spots) and the 'Connecticut King' (no spots) showed that the segregation ratio of individuals with and without spots was 1:3, which suggested that a pair of alleles suppressed spot formation [37]. However, Zhao et al. reported that dominant genes promote spot formation in Asiatic hybrid lilies [38]. Additionally, the raised spot patterns on the perianths of Asiatic hybrid lilies are multifarious. They range in distribution from the basal part and marginal zone to half the tepals and almost the full tepals. Therefore, to clarify the intricate inheritance patterns of raised spots in lilies, more hybrid combinations and larger F1 populations are imperative.

In the present research, four Asian hybrid cultivars with different flower colors were selected as parents to design three cross combinations. The pink-white-flowered cultivar 'Easy Waltz' (no carotenoids, medium anthocyanins, few spots) was used as the maternal parent in three combinations. The orange-flowered cultivar 'Tresor' (high carotenoids, no anthocyanins, intermediate spots), the red-flowered cultivar 'Red Life' (high carotenoids, medium anthocyanins, many spots), and the purple-red-flowered cultivar 'Pearl Loraine' (low carotenoids, high anthocyanins, intermediate spots) were all used as paternal parents

(pigment composition and content from Wang et al., 2021 [39]). The flower color (color hues and raised spots) of hybrid plants was determined. Then, according to the color phenotype, a cluster analysis and a statistical analysis were used to explore the heredity and variation of these decorative features for the three combinations. The objective of this study was to better understand the flower color changes from the different combinations of pigment composition and content in Asiatic hybrid lilies. The results could provide some insights into how to select parents to develop more lily cultivars with high aesthetic value.

2. Materials and Methods

2.1. Plant Materials

Four Asiatic hybrid cultivars with different flower colors were used as parents to obtain hybrid populations (Figure 1). Three cross combinations, ‘Easy Waltz’ × ‘Tresor’, ‘Easy Waltz’ × ‘Red Life’, and ‘Easy Waltz’ × ‘Pearl Loraine’, were performed, and 65, 289 and 125 seedlings were obtained, respectively (Table 1). All the cultivars were tetraploid ($2n = 4x = 48$). The hybrid seeds with embryos were stored in sand at 4 °C for a month. Then, they were sown and grown for three years until blossoming occurred. All these materials were grown in the germplasm resource nursery of Beijing Forestry University.



Figure 1. The parents of hybridizations. ♀: maternal parent; ♂: paternal parent.

Table 1. Pigment composition and content of parents and number of F1 hybrids in three cross combinations.

No.	Cross Combination	♀	Pigment Composition and Content (µg/g, DW)	♂	Pigment Composition and Content (µg/g, DW)	Hybrid Number
1	EW01	‘Easy Waltz’	no carotenoid medium anthocyanin (94.36 ± 7.36)	‘Tresor’	high carotenoid (141.38 ± 5.32) no anthocyanin	65
2	EW02	‘Easy Waltz’	no carotenoid medium anthocyanin (94.36 ± 7.36)	‘Red Life’	high carotenoid (120.78 ± 9.56) medium anthocyanin (91.33 ± 3.94)	289
3	EW03	‘Easy Waltz’	no carotenoid medium anthocyanin (94.36 ± 7.36)	‘Pearl Loraine’	low carotenoid (4.66 ± 0.16) high anthocyanin (144.43 ± 2.51)	125
Total						479

Note: pigment composition and content from Wang et al., 2021 [39]. DW, Dry weight.

2.2. Flower Color Measurement

The fresh tepal colors of all hybrids and parents were measured using a colorimeter (NF555, Nippon Denshoku Industries Co., Ltd., Tokyo, Japan) under a C/2° light source.

The inner tepals were placed on clean white paper, and the light source was aligned to the central part of the adaxial surface (Ad. 24), based on The International Union for the Protection of New Varieties of Plants [40]. The measurement was repeated three times. The average values of the color parameters, L^* , a^* , and b^* , of the CIELAB system were obtained. In this system, the parameter L^* value indicates lightness, ranging from 0 (black) to 100 (white). The parameter a^* value represents redness, with positive values for red colors and negative values for green. The parameter b^* value represents yellowness, with positive values for yellow colors and negative values for blue [20,41]. In addition, the total numbers of raised spots (abbreviated as SN) on the inner and outer tepals were counted in sequence. For the parents, the average of the spot numbers of the three individuals was calculated.

2.3. Statistical Analysis

Basic data management and statistics were performed using Microsoft Office Excel 2016. Maximum, minimum, and mean values, and the standard deviation of crossing parents and hybrids were calculated using the SPSS 25 software. The following formulae were used to determine the mid-parent value (MPs), index of heterosis (Hi), and coefficient of variation (CV):

$$MPs = \frac{P_1 + P_2}{2}$$

$$Hi = \frac{\bar{X}}{MPs} \times 100\%$$

$$CV = \frac{\sigma}{\bar{X}} \times 100\%$$

where P_1 and P_2 represent the values of the parents, \bar{X} represents the mean value of the hybrids, and σ is the standard deviation. Then, cluster analysis (between-group linkage, squared Euclidean distance) with L^* , a^* , and b^* values of all hybrids was carried out using SPSS 25 software. Finally, box plots, three-dimensional coordinate scatterplots, and frequency distribution histograms were all created using the Origin 2021 software.

3. Results

3.1. Colorimetric Evaluation and Numerical Classification of the Hybrids

The cluster analysis with L^* , a^* , and b^* values for all 479 F1 hybrids divided them into 6 flower color groups (Euclidean distance = 7.5), including orange (236 individuals), pink (18 individuals), pink-white (27 individuals), yellow (43 individuals), orange-pink (134 individuals), and orange-red (21 individuals) (Figure 2). The flower color groups accounted for 49.27%, 3.76%, 5.63%, 8.98%, 27.97%, and 4.38% of the total hybrids, respectively.

Subsequently, to clearly describe and distinguish the flower color characteristics of the 6 groups, box plots were created (Figure 3). The color coordinates showed that the L^* values of hybrids ranged from 30.71 to 83.19. The L^* values of the pink-white and yellow flower color groups were higher than those of the other groups, while that of the orange-red flower color group was the lowest. The a^* values varied from -2.68 to 38.41 ; the orange-red flower color group had the highest a^* value, while the yellow and pink-white groups had the lowest. The b^* values ranged from 2.98 to 73.35, and that of the orange flower color group was the highest, with an order of orange > yellow > orange-red > orange-pink > pink > pink-white. Furthermore, although the distribution areas of the b^* values of the pink-white and pink flower color groups overlapped, their a^* value distributions were obviously different. Similarly, the orange-red and orange-pink flower color groups could be distinguished based on the a^* value regions, even though their b^* values overlapped.

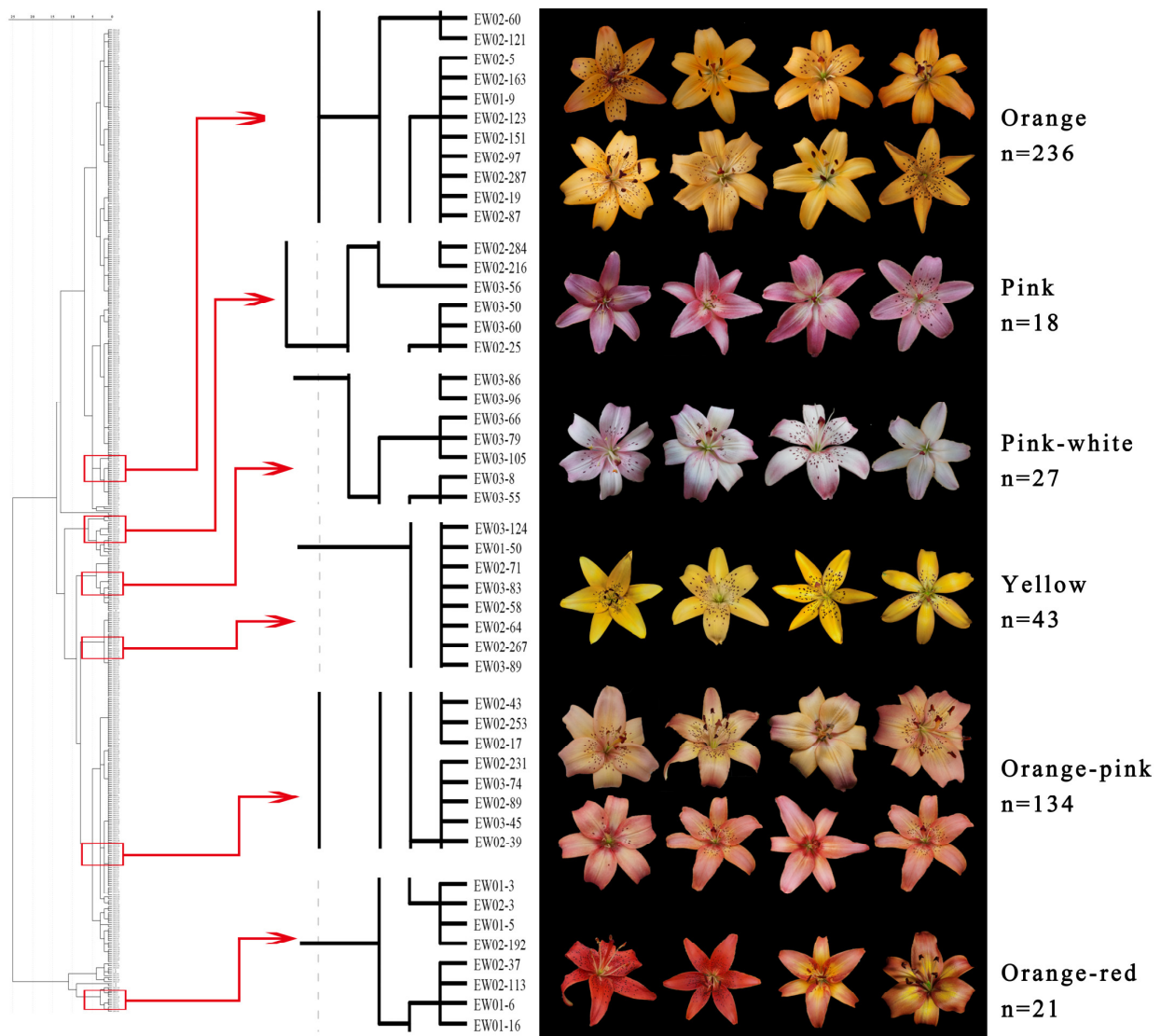


Figure 2. The cluster analysis with L^* , a^* , and b^* values for 479 F1 hybrids. The left part of this figure represents the cluster graph based on the flower color data (L^* , a^* , and b^* values) of each F1 individual; the middle part of this figure represents an enlargement of the partial branches of the cluster graph; the right part of this figure displays typical pictures of the 6 color groups (n represents the number of individuals in each group).

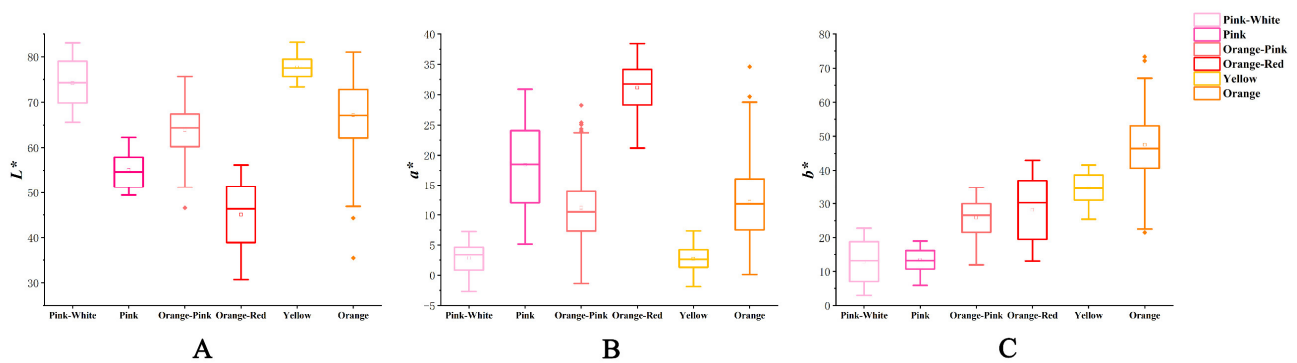


Figure 3. Box plots of different color groups for F1 hybrids with L^* (A), a^* (B), and b^* (C) values. X-coordinate: 6 color groups; Y-coordinate: color space value L^* , a^* , and b^* .

3.2. Variation in Flower Color of the Cross Combinations

To clarify the flower color diversity of F1 hybrids among the three combinations, we determined the sizes of the flower color groups for each cross (Table 2). The typical pictures of different color groups in each combination were shown (Figure 4). Meanwhile, the color space values, L^* , a^* , and b^* , of the progeny from each combination were assigned three-dimensional coordinates (Figure 5).

Table 2. The flower color segregation of F1 plants for three cross combinations.

Cross Combination	Pink-White	Pink	Orange-Pink	Orange-Red	Yellow	Orange
EW01	0	0	0	6	2	57
EW02	4	11	80	14	21	159
EW03	23	7	54	1	20	20

For the EW01 combination, there were three flower color groups (Figures 4A and 5A), including orange-red, yellow, and orange. The orange flower color group was the largest (Table 2). The offspring of EW02 were divided into 6 flower color groups (Figures 4B and 5B), which were orange, orange-pink, yellow, orange-red, pink and pink-white in order of proportion. Among them, the pink and pink-white groups had the lowest numbers of individuals, 11 and 4, respectively (Table 2). Progenies of EW03 were also divided into the same 6 flower color groups (Figures 4C and 5C). The orange-pink group accounted for the largest proportion, followed by the pink-white group, while the orange-red group accounted for the smallest proportion (Table 2).

Thus, compared with those of EW01, the progeny plants of EW02 and EW03 had more extensive and abundant variation in flower color. Comparison between EW02 and EW03 showed that the proportions of offspring in the six flower color groups were different.

To elucidate the inheritance of flower color, we investigated the variation and distribution of floral color measurement indices (L^* , a^* , and b^* values) for the parents and their offspring (Figure 6). Comparing the L^* values of the three combinations, we discovered that the averages of the L^* value for the offspring (66.45, 65.22, and 68.12, respectively) were closer to that of the maternal parents (68.25). In contrast, the a^* value distributions of the hybrids from the three hybridizations ($-1.14\sim 38.41$, $-1.15\sim 38.41$, and $-2.68\sim 28.77$, respectively) fell within those of the parents, and the average a^* values (12.02, 13.09, and 8.1, respectively) were closer to that of the maternal parent (the low-value parent). The average b^* values for the three combinations (51.16, 37.6, and 25.66, respectively) were higher than those of the paternal parents (the high-value parent). More than half of the individuals in each cross combination (75.38%, 84.43%, and 69.60%) showed transgressive segregation of b^* values.

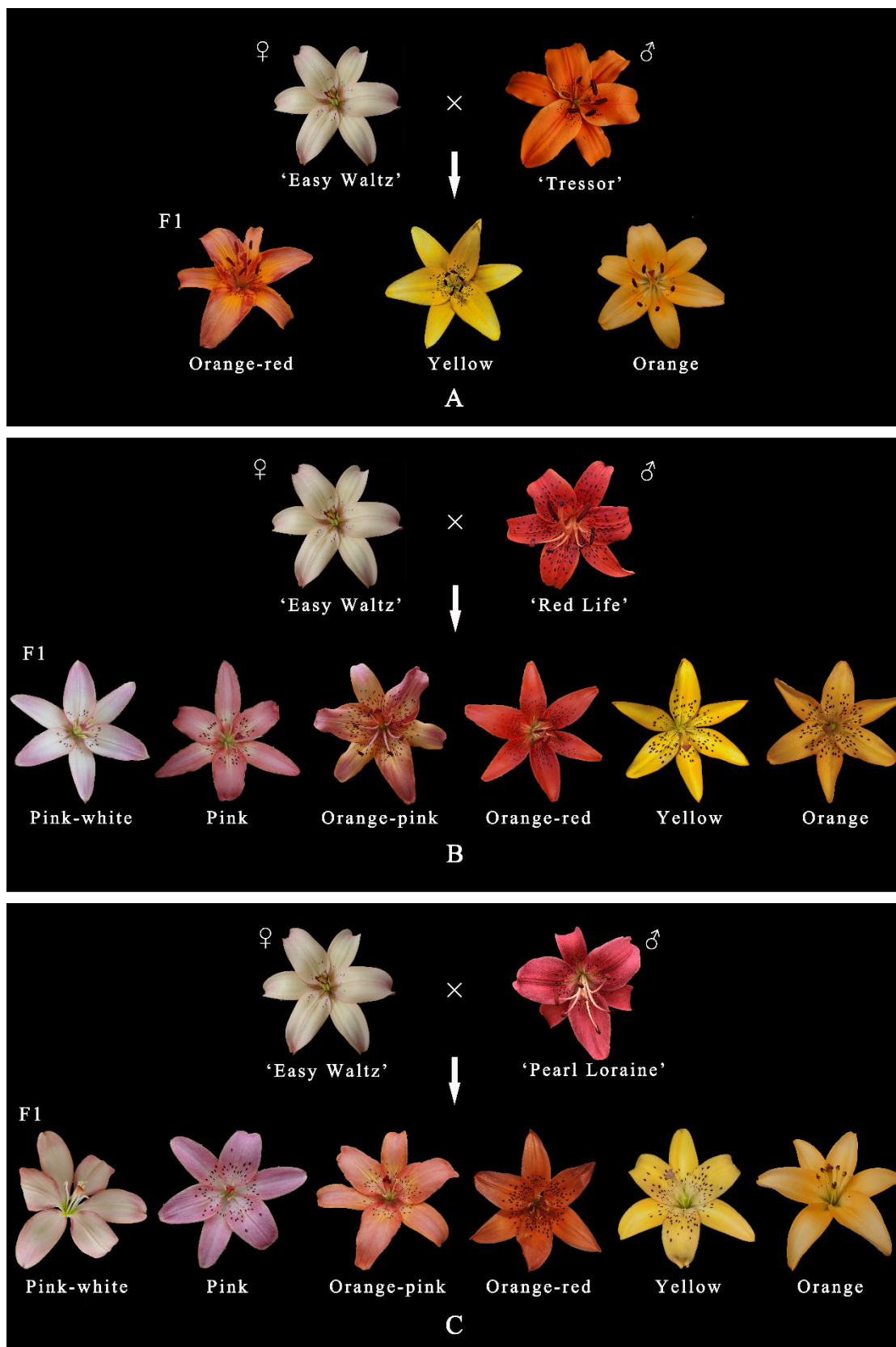


Figure 4. The typical pictures of different color groups in each combination. (A–C): F1 plants of EW01, EW02 and EW03; The F1 plants color groups are arranged from low to high according to the b^* value.

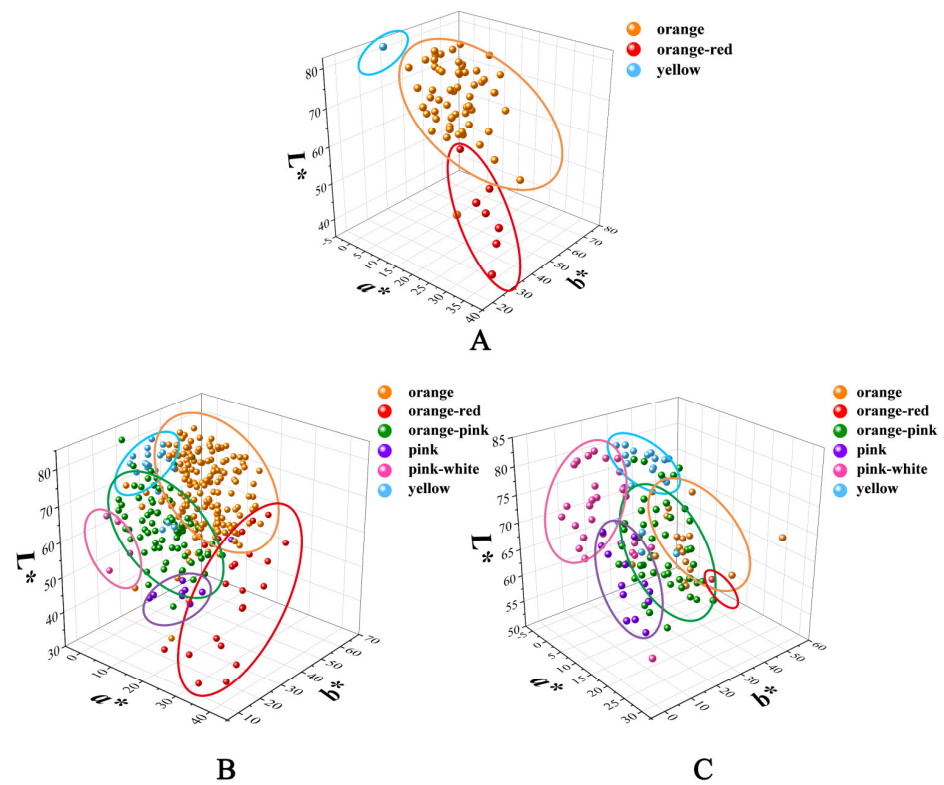


Figure 5. Three-dimensional (L^* , a^* and b^*) coordinates of F1 plants in each combination. (A–C): F1 plants of EW01, EW02, and EW03, respectively.

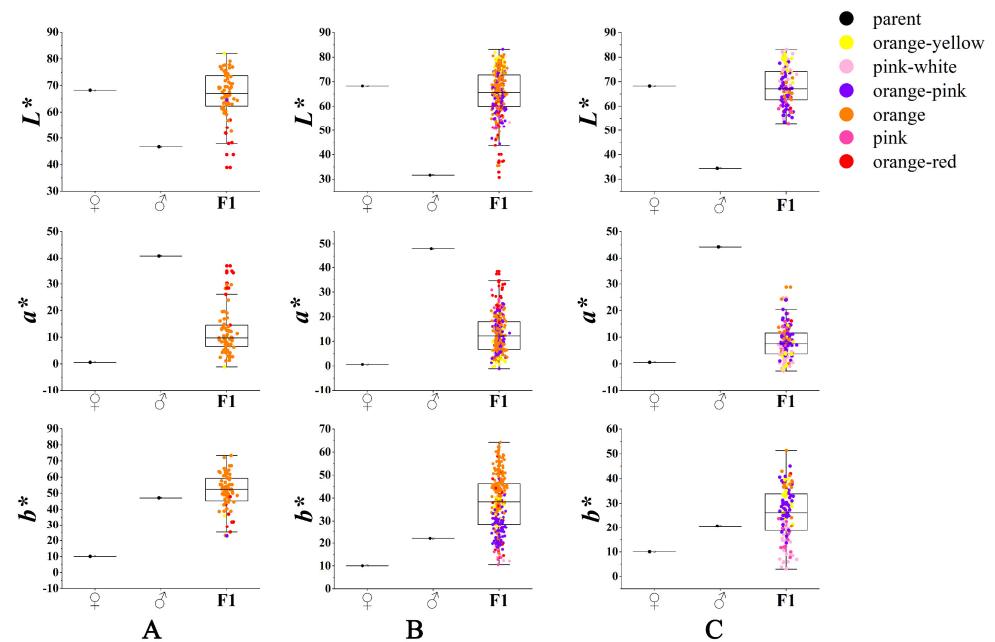


Figure 6. Box plots showing the variation and distribution of flower color data (L^* , a^* , and b^* value) of parents and hybrids in three combinations. (A–C): L^* , a^* , and b^* value distribution in combinations of EW01, EW02, and EW03; X-coordinate: parents (♀, maternal parent; ♂, paternal parent) and F1 plants; Y-coordinate: L^* , a^* , and b^* value, respectively.

3.3. Variation in Raised Spots on Tepals of Hybrids from Different Cross Combinations

Spot patterns constitute an important trait affecting flower color. In the present study, the tepal spot type for all parents and hybrids was raised spots. To understand the inheritance patterns of raised spots, we measured and evaluated the spot numbers of the parents and hybrids in each combination.

For the maternal parent, the average number of spots was 15, while that of the paternal parent was variable. In the EW01 and EW03 hybridizations, ‘Tresor’ and ‘Pearl Loraine’ had similar average numbers of spots, 147 and 164, respectively, which were distributed mainly in the basal area and marginal zone of the perianths (Figure 1) (Ad. 28) [34]. For F1 plants of these two combinations, the presence and absence of raised spots were separated at a ratio of 5:1 (54:11) and 4:1 (98:27), respectively (Table 3). However, in the EW02 cross, ‘Red Life’ had an average of 437 spots, which almost covered the entire perianth (Figure 1). The tepals of all EW02 hybrids exhibited raised spots (Table 3).

Table 3. The spot numbers of cross parents and different hybrid populations.

Cross Combination	The Average Spots Number of Parents				Hybrids		
	♀	SN	♂	SN	No. without Spots	No. with Spots	Proportion
EW01	‘Easy Waltz’	15	‘Tresor’	147	11	54	1:5
EW02	‘Easy Waltz’	15	‘Red Life’	437	0	289	—
EW03	‘Easy Waltz’	15	‘Pearl Loraine’	164	27	98	1:4

Note: SN, spots number.

Afterward, to investigate the effect of the parents’ spot number on genetic segregation, the frequency distribution of spot numbers in the F1 generation were analyzed (Figure 7), and a heterosis analysis of the spot numbers in the three cross combinations was performed (Table 4). For all three combinations, the number of raised spots on tepals was continuously separated in the F1 generation (Figure 7), which showed clear quantitative genetic characteristics. The mean values of the offspring of all combinations were close to the mid-parent values. However, for the CV index, EW02 had the lowest value, followed by EW01 and then EW03 (Table 4). In EW02, spot number showed a normal distribution, and the numbers of almost all F1 plants were within the ranges of their parents’ numbers (Figure 7B), i.e., 98.27% of progenies showed a distribution concentrated between those of their parents (Table 4). In EW01 and EW03, spot number showed an obviously skewed distribution (Figure 7A,C). For the indices of BLP and OHP, 20.00% and 24.62% of the progenies of EW01 and 29.60% and 17.60% of the progenies of EW03 exhibited transgressive segregation (Table 4).

Table 4. Heterosis analysis of spots number in three cross combinations.

Cross Combination	Parent			F1			Hi/%	Hybrid Ratio/%		
	♀	♂	MPs	$\bar{X} \pm \sigma$	Range	CV/%		BLP/%	BP/%	OHP/%
EW01	15.00	147.00	81.00	88.14 ± 84.04	0.00~339.00	95.35	108.81	20.00	55.38	24.62
EW02	15.00	437.00	226.00	219.38 ± 89.92	2.00~526.00	40.99	97.07	0.35	98.27	1.38
EW03	15.00	164.00	90.00	81.49 ± 82.98	0.00~341.00	101.83	90.54	29.60	52.80	17.60

Note: MPs, mid-parent value; \bar{X} , mean value; σ , standard deviation; range, from minimum to maximum; CV, Coefficient of Variation; Hi, index of heterosis; BLP, percentage of F1 individuals below low-value parent; OHP, percentage of F1 individuals over high-value parent; BP, percentage of F1 individuals distributed between parents.

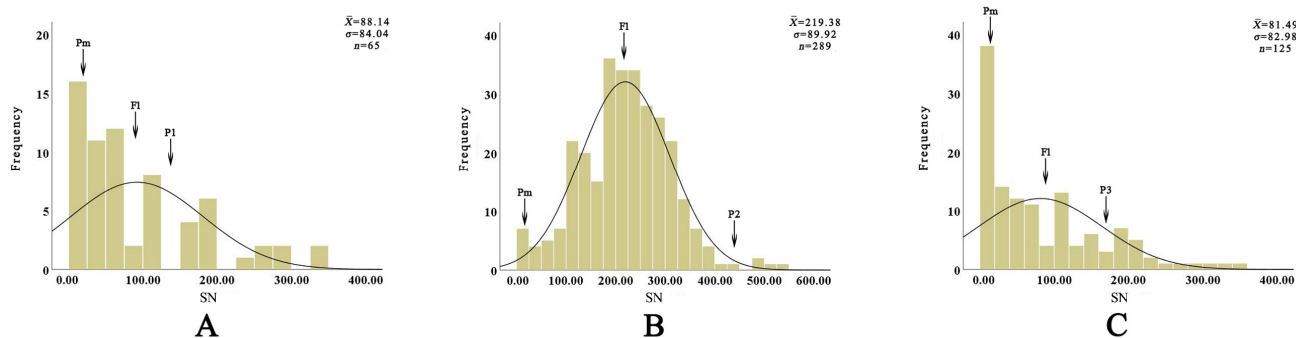


Figure 7. Frequency distribution diagrams of raised spots number (SN) for F1 plants. (A–C): spots number of F1 plants in combinations EW01, EW02, and EW03; X-coordinate: spots number (SN); Y-coordinate: frequency; F1 and \bar{X} : mean value of hybrids; σ : standard deviation; n: number of individuals; Pm: spots number of maternal parent; P1, P2, and P3: spots number of paternal parents in EW01, EW02, and EW03.

4. Discussion

Conventional crosses, including interspecific and intraspecific hybridization, are the main way to create new lily cultivars with distinctive flower colors. Thus, understanding the heredity of flower color can save lily breeders much time, trouble, and effort in selections among several thousand plants [16]. It is well known that the composition and content of anthocyanins and carotenoids mainly affect the flower color hues of Asiatic hybrid lilies [8]. Therefore, cultivars with four different flower colors and different types and concentrations of pigments were selected as parents. For the cross EW01 between parents with pink-white (no carotenoids, medium anthocyanins) and orange (high carotenoids, no anthocyanins) flowers, the primary flower color of F1 plants was orange. The similarity of this finding with those of previous studies [17,18] suggested that high carotenoid levels were highly heritable and that the progenies were less segregated in flower color. For the crosses EW02 and EW03, the simultaneous presence of both carotenoids and anthocyanins in the paternal parent's tepals produced offspring with extensive flower color segregation (Figure 4B,C). Meanwhile, different contents of anthocyanins and carotenoids resulted in variable levels of progeny isolation. As the carotenoid content decreased and the anthocyanin content increased in the parents, the proportions of the pink and pink-white flower color groups increased in the offspring, while that of the orange flower color group decreased (Table 2). Therefore, these results provide guidance on the selection of parents in cross-breeding practices, e.g., if our desired character is light-colored flowers, such as pink or pink-white flowers, varieties with low carotenoids and high anthocyanins should be selected as parents. When we wish to create deep-colored flowers, such as orange-red flowers, varieties with high levels of carotenoids and anthocyanins should be chosen for artificial crosses. Furthermore, if a cross between cultivars with high carotenoids is conducted, the majority of the first generation (F1) seedlings will predominantly produce flowers that are orange in hue, and very few will display yellow flowers.

Moreover, numerous studies have reported close relationships between color space values (L^* , a^* , and b^*) and the contents of anthocyanins and carotenoids. In *Chrysanthemum*, there was a significant negative correlation between L^* and a^* [27,42], which was consistent with the results presented in Figure 3A,B from our research. The pink-white and pink flower color groups had the highest L^* value and the lowest a^* value, whereas the orange-red group had the lowest L^* value and the highest a^* value. In addition, it was discovered that a^* and b^* were strongly positively correlated with total anthocyanins and total carotenoids, respectively [27,43]. In sweet-orange, there was a significant positive correlation between a^* and total carotenoids [44]. According to these findings, the variation and distribution of the flower color measurement indices, L^* , a^* , and b^* , of parents and offspring were investigated to determine the inheritance patterns of the pigments (Figure 6). Among the three combinations, the average b^* value in the hybrids was higher than that in the high-value

parent (Figure 6, b^*), suggesting that carotenoids exhibited transgressive segregation. The offspring of EW01 had the largest b^* value (Figure 6, b^*), followed by those of EW02 and then EW03, which may explain why the last two hybridizations produced offspring with more flower colors. The average a^* value of the F1 plants was closer to that of the low-value parent than to that of the high-value parent (Figure 6, a^*), indicating that the effect of anthocyanins on progeny flower color separation was weaker than that of carotenoids. Furthermore, the discovery that L^* was negatively correlated with total anthocyanins and total carotenoids [44,45] may also explain why the average L^* value of F1 plants was closer to that of the maternal parent (Figure 6, L^*). Although our research provides fundamental information on the inheritance of pigments, how the metabolic pathways of anthocyanins and carotenoids interact to regulate flower color requires in-depth investigation in future studies.

In addition to color hues, raised spots on tepals are a significant component influencing flower color [8,32]. Investigating the inheritance patterns of raised spots in lilies is an important issue. Several studies have reported that the presence or absence of raised spots is regulated by a single locus [32,35]; however, whether dominant or recessive alleles regulate spot formation remains unclear [37,38]. In the present research, the crosses 'Easy Waltz' (with few spots, $n = 15$) \times 'Tresor' (with intermediate spots, $n = 147$), and 'Easy Waltz' (with few spots, $n = 15$) \times 'Pearl Loraine' (with intermediate spots, $n = 164$) yielded F1 plants with and without raised spots separated at a ratio of 5:1 (54:11) and 4:1 (98:27), respectively. For the cross 'Easy Waltz' (with few spots, $n = 15$) \times 'Red Life' (with many spots, $n = 437$), the tepals of all F1 hybrids exhibited raised spots. It follows that dominant genes promote spot formation in Asiatic hybrid lilies. The discrepancy with previous results [37] may be due to the complex chromosomal ploidy and genetic background of lilies. Additionally, the number of the F1 plant spots segregated continuously, showing quantitative genetic characteristics (Figure 7) consistent with the findings of previous studies [32,35]. These results have implications for breeding, i.e., if varieties with few/intermediate spots are selected as parents, the progeny will be spotless or will have more spots than the parents. If our goal is to select new cultivars with numerous spots covering the full tepals, multi-spotted varieties are a better choice as parents.

5. Conclusions

In conclusion, anthocyanins and carotenoids are responsible for regulating the complex flower color of Asiatic hybrid lilies. It is crucial to clarify how parental pigment composition and content influence the separation of flower colors in offspring. Our research revealed that a high carotenoid content was highly heritable and that cultivars with this trait generally generated offspring with orange flowers. When the parents have differing levels of carotenoids and anthocyanins, the progenies exhibit more extensive color segregation and variable proportions of color groups. Additionally, the number of raised spots was a quantitative trait regulated by multiple genes, and dominant alleles promoted spot formation. These patterns of inheritance can help breeders predict the outcomes of particular crosses. Meanwhile, they will contribute to the breeding of novel lily cultivars with high aesthetic value.

Author Contributions: Conceptualization, G.J.; Methodology, G.J., J.L. and J.C.; Data curation, J.L. and J.C.; Investigation, J.L. and J.C.; Formal analysis, P.Y. and Y.Z.; Writing—original draft preparation, J.L., Q.Z. and P.Y.; Writing—review and editing, J.L., Y.Z. and G.J.; Supervision, G.J.; Project administration, G.J.; Funding acquisition, G.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Key R&D Program of China (Grant No.2019YFD1000400) and the Key R&D Program of Hebei Province (Grant No. 21326317D).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the results are already mentioned in the main text.

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

References

- Comber, H.F. A new classification of the genus *Lilium*. In *Royal Horticultural Society Lily Year Book*; Chittenden, F.J., Ed.; The Royal Horticultural Society: London, England, 1949; pp. 85–105.
- Smyth, D.R.; Kongsuwan, K.; Wisudharomn, S. A survey of C-band patterns in chromosomes of *Lilium* (Liliaceae). *Plant Syst. Evol.* **1989**, *163*, 53–69. [[CrossRef](#)]
- Du, Y.P.; He, H.B.; Wang, Z.X.; Wei, C.; Li, S.; Jia, G.X. Investigation and evaluation of the genus *Lilium* resources native to China. *Genet Resour. Crop. Ev.* **2014**, *61*, 395–412. [[CrossRef](#)]
- Leslie, A.C. *The International Lily Register*, 3rd ed.; The Royal Horticultural Society: London, England, 1982.
- Lim, K.B.; van Tuyl, J.M. Lily: *Lilium* hybrids. In *Flower Breeding and Genetics: Issues, Challenges and Opportunities for the 21st Century*; Anderson, N.O., Ed.; Springer: Berlin, Germany, 2006; pp. 513–532.
- Barba-Gonzalez, R.; Van Silfhout, A.A.; Visser, R.G.F.; Ramanna, M.S.; Van Tuyl, J.M. Progenies of allotriploids of Oriental × Asiatic lilies (*Lilium*) examined by GISH analysis. *Euphytica* **2006**, *151*, 243–250. [[CrossRef](#)]
- Van Tuyl, J.M.; Arens, P.; Shahin, A.; Marasek-Ciołakowska, A.; Barba-Gonzalez, R.; Kim, H.T.; Lim, K.-B. *Lilium*. In *Ornamental Crops, Handbook of Plant Breeding*; Van Huylbroeck, J., Ed.; Springer: Berlin, Germany, 2018; pp. 481–512. [[CrossRef](#)]
- Yamagishi, M. How genes paint lily flowers: Regulation of colouration and pigmentation patterning. *Sci. Hortic.* **2013**, *163*, 27–36. [[CrossRef](#)]
- Nørbæk, R.; Kondo, T. Anthocyanins from flowers of *Lilium* (Liliaceae). *Phytochemistry* **1999**, *50*, 1181–1184. [[CrossRef](#)]
- Yamagishi, M.; Kishimoto, S.; Nakayama, M. Carotenoid composition and changes in expression of carotenoid biosynthetic genes in tepals of Asiatic hybrid lily. *Plant Breed.* **2010**, *129*, 100–107. [[CrossRef](#)]
- Jeknić, Z.; Morré, J.T.; Jeknić, S.; Jevremović, S.; Subotić, A.; Chen, T.H.H. Cloning and functional characterization of a gene for capsanthin-capsorubin synthase from tiger lily (*Lilium lancifolium* Thunb. ‘Splendens’). *Plant Cell Physiol.* **2012**, *53*, 1899–1912. [[CrossRef](#)]
- Wang, X.; Yamagishi, M. Mechanisms suppressing carotenoid accumulation in flowers differ depending on the hybrid groups of lilies (*Lilium* spp.). *Sci. Hortic.* **2019**, *243*, 159–168. [[CrossRef](#)]
- Deli, J.; Molnár, P.; Matus, Z.; Tóth, G.; Steck, A.; Pfander, H. Isolation and characterization of 3,5,6-trihydroxy-carotenoids from petals of *Lilium tigrinum*. *Chromatographia* **1998**, *48*, 27–31. [[CrossRef](#)]
- Lai, Y.S.; Shimoyamada, Y.; Nakayama, M.; Yamagishi, M. Pigment accumulation and transcription of *LhMYB12* and anthocyanin biosynthesis genes during flower development in the Asiatic hybrid lily (*Lilium* spp.). *Plant Sci.* **2012**, *193*, 136–147. [[CrossRef](#)]
- Yamagishi, M. White with partially pink flower color in *Lilium cernuum* var. *album* is caused by transcriptional regulation of anthocyanin biosynthesis genes. *Sci. Hortic.* **2020**, *260*, 108880. [[CrossRef](#)]
- Balode, A. Color analysis of flowers in *Lilium* sp. breeding. *Acta Hortic.* **2007**, *755*, 213–218. [[CrossRef](#)]
- Hwang, Y.J.; Kang, S.Y.; Rhee, H.Y.; Lim, K.B. The characteristics of progenies derived from *Lilium lancifolium* and Asiatic hybrid ‘Dreamland’. *Korean J. Breed. Sci.* **2009**, *41*, 451–455.
- Jo, Y.K.; Ramzan, F.; Son, B.G.; Kim, H.Y.; Lim, K.B. Crossing of Asiatic hybrids for breeding of new Lily cultivars. *Korean J. Breed. Sci.* **2018**, *50*, 1–12. [[CrossRef](#)]
- Wang, H.; Kong, Y.; Dou, X.Y.; Lang, L.X.; Bai, J.R. Genetic variation of phenotypic traits in F1 generation between Asiatic hybrid lily ‘Renoir’ and *Lilium davidii* var. *willmottiae*. *Mol. Plant Breed.* **2021**, *19*, 6111–6119. [[CrossRef](#)]
- Hanbury, A.; Serra, J. Mathematical morphology in the CIELAB space. *Image. Anal. Stereol.* **2002**, *21*, 201–206. [[CrossRef](#)]
- Afonso, T.; Moresco, R.; Uarrota, V.G.; Navarro, B.B.; Nunes, E.D.C.; Maraschin, M.; Rocha, M. UV-vis and CIELAB based chemometric characterization of Manihot esculenta carotenoid contents. *J. Integr. Bioinform.* **2017**, *14*, 20170056. [[CrossRef](#)]
- Wang, N.H.; Dai, M.Y.; Zheng, G.; Chang, P.J.; Xuan, L.J.; Liu, Z.G.; Wang, Y.L.; Cheng, S.Y.; Wang, Z.W.; Wang, H.L. Flavonoid components and gene expression analysis reveal flower pigmentation difference between *Magnolia biondii* and its variety *M. biondii* var. *purpurascens*. *Trees-Struct. Funct.* **2021**, *36*, 583–591. [[CrossRef](#)]
- Donoso, A.; Rivas, C.; Zamorano, A.; Pena, A.; Handford, M.; Aros, D. Understanding *Alstroemeria pallida* flower colour: Links between phenotype, anthocyanins and gene expression. *Plants* **2021**, *10*, 55. [[CrossRef](#)]
- Li, X.; Lu, M.; Tang, D.Q.; Shi, Y.M. Composition of carotenoids and flavonoids in *Narcissus* cultivars and their relationship with flower color. *PLoS ONE* **2015**, *10*, e0142074. [[CrossRef](#)]
- Liu, L.; Zhang, L.Y.; Wang, S.L.; Niu, X.Y. Analysis of anthocyanins and flavonols in petals of 10 *Rhododendron* species from the Sygera Mountains in Southeast Tibet. *Plant Physiol. Bioch.* **2016**, *104*, 250–256. [[CrossRef](#)]
- Cui, H.L.; Zhang, Y.A.; Shi, X.L.; Gong, F.F.; Xiong, X.; Kang, X.P.; Xing, G.M.; Li, S. The numerical classification and grading standards of daylily (*Hemerocallis*) flower color. *PLoS ONE* **2019**, *14*, e0216460. [[CrossRef](#)]
- Lu, C.F.; Li, Y.F.; Wang, J.Y.; Qu, J.P.; Chen, Y.; Chen, X.Y.; Huang, H.; Dai, S.L. Flower color classification and correlation between color space values with pigments in potted multiflora chrysanthemum. *Sci. Hortic.* **2021**, *283*, 110082. [[CrossRef](#)]
- Lei, T.; Song, Y.; Jin, X.H.; Su, T.Y.; Pu, Y.W. Effects of pigment constituents and their distribution on spathe coloration of *Zantedeschia hybrida*. *Hortscience* **2017**, *52*, 1840–1848. [[CrossRef](#)]

29. Zhou, Y.W.; Yin, M.; Abbas, F.; Sun, Y.; Gao, T.; Yan, F.L.; Li, X.Y.; Yu, Y.Y.; Yue, Y.C.; Yu, R.C. Classification and association analysis of Gerbera (*Gerbera hybrida*) flower color traits. *Front. Plant Sci.* **2022**, *12*, 779288. [CrossRef]
30. Wang, H.; Fan, Y.W.; Yang, Y.; Zhang, H.; Li, M.F.; Sun, P.; Zhang, X.Z.; Xue, Z.; Jin, W.M. Classification of rose petal colors based on optical spectrum and pigment content analyses. *Hortic. Environ. Biotechnol.* **2022**. [CrossRef]
31. Wang, J.; Xu, L.F.; Wang, L.; Qi, X.Y.; Song, M.; Cao, Y.W.; He, G.R.; Tang, Y.C.; Yang, P.P.; Ming, J. The numerical classification of flower color phenotype in lily. *Acta Horticult. Sin.* **2021**, *49*, 571–580. [CrossRef]
32. Abe, H.; Nakano, M.; Nakatsuka, A.; Nakayama, M.; Koshioka, M.; Yamagishi, M. Genetic analysis of floral anthocyanin pigmentation traits in Asiatic hybrid lily using molecular linkage maps. *Theor. Appl. Genet.* **2002**, *105*, 1175–1182. [CrossRef]
33. Yamagishi, M. MYB19LONG is involved in brushmark pattern development in Asiatic hybrid lily (*Lilium* spp.) flowers. *Sci. Horticult.* **2020**, *272*, 109570. [CrossRef]
34. He, X.F.; Xu, S.F.; Leng, P.S.; Wang, W.H. Transcriptome sequencing reveals genes involved in petal spot formation of Asiatic hybrid lily cultivar ‘Easy Dance’. *Int. J. Agric. Biol.* **2018**, *20*, 939–944. [CrossRef]
35. Yamagishi, M.; Akagi, K. Morphology and heredity of tepal spots in Asiatic and Oriental hybrid lilies (*Lilium* spp.). *Euphytica* **2013**, *194*, 325–334. [CrossRef]
36. Yamagishi, M. Isolation and identification of MYB transcription factors (*MYB19Long* and *MYB19Short*) involved in raised spot anthocyanin pigmentation in lilies (*Lilium* spp.). *J. Plant Physiol.* **2020**, *250*, 153164. [CrossRef]
37. Shahin, A.; Arens, P.; Van Heusden, A.W.; Van Der Linden, G.; Van Kaauwen, M.; Khan, N.; Schouten, H.J.; Van De Weg, W.E.; Visser, R.G.F.; Van Tuyl, J.M. Genetic mapping in *Lilium*: Mapping of major genes and quantitative trait loci for several ornamental traits and disease resistances: Genetic mapping in *Lilium*. *Plant Breed.* **2011**, *130*, 372–382. [CrossRef]
38. Zhao, X.Y.; Wang, S.D.; Wang, W.H.; Zhang, K.; Liu, C.Y. *Tingyuan Baihe Shiyong Jishu*, 1st ed.; China Agriculture Press: Beijing, China, 2016; pp. 69–78.
39. Wang, Q.; Lv, T.; Lv, Y.M. The impact of tepal structure and pigment composition on the flower colour of lily. *Acta Horticult. Sin.* **2021**, *48*, 1873–1884. [CrossRef]
40. UPOV. Guidelines for the conduct of tests for distinctness, uniformity and stability, *Lilium*, TG/59/7. In Proceedings of the International Union for the Protection of New Varieties of Plants, Geneva, Switzerland, 24 March 2010; Available online: <https://www.upov.int/portal/index.html.en> (accessed on 10 October 2022).
41. Schmitzer, V.; Veberic, R.; Osterc, G.; Stampar, F. Color and phenolic content changes during flower development in groundcover rose. *J. Am. Soc. Horticult. Sci.* **2010**, *135*, 195–202. [CrossRef]
42. Akbari, R.; Hatamzadeh, A.; Sariri, R.; Bakhshi, D. Relationship of flower color parameters and metal ions of petal tissue in fully opened flowers of gerbera. *J. Plant Stud.* **2013**, *2*, 89–96. [CrossRef]
43. Itle, R.A.; Kabelka, E.A. Correlation between L*a*b* color space values and carotenoid content in pumpkins and squash (*Cucurbita* spp.). *Hortscience* **2009**, *44*, 633–637. [CrossRef]
44. Abdelaali, S.B.; Rodrigo, M.J.; Saddoud, O.; Zacarías, L.; Hajlaoui, M.R.; Mars, M. Carotenoids and colour diversity of traditional and emerging Tunisian orange cultivars (*Citrus sinensis* (L.) Osbeck). *Sci. Horticult.* **2018**, *227*, 296–304. [CrossRef]
45. Xue, L.; Wang, Z.G.; Zhang, W.; Li, Y.X.; Wang, J.; Lei, J. Flower pigment inheritance and anthocyanin characterization of hybrids from pink-flowered and white-flowered strawberry. *Sci. Horticult.* **2016**, *200*, 143–150. [CrossRef]