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Research Paper

Quantifying colour and spot characteristics for the ventral petals in *Sinningia speciosa*

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This study examined the colour and spot patterns for the ventral petals of a cross line in *Sinningia speciosa*. The second-generation individuals of the cross line exhibited phenotypic segregation in floral colour and spot patterns. Three colour traits (colour region ratio, region of interest colour, and colour gradient) and five spot traits (spot quantity, spot density, spot area ratio, spot colour, and background colour) were defined and quantified using image processing techniques. The variation in the traits and the correlations between the traits were also investigated. The results indicated a considerable degree of variation among the traits. The proposed approach can quantify petal traits more objectively and precisely compared with conventional naked eye examination. Thus, it can be used for applications, such as new flower variety determination, that require precise trait quantification.

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1. Introduction

Quantifying the colour and spot patterns of flowers is intriguing to horticulturists, botanists, and florists. *Sinningia speciosa* (Lodd.) Hiern, commonly known as "Florist's Gloxinia", is a popular ornamental houseplant with flowers that are highly diverse in colour and spot patterns. A wild variety of *S. speciosa*, 'Carangola', blooms flowers with pale petals and a purple block at the base (Fig. 1). In contrast, a peloric *S. speciosa*, 'Peridots Darth Vader', yields blossoms with magenta lobes and a spotty pattern. With the advantage of distinct colour and spot patterns, the second-generation (F_2) crossing of these two varieties has considerable distinctiveness in floral colour and spot patterns; thus, it was ideal for studying floral colour and spot variations. This study examined and quantified the colour and spot patterns of the ventral petals of F_2 flowers by using image processing approaches.

Quantitatively assessing the colour and spot patterns of flowers is required in hypothesis-based studies, such as those

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Nomenclature

a*	Redness
b*	Blueness
ΔE	Euclidean distance between 2 colours in CIE L*a*b* colour space
DUS test	The distinctness, uniformity, and stability test
F_1	The first generation of crossing lines
F_2	The second generation of crossing lines
GLCM	Grey-level co-occurrence matrix
L*	Lightness
PCA	Principal component analysis
r	Correlation coefficient
RHS	The Royal Horticultural Society
ROI	Region of interest
UPOV	International Union for the Protection of New Varieties of Plants

on breeding selection (Nakatsuka et al., 2008), new flower variety examination (UPOV, 2002), perception of pollinators (Goyret, 2010), and genotype–phenotype association (De Keyser, Lootens, Van Bockstaele, & De Riek, 2013). Conventionally, the colour and spot patterns of flowers are evaluated by the naked eye. Flower colours are manually determined as the major colours observed (Alexandre, Vrignaud, Mangin, & Joly, 2015) and are categorised by referring to the Royal Horticultural Society's (RHS) colour chart (Sun, Li, & Zhang, 2006). Floral spots are described on the basis of the observed appearance of nectar guides (i.e., the spot patterns of petals; Dafni & Kevan, 1996; Hansen, Van der Niet, & Johnson, 2012). Flower patterns are, however, sophisticated and diverse. Naked eye examination is subjective and constrains the interpretation of floral pattern variations (Voss, 1992).

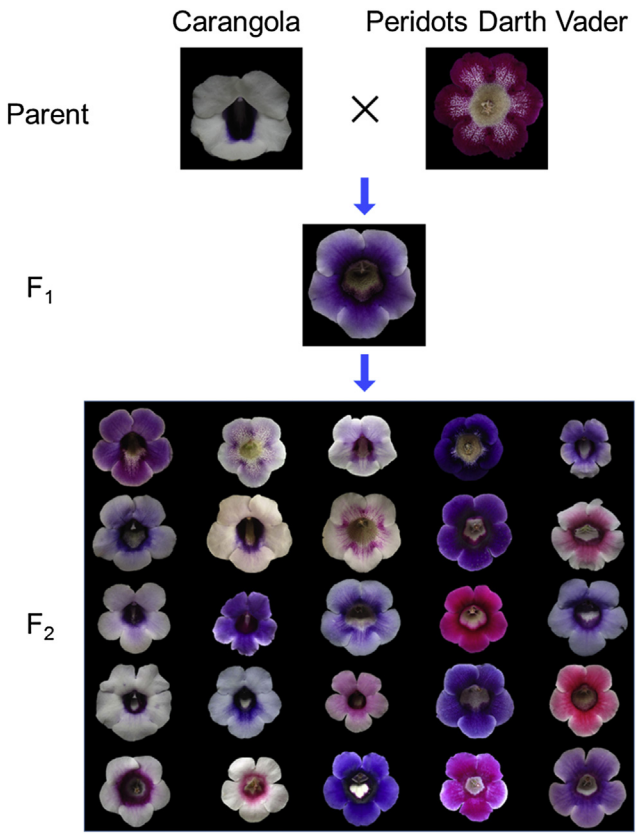

Fig. 1 – Interbreeding of *S. speciosa*.

Image-based approaches, by contrast, provide a quantitative and objective solution for evaluating colour and spot patterns. Several studies have examined floral colour and spot patterns using image-related techniques. Yoshioka, Ohsawa, Iwata, Ninomiya, and Fukuta (2006) quantified picotee colour proportions of the petals of *Eustoma grandiflorum* using image processing. Wassink and Caruso (2013) assessed the petal brightness, hue, and chroma of *Lobelia siphilitica* using a spectrophotometer. Guru, Kumar, and Manjunath (2011) quantified the spot features of the corollas of several species using the grey-level co-occurrence matrix (GLCM) and Gabor filter methods. Another study measured the variations in petal colour contrast and the proportion of the nectar guide area in *Anacamptis morio* (Sletvold, Trunschke, Smit, Verbeek, & Ågren, 2016).

This study quantified the colour and spot patterns of the ventral petals of F_2 *S. speciosa* flowers using image processing algorithms. The ventral petals were selected as the target material because they exhibited a high degree of variation in colour and spot patterns. Ventral petal also serves as the visual cues for flower–pollinator interaction. The specific objectives of this study were to (1) acquire images of the ventral petals of F_2 flowers, (2) define and quantify the colour and spot traits of the ventral petals, (3) identify variations in the traits, and (4) compare the proposed method with the trait evaluation guideline published by International Union for the Protection of New Varieties of Plants (UPOV) for flower variety examination.

2. Materials and methods

2.1. Flower materials

Two cultivars of *S. speciosa*, 'Carangola' and 'Peridots Darth Vader', were crossed to breed first generation (F_1) plants. One individual among the F_1 plants was then selfed to generate the F_2 population (Fig. 1). Please refer to Hsu, Wang, Liang, Wang, and Kuo (2017) for details of the plant cultivation. The plants were grown in a greenhouse at 22–28 °C under natural lighting with 20% shading and at 70%–80% relative humidity. The photosynthetic photon flux density ranged between 140 and 350 μmol m^{-2} s^{-1}. Forty-seven F_2 plants were used. Three flowers were collected from 47 plants, resulting in a total of 141 specimens.

2.2. Image acquisition

Once at full bloom, the flowers were cut from the peduncles, and the sepals were removed. The flower was dissected along the boundaries between the dorsal and lateral petals, and the section containing lateral and ventral petal was retained. Images of the ventral and lateral petals were subsequently

Fig. 2 – Flattened petals of *S. speciosa*. The dashed lines enclose the ventral petals, the targets in the present study.

acquired using flatbed scanners (V37, Epson; Suwa, Japan) at a resolution of 600 dpi (Fig. 2). The petals were pressed to maintain their optimal original shapes during scanning. Black cloth was used to shelter the petals and eliminate stray light. The scanners were calibrated using a standard colour reference board (ColourChecker Passport, X-Rite; Grand Rapids, USA). The reference board consists of colour patches with given device-independent Commission Internationale de l'Éclairage lightness, redness, blueness (CIE L*a*b*) (Hanbury & Serra, 2001) colour parameters. The conversion from the scanner red, green, and blue (RGB) colour parameters to CIE L*a*b* colour parameters was then calculated using multiple regression, so that the CIE L*a*b* colour parameters of the petal images could be obtained. Once the images were acquired, the ventral petals were manually segmented using a polygon tool developed in MATLAB (The MathWorks; Natick, USA).

2.3. Colour and spot-region identification

Each ventral petal image was segmented into a colour region (Fig. 3e) and a spot region (Fig. 3f). The spot region was defined as the region exhibiting spots, whereas the colour region was defined as the complement of the spot region. The spot region was associated with spot features, whereas the colour region was associated with colour features. Spot and colour regions were identified using a series of image processing algorithms. First, the GLCM prominence image (Fig. 3b) of a ventral petal was calculated. GLCM prominence is a measure of greyscale variation in an image. To obtain the GLCM prominence image, a ventral petal image (Fig. 3a) was first converted into a greyscale image of 3-bit word length. A sliding window of 51 × 51 pixels scanned the greyscale image from the top-left corner to the bottom-right corner using a sliding step of 1 pixel. Each sliding window centred at a pixel in the greyscale image. At each centre pixel, the GLCM of the sliding window was computed using a displacement of 1 pixel and an angle of 0°. The prominence value of the GLCM was calculated following the definition given by Trivedi, Harlow, Conners, and Goh (1984). The prominence image (Fig. 3b), comprising the prominence values at every pixel, was then formed after the sliding window had scanned the entire greyscale image. Next, the prominence image was binarised using Otsu's method (Otsu, 1979). The largest object (Fig. 3c) in the binary image, which was typically the spot region, was identified using connected-component labelling (Dillencourt, Samet, & Tamminen, 1992). A spot-region mask (Fig. 3d) was then created after applying a morphological closing operation (Gonzalez & Woods, 2007) to the largest object. Finally, the spot region (Fig. 3f) was identified using the spot-region mask (Fig. 3d) and the petal image (Fig. 3a). The colour region (Fig. 3e) was subsequently identified as the complement of the spot

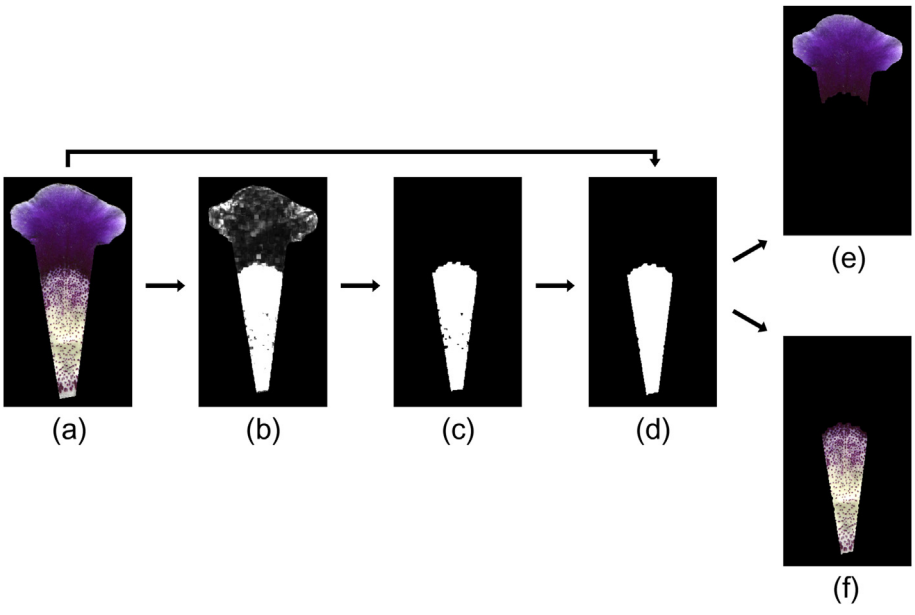

Fig. 3 – Colour and spot-region identification process: (a) ventral petal image; (b) prominence image; (c) largest connected component in the prominence image; (d) spot-region mask; (e) colour region; and (f) spot region.

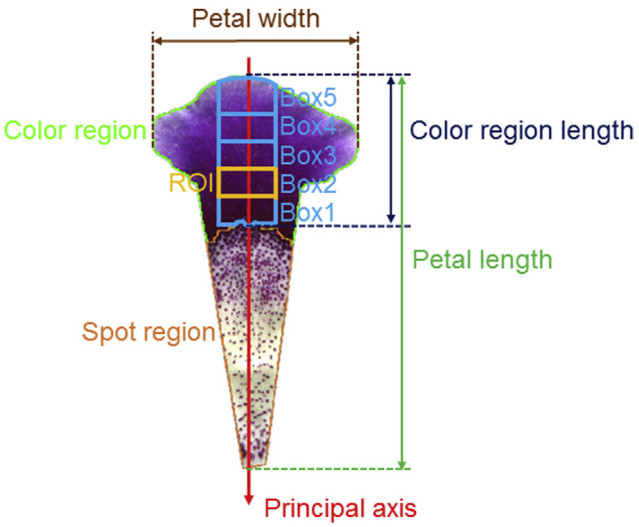

Fig. 4 – Colour trait definitions.

region. The image processing algorithms were implemented in MATLAB.

2.4. *Colour trait quantification*

Three traits were quantified for the colour region: colour region ratio, colour gradient, and region of interest (ROI) colour. The colour region ratio was defined as the ratio of the colour region area to the ventral petal area. The Boxes were defined as five adjacent rectangles labelled in descending order from the petal distal. The rectangles were centred down the principal axis of the petal. The principal axis was determined using principal component analysis (PCA) and passed through the centre of the ventral petal image. Each rectangle had a length one-third the width of the petal and a width of one-fifth the colour region length (Fig. 4). The colour region length was defined as the segment length of the principal axis intersecting the colour region. The background area and spot region were excluded from use for calculating the colour parameters of Box 5 and Box 1, respectively. Box 2 was defined as the ROI because it contained the largest variation in colour among the specimens. All the colours were represented using CIE L*a*b* parameters. Colour gradient was defined as the colour of Box 1 subtracting the colour of Box 5 (i.e., ΔL^*, Δa^*, and Δb^*). The magnitude of colour gradient and the colour difference were computed using Eq. (1) (Backhaus, Kliegl, & Werner, 1998).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

Colour trait quantification was performed automatically using MATLAB.

2.5. *Spot trait quantification*

Five traits were quantified for the spot region: spot quantity, spot density (spots cm^{-2}), spot area ratio, spot colour, and background colour. Spot quantity was defined as the number of spots in the spot region. Spot density was defined as the spot quantity per unit area. Spot area ratio was defined as the ratio of the total area of the spots to the total area of the spot region. Spot colour was defined as the mean colour of the spots. Background colour was defined as the mean colour of the complement area to the spots. The spots were identified using a series of image processing algorithms. An image of the spot region was first converted into a greyscale image (Fig. 5a). The complement image of the greyscale image was calculated (Fig. 5b). Subsequently, an image (Fig. 5c) was obtained by adjusting the contrast of the complement image to span the greyscale dynamic range. Another image (Fig. 5d) was obtained by applying a morphological opening operation (disc structuring element of size 15 pixels; Vincent, 1993) to the complement image. The subtraction of the contrast-adjusted image from the opening-applied image provided the spot-emerged image (Fig. 5e). The mask of the spots (Fig. 5f) was then obtained by binarising the spot-emerged image using Otsu's

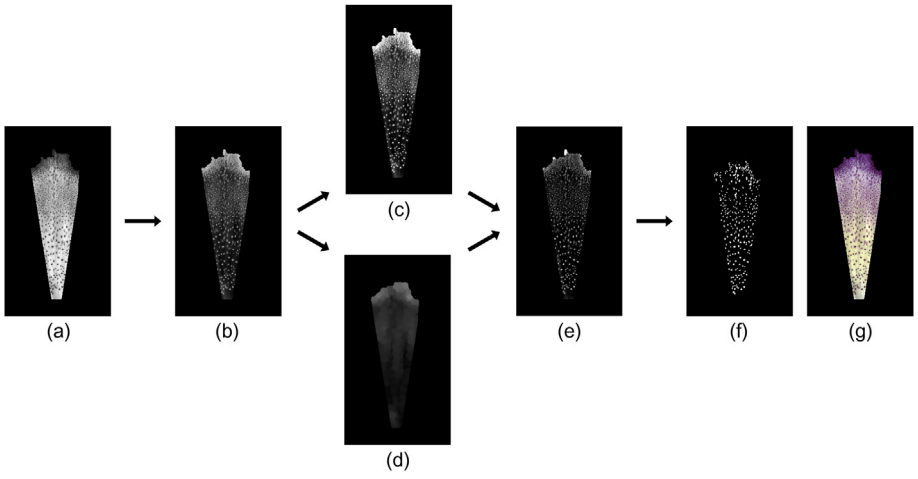

Fig. 5 – Spot identification process: (a) greyscale spot-region image; (b) complement image of (a); (c) contrast-adjusted image of (b); (d) opening-applied image of (b); (e) image resulting from subtracting (d) from (c); (f) spot mask; and (g) spot-region image.

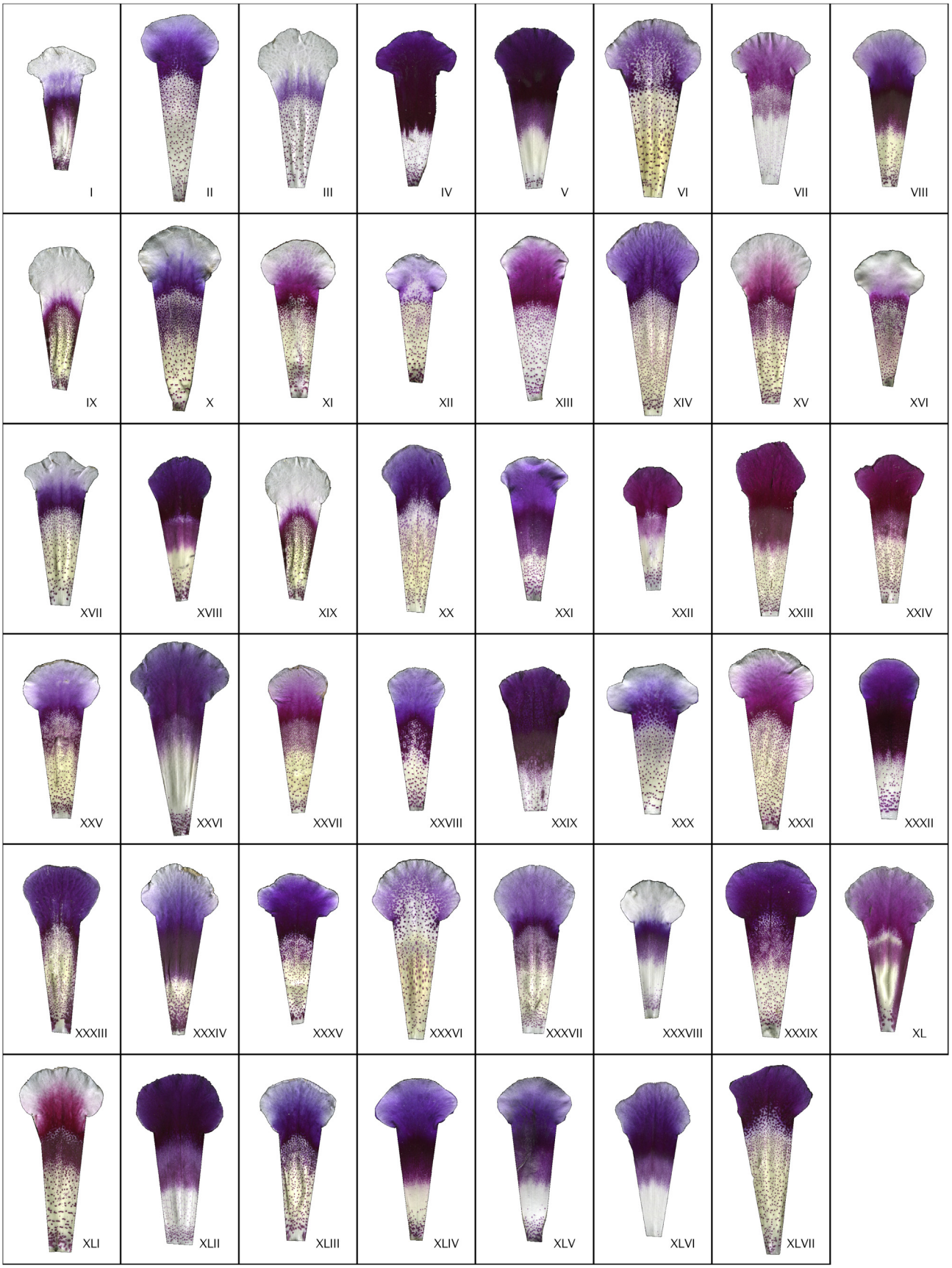

Fig. 6 – Petals of the 47 F_2 plants.

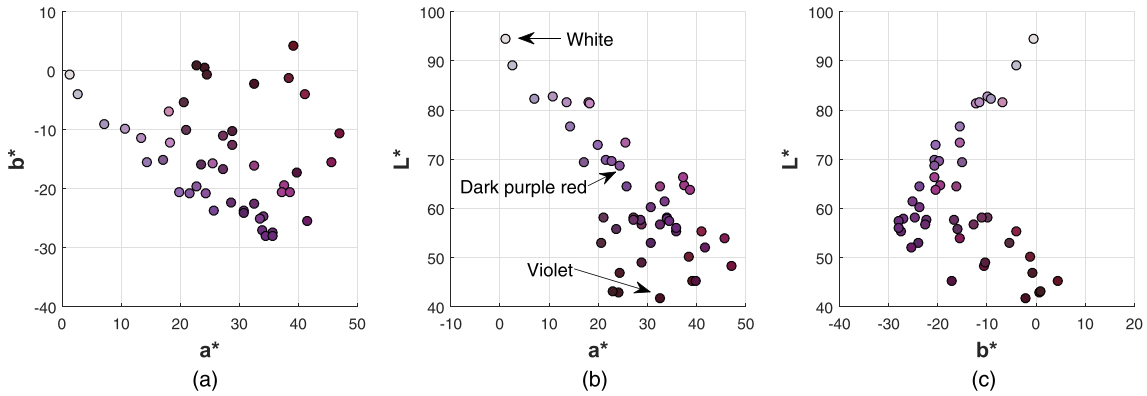

Fig. 7 – Distributions of ROI colours in (a) a*–b*, (b) a*–L*, and (c) b*–L* planes. The marker colours are the ROI colours.

method. The spots were consequently identified using the spot mask. The processing of spot trait quantification was performed automatically by running a script in MATLAB.

3. Results and discussion

3.1. *Petal images*

Figure 6 displays 47 petal images from different individuals. Considerable variations in size, colour, and spot were observed between the petals. Petal lengths ranged from 4.10 cm (Fig. 6I) to 6.91 cm (Fig. 6II). Some petals appeared pale (Fig. 6III), whereas other petals appeared dark purple (Fig. 6IV and V). Some petals had grainy spot patterns (Fig. 6VI). Conversely, the spot patterns of some petals were nearly invisible (Fig. 6VII). Spot quantity ranged from 11 (Fig. 6VIII) to 433 (Fig. 6II).

3.2. *Colour traits*

The ROI colours are illustrated in Fig. 7. Each point in the figure is the mean ROI colour for the three specimens of an

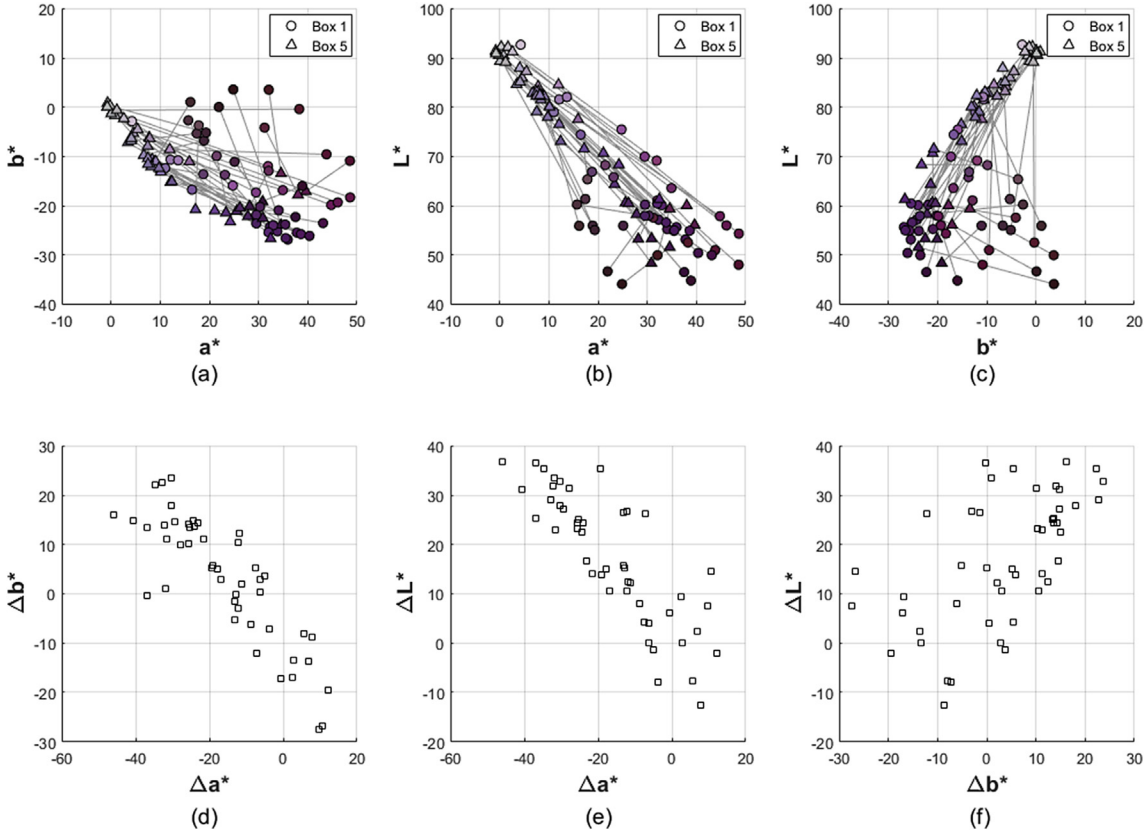

Fig. 8 – Mean box colours and colour gradients of the petals in (a) a*–b*, (b) a*–L*, (c) b*–L*, (d) Δa*–Δb*, (e) Δa*–ΔL*, and (f) Δb*–ΔL* planes. The circles and the triangles, respectively, represent the Boxes 1 and 5 colours. The squares represent the colour gradients.

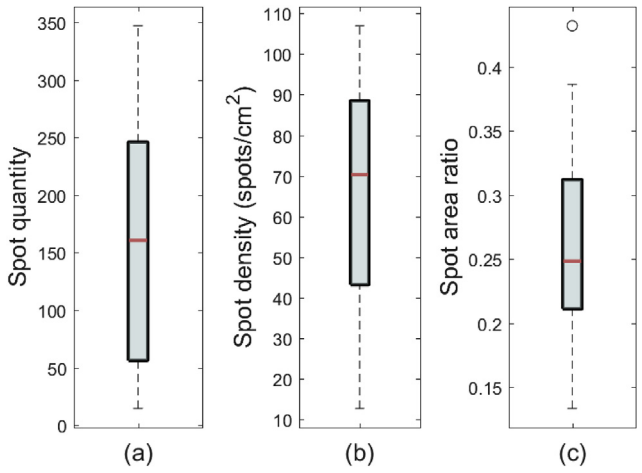

Fig. 9 – Boxplots of (a) spot quantity, (b) spot density, and (c) spot area ratio.

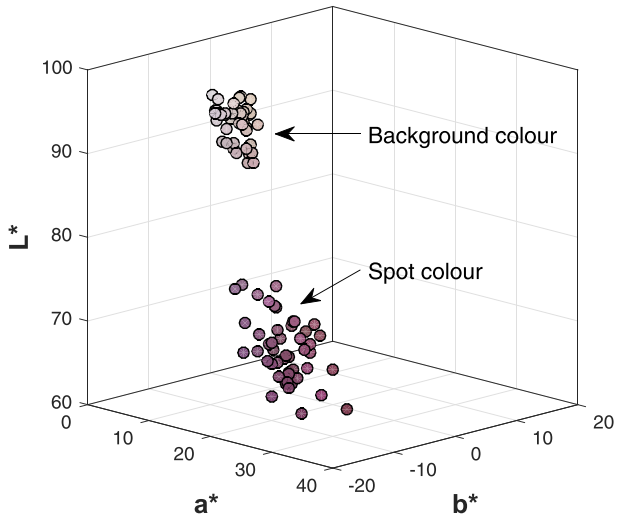

Fig. 10 – Spot and background colours of the petal samples in L*a*b* colour space. The marker colours are the spot or background colours.

individual. ROI colour variations were considerable. L* ranged from 41.79 to 94.51, a* ranged from 1.25 to 46.98, and b* ranged from −28.03 to 4.32. The root-mean-squared deviation of ROI colours among the 47 F_2 plants was 18.71 ΔE units. The correlation between the L* and a* values was strong (r = −0.73; Figs. 8b and 13). Figure 7b indicates ROI colours of white (colour 155C of RHS colour chart; Fig. 6IX), dark purple red (colour 81C of RHS colour chart; Fig. 6X), and violet (colour 59A of RHS colour chart; Fig. 6IV). The colour variation of the petals may have been caused by differences in their amount of delphinidin (a blue–red colour anthocyanidin; Asen, Stewart, Norris, 1972; Goto & Kondo, 1991).

Figure 8a–c illustrates the colours of Boxes 1 and 5. Each point in the figure is the mean colour of Box 1 or Box 5 for the three specimens of an individual. The markers were coloured using the measured box colours. The colours of the same individual were connected using a grey line. Figure 8d–f illustrates the colour gradients. The mean magnitude of the colour gradients was 30.10 ΔE units, and the standard deviation of the colour gradient magnitude was 14.67 ΔE units. Figure 8b and e indicates that Box 1 colours were typically darker than Box 5 colours (i.e., mean $\Delta L^* > 0$ in Fig. 8e). Also, Box 1 colours were associated with a greater degree of redness (a*) compared with Box 5 colours (i.e., mean $\Delta a^* < 0$ in Fig. 8e). The mean L* and a* values of Box 1 were 59.79 and 29.41, respectively, whereas those of Box 5 were 76.85 and 13.24. Student's t-test revealed significant differences in mean L* and a* values between Boxes 1 and 5 ($P < 0.01$). We also observed that Δa* values were linearly correlated with ΔL* values (r = −0.84) and Δb* values (r = −0.85), respectively.

The petals with the highest and lowest levels of colour gradient are those presented in Fig. 6XI and 6XII, respectively. Box 1 of petals with high levels of colour gradient was typically associated with a higher degree of redness (Fig. 8b and e). By contrast, the colours of the petals with low levels of colour gradient were homogeneous in the colour region. The Box 1 colour and colour gradient can be used as indices of consumer preference. Typically, flowers with high levels of colour gradient are preferred by consumers compared with flowers with homogeneous colours (Berghage & Wolnick, 2000). Additionally, among the flowers with homogeneous colours (i.e., low levels of colour gradient), those with higher degrees of colour saturation (i.e., high redness a*) are generally more

	(a) Primary petal colour		(b) Petal colour gradient		(c) Spot region size		(d) Number of spots	
	DUS test (RHS colour code)	Proposed method (L*a*b*)	DUS test	Proposed method (ΔE unit)	DUS test	Proposed method (mm^2)	DUS test	Proposed method (count)
 XV	Blue pink (72D)	Box 5: [91.70, 1.78, -0.74] Box 4: [89.14, 7.03, -2.62] Box 3: [81.99, 18.59, -6.49] Box 2: [72.28, 30.10, -10.99] Box 1: [62.28, 39.74, -14.37]	Present	48.02	Large	280.46	Many spots	266
 XVI	Blue pink (N74D)	Box 5: [92.36, -1.02, 0.73] Box 4: [93.37, 1.49, -1.06] Box 3: [90.55, 5.10, -3.44] Box 2: [86.16, 9.96, -6.81] Box 1: [79.69, 17.67, -12.05]	Present	31.98	Large	186.66	Many spots	183

Fig. 11 – Comparison of trait evaluation results using DUS and the proposed methods of the same petal.

attractive to consumers than are those with lower degrees of colour saturation (Berghage & Wolnick, 2000).

3.3. *Spot traits*

Figure 9 presents boxplots of (a) spot quantity, (b) spot density, and (c) spot area ratio. The standard deviation of spot quantity, spot density, and spot area ratio were 103.93, 27.56, and 0.07, respectively. Figure 6VI and VII shows the petals that had the least (11) and most (443) spots, respectively.

Figure 10 shows the profiles of spot and background colours in the L*a*b* colour space. A considerable difference between the spot and background colours was observable. The mean difference between the spot and background colours was 91.81 ΔE units. Typically, the spot colours were violet (colour 78C of RHS colour chart; mean L* = 67.76, a* = 19.08,

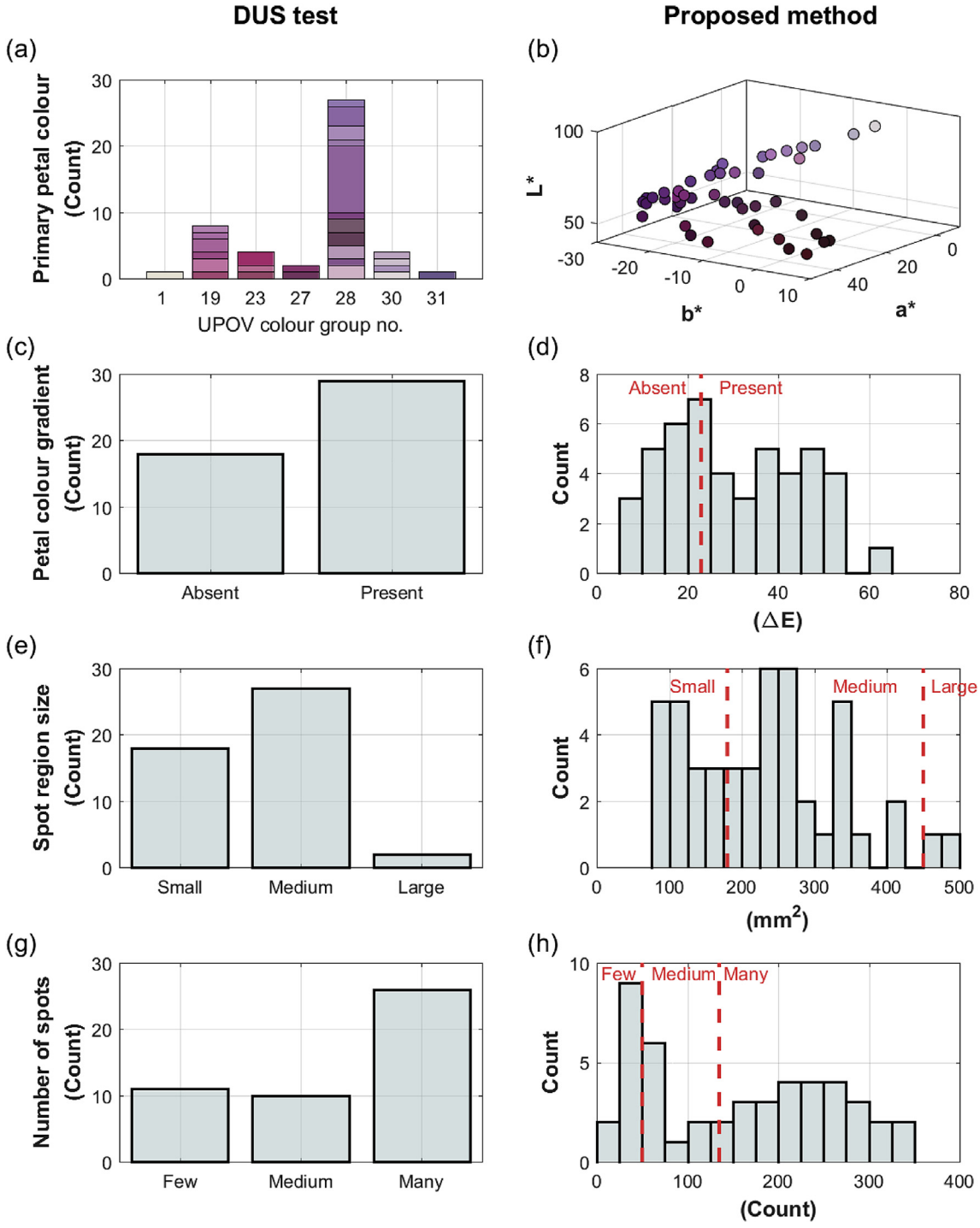

Fig. 12 – Summary of the trait distributions for evaluating the 47 petals using DUS test (a, c, e, and g) and the proposed method (b, d, f, and h). The red dotted lines in (d), (f), and (h) indicate thresholds used in DUS test.

and b* = −6), whereas the background colours were grey (colour 156D of RHS colour chart; mean L* = 90.09, a* = 2.71, and b* = 1.63). The variation in spot colour was larger than that in background colour. The root-mean-squared deviations of the spot and background colours were 5.86 and 3.05 ΔE units, respectively (Fig. 10).

3.4. *Application to new flower variety examination*

The proposed approach for quantifying colour and spot traits can improve the procedure for examining new flower varieties. Conventionally, new flower varieties are determined by following the protocols of the distinctness, uniformity, and stability (DUS) test (UPOV, 2002). DUS test for flowers of ornamental plants (e.g., *Alstroemeria* L.; UPOV, 2006) concerns primary petal colour (in the web version), petal colour gradient (in the web version), spot region size, and number of spots (Fig. 11). In DUS test, primary petal colour is determined by manually referring the primary colour of a petal to RHS colour chart. By contrast, the proposed method examines and quantifies five colours (i.e., colours of Boxes 1 to 5 (in the web version)) of a petal (Fig. 11a). In DUS test, colour gradient is determined by naked eye examination as presented or not presented in a petal. By contrast, the proposed method quantifies the magnitude of colour gradient in a petal in CIE L*a*b* ΔE units (Fig. 11b). In DUS test, spot region size of a petal is manually categorised into small, medium, and large. By contrast, the proposed method measures the area of the spot region using image processing algorithms (Fig. 11c). In DUS test, number of spots is categorised into absent, few spots, medium spots, or many spots by naked eye examination. By contrast, the proposed method determines the number of spots in a petal (Fig. 11d).

The proposed method is more precise and objective in trait quantification compared to manually conducted DUS test. Figure 12 summarises the trait distribution for evaluating the petals of 47 individuals using DUS test and the proposed method. For DUS test, the primary colours of the petals were assigned to 7 UPOV colour groups (in the web version) (1: white, 19: blue pink, 23: purple red, 27: purple, 28: violet, 30: light blue violet, 31: blue violet; Fig. 12a) according to their RHS codes. By contrast, the ROI colours (in the web version) were

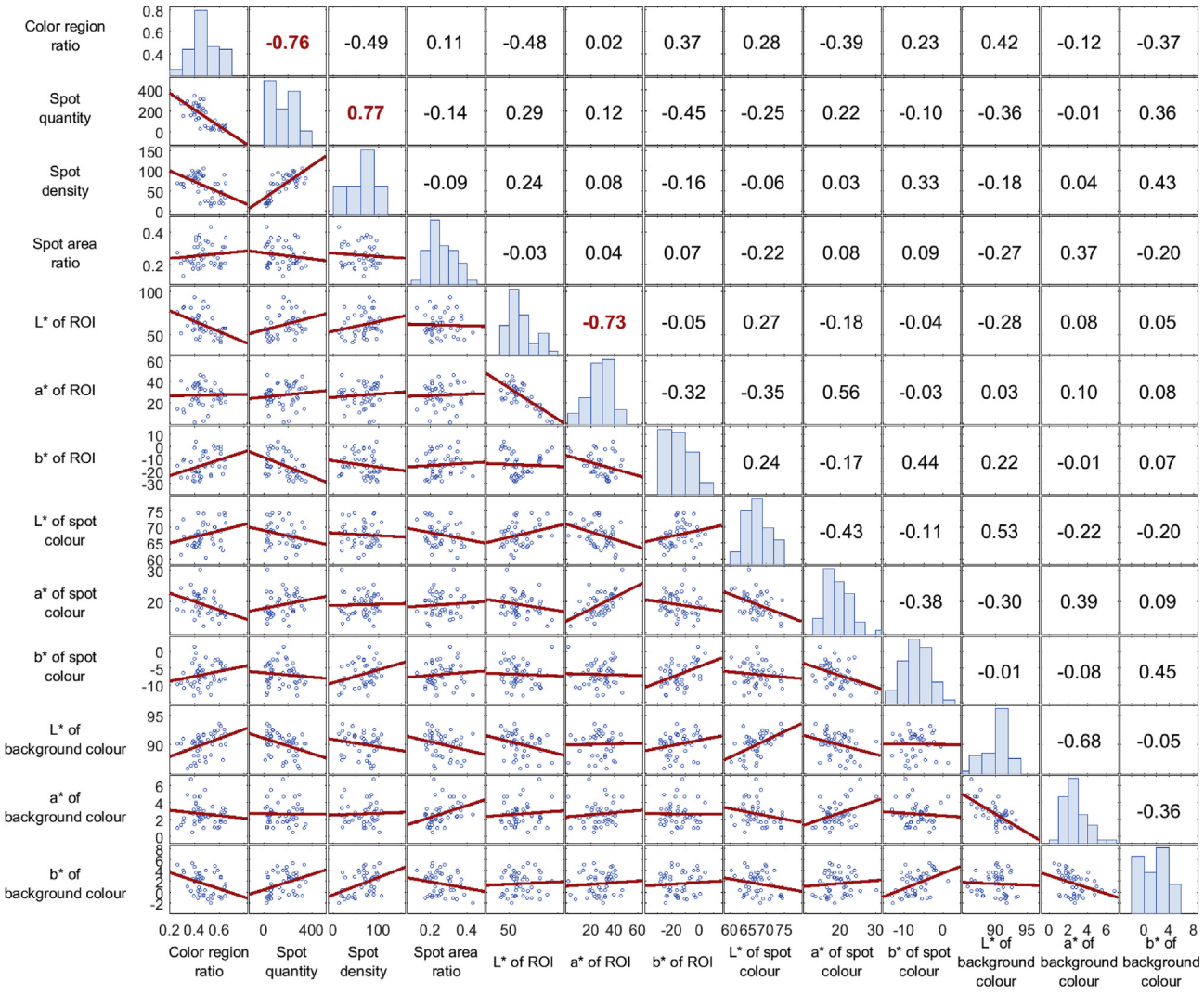

Fig. 13 – Distributions of pairwise correlations between traits. Strong correlation coefficients are indicated in red.

quantified by the proposed method as distinct L*, a*, and b* parameters (Fig. 12b). For DUS test, the petal colour gradient (in the web version) (Fig. 12c), spot region size (Fig. 12e), and number of spots (Fig. 12g) were categorised. Conversely, for the proposed method, the traits of each specimen were quantified as distinct numbers (Fig. 12d, f, and h).

3.5. *Trait correlation*

The pairwise correlations between colour region ratio, spot quantity, spot density, spot area ratio, ROI colour, spot colour, and background colour were examined. Figure 13 displays the distributions of and correlations between traits. The high degree of correlation between colour region ratio and spot quantity ($r = -0.76$), spot density and spot quantity ($r = 0.77$), and L* and a* of the ROI colour ($r = -0.73$) was detected. The spot densities of the petals with larger spot quantities were typically higher than the spot densities of the petals with fewer spots (Fig. 13). Petal spots serve as visual nectar guides for pollinators (Medel, Botto-Mahan, & Kalin-Arroyo, 2003; Sprengel, 1793). Spot quantity and density can thus be used as preference indices for pollinators. In general, pollinators prefer flowers with high spot quantity and density (Hansen et al., 2012; Kelber, 1997).

4. Conclusions

This study demonstrated an approach to objectively evaluating traits in petals. The variation in colour and spot patterns in the ventral petal was evaluated for the F_2 cross between two varieties of *S. speciosa*. One parent bloomed pale petals with dark block, whereas the other parent bloomed magenta petals with spots. Thus, the cross population exhibited an inherited high degree of variation in colour and spot patterns. The colour and spot traits of the ventral petal images were quantified using image processing. The root-mean-squared deviation of the ROI colour was 18.71 ΔE units. The mean magnitude of colour gradient was 30.10 ΔE units. The number of spots per petal ranged between 12 and 443. Analysis also showed that the major colour variation in the petals was more considerable at the petal distal. The proposed approach can quantify petal traits more objectively and precisely compared with conventional naked eye examination methods. Thus, the proposed approach has a potential to be used for DUS tests and some other applications.

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