Spathe Color Variation in *Anthurium andraeanum* Hort. and Its Relationship to Vacuolar pH

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Abstract. The relationship between vacuolar pH in *Anthurium andraeanum* (Hort.) and spathe color, cultivar, developmental stage, spathe location, spathe surface differences, and time after harvest was investigated with the overall long-term objective of developing a methodology for engineering blue-colored spathes. Chromospectral analysis of the pigmentation was also studied. Six experiments were conducted involving 23 cultivars of anthurium with each experiment arranged in a randomized complete block design with five replications. Spathe color was associated with vacuolar pH with the whites and greens having the highest pH (average 5.65) followed by corals (5.38), pinks (5.20), reds (5.10), and oranges (4.5). In general, there was correspondence between the lightness of the pigmentation (L^*) and the pH values with the lighter colors having higher pH values. There were, however, significant differences among cultivars within the color groups. Whereas spathe pH decreased with aging, there was no difference in the spectral data, suggesting that factors other than anthocyanin content may be contributing to the pH difference. There were no differences in pH between locations sampled on the spathe nor between the spathe surfaces provided there were no differences in color intensity (L^*). The pH increased with vase life in two of three cultivars tested with pH values showing an association with increases in a^* and b^* (chromospectral data) reflecting a bluing effect. The importance of the results to engineering blue-colored spathes in anthurium is discussed.

Flower color in plants is determined by pigments such as aurones, anthocyanins, and carotenoids (Davis and Schwinn, 1997; Schijlen et al., 2004). Anthocyanins, the major pigment in *Anthurium andraeanum* (Hort), are the product of flavonoid biosynthesis and are divided into cyanidins and their derivatives that produce colors ranging from red to purple (Griesbach, 1996); pelargonidins and their derivatives that produce colors ranging from coral to orange (Iwata et al., 1979); and delphinidins and their derivatives that produce colors from blue to deep red (Asen and Siegelman, 1957).

Researchers have stated that the ultimate color displayed is dependent not only on the pigment present, but also on a number of factors including cell shape (Noda et al., 1994), presence of various metal ions (Shoji et al., 2007), stacking of anthocyanins with copigments such as flavones and flavonols (Goto and Kondo, 1991), and pH (Harborne, 1988; Katsumoto et al., 2007; Stewart et al., 1975). As a result, in some species, the flower color is not corre-

lated with pigment composition. For instance, in rose cultivars that contained cyanidin, flowers varied from red to lavender and those with peonidin varied from red to purple (Griesbach, 1996). Similar observations were made in tulip (Nieuwhof et al., 1989) and hydrangea (Yoshida et al., 2003).

The role of pH on flower color has been well established in many plant species, including hydrangea, petunia, morning glory, orchids, and rose. Although the sepals of hydrangea have only one anthocyanin, delphinidin-3-glucoside, the color displayed varies from red to blue (Asen and Siegelman, 1957) with corresponding changes in vacuolar pH from 3.3 to 4.1 (Yoshida et al., 2003). In petunia, the inheritance of flower color was attributed to the combined effect of anthocyanin pigmentation and pH, the latter being controlled by two independent codominant genes, *Ph1* and *Ph2* (Griesbach, 1996). In morning glory, flower color varied from reddish purple buds to blue flowers with an increase in vacuolar pH from 6.6 to 7.7, a change believed to be driven by a $\text{Na}^+(\text{K}^+)/\text{H}^+$ exchanger (Yamaguchi et al., 2001; Yoshida et al., 2005). In *Phalaenopsis pulcherrima*, blue-flowered cultivars had a pH more alkaline (pH 5.7) compared with the purple form of the species (pH 4.9) with high pH being governed by a single recessive gene (Griesbach, 1997).

Although roses exhibit a variety of colors, they lack blues. Early attempts to generate blues

in roses through the introduction of the flavonoid 3', 5'-hydroxylase (F3'5'H) gene were unsuccessful. Katsumoto et al. (2007) generated blue roses by placing the F3'5'H gene into a genetic background with higher vacuolar pH and high flavonol content. Griesbach (2005) observed that although flavonols and an appropriate pH are important in obtaining blue orchids, the more important of the two factors was vacuolar pH. Creation of blue orchids, he suggested, would therefore require the screening of germplasm for high floral pH and combining the independent codominantly inherited high pH genes into a single genotype (Griesbach, 2005).

Anthurium andraeanum Linden ex André is a tropical ornamental species with a limited range of spathe colors based on anthocyanin pigments that vary from orange to red. The color range has been expanded through interspecific hybridization with species belonging to the section Calomystrum (Kamamoto and Kuehnle, 1996) through the creation of a species complex referred to as *A. andraeanum* (Hort.).

Iwata et al. (1979) reported that the major spathe colors, red to pink and orange to coral, are determined by two anthocyanins, pelargonidin 3-rutinoside (pelargonidin 3-rhamnosyl-glucoside) and cyanidin 3-rutinoside (cyanidin 3-rhamnosyl glucoside) found exclusively in the hypodermal layers of abaxial and adaxial surfaces of the spathe (Ehrenberger and Kuehnle, 2003; Higaki et al., 1984). Whereas pelargonidin 3-rutinoside is responsible for orange and coral spathes, both pelargonidin 3-rutinoside and cyanidin 3-rutinoside are found in red and pink spathes. Coral and pink spathes have lower concentrations of anthocyanins in comparison with orange and red counterparts (Iwata et al., 1985). The white spathes lack both anthocyanins but contain colorless flavone C-glycosides (Williams et al., 1981). Conspicuous lack of delphinidins or peonidin (a cyanidin derivative) account for the lack of mauves, purples, and blues (Iwata et al., 1985).

Recently the genetics and biochemistry of the anthurium flavonoid biosynthetic pathway have been characterized with the intention of creating colors in the blue range (Collette et al., 2004; Elibox and Umaharan, 2008a). However, no studies exist with regard to variation in pH in anthurium cultivars. With the objective of identifying suitable candidates for transformation toward generating blues in anthurium, this study investigates the relationship between epidermal vacuolar pH and a number of plant factors, including cultivar, spathe color, developmental stage of the spathe, location of anthocyanin within the spathe, differences between the abaxial and adaxial surfaces, and postharvest changes.

Materials and Methods

Plant material. Spathes were collected from 23 cultivars of *Anthurium andraeanum* (Hort.) maintained at Kairi Blooms Ltd., a commercial anthurium farm situated in Carapo Village, Arima, Trinidad. The collected

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samples were placed in a cooler and transported to the laboratory. All blooms were harvested between 0700 to 0800 HR during the months of January to February. Cultivars Pierrot, Tropical, and Success were harvested at three developmental Stages 6-1 (newly opened), 6-2 (spadix $\frac{1}{2}$ mature), and 6-3 (spadix $\frac{3}{4}$ mature), as defined by Collette (2002). Cultivar Tropical was harvested at five stages (Stages 2 to 6) to determine the effect of stage on color formation.

pH measurement. A random sample of five discs from 10 discs (50 mm²) obtained from each spathe using a cork borer was used as an experimental unit in the studies. The epidermal peels from the adaxial surface of these discs were obtained, ground in 0.4-mL Millipore water, pipetted into an enzyme-linked immunosorbent assay plate, and the pH determined using a pH meter (Corning pH meter Model 220; Vernon Hills, IL) carrying a general purpose combination electrode (Griesbach, 2005; Katsumoto et al., 2007).

Color determination. Color values based on the CIE L*a*b* system for the various spathe colors of cultivars were determined using the Chroma Meter CM-200b (Minolta® Company Ltd., Tokyo, Japan). In this system, “L” refers to lightness, whereas “a” and “b” represent the color opponent dimensions. The equipment was calibrated against a white Minolta® Calibration plate before use. Five random measurements were made per spathe. Each cultivar was represented by three spathes.

Experimentation. In the first experiment, 17 cultivars belonging to the white (six), orange (five), and red groups (six) were evaluated for the vacuolar pH. Each cultivar was replicated five times with each replicate represented by a single spathe. The white group consisted of white (three: ‘Cuba’, ‘Pierrot’, ‘Cotopaxy’) and green (three: ‘Midori’, ‘Pistache’, ‘KAIRI709’) spathes, both of which lack anthocyanins (Williams et al., 1981). The orange group consists of coral cultivars (two: ‘Venus’, ‘KAIRI899’) and orange cultivars (three: ‘Hawaii’, ‘KAIRI3827’, ‘KAIRI1767’), both of which contain pelargonidin 3-rutinoside but in different quantities (Iwata et al., 1979). The red group consists of pink (three: ‘Spirit’, ‘Lydia’, ‘Rosa’) and red (three: ‘Mirjam’, ‘Success’, ‘Tropical’) cultivars, both of which contain mainly cyanidin 3-rutinoside but at different levels (Iwata et al., 1979).

The effect of developmental stage of the spathe on pH was determined in three anthurium cultivars (Pierrot, Success, and Tropical) in Expt. 2 at three stages, as described. The nine treatment combinations were arranged in a factorial structure and were replicated five times. Peeling, extraction, and pH measurements were carried out as described. In a separate study, the chromospectral parameters were investigated in anthurium cut flowers from Stage 2 to Stage 6 to investigate the effect of developmental stage on color formation.

In the third experiment, leaf discs were obtained from the tip of the spathe versus the base of the spathe in five cultivars representing four spathe colors (two red, one pink, one orange, and one white) to determine the effect

of tissue maturity on pH. The experiment was replicated five times.

In the fourth experiment, epidermal peels from three discs each from the abaxial and adaxial surfaces were investigated with five replications in two cultivars (Tropical, Sonata) as described.

In the fifth experiment, three cultivars (President, Senator, and KAIRI3674) with “obake” (bicolored) spathes were used. These cultivars produce bicolored spathes with various anthocyanins at the center of the spathe and no anthocyanins at the periphery. ‘President’ and ‘KAIRI3674’ have a dark pink center, whereas ‘Senator’ has a coral center. In this study, the central pigmented portion was represented by five discs (50 mm²) and the anthocyaninless peripheral regions represented by five discs. The epidermal peels from the adaxial surface for each region were extracted and pH measurement taken as described.

In the last experiment, changes to spathe pH were monitored in four cultivars (Pierrot, Mirjam, Success, Tropical) after harvest at 0 d after picking (DAP), 8 DAP, 16 DAP, 24 DAP, and 32 DAP. The 16 combinations were replicated five times in a completely randomized design. Cut flowers with no scratches, no deformities, and straight peduncles were harvested at the $\frac{3}{4}$ mature stage of the spadix (Kamemoto, 1962). Twenty-five cut flowers per cultivar were harvested and brought to the laboratory in a cooler. The experiment was conducted in sterile, 250-mL measuring cylinders in a laboratory (11 h white fluorescent light; 23.8 °C; 73.5% relative humidity). Each cylinder contained five cut flowers placed in 210 mL of sterile distilled water and was covered with a cellophane wrap to prevent evaporation. Before placing the cut flowers into the cylinder, the base of the peduncles was cut under water at an angle of 45° using a sterile scalpel.

Data analysis. Data on pH as well as color (L*a*b*) were analyzed using NCSS (NCSS, 2001, Kaysville, UT). The relationship between color intensity and pH was investigated using

Pearson’s product moment correlation coefficient or regression analysis (NCSS, 2001).

Results

There were significant ($P < 0.05$) differences in pH between the various color groups (Table 1) with the highest pH recorded for green-spathed cultivars followed by white, coral, pinks/reds, and orange, in that order. There was no significant difference ($P > 0.05$) between red- and pink-spathed cultivar groups. Cultivar differences in vacuolar pH within the various colors were significant ($P < 0.05$) for green, coral, red, and pink (Table 1), but were not as large as between colors.

Chromospectral analysis, apart from showing expected differences between the various color groups, also showed significant ($P < 0.05$) differences in the L*a*b* space parameters among cultivars (Table 1). Among the red-spathed cultivars, ‘Tropical’ had a significantly higher pH value ($P < 0.05$) compared with ‘Mirjam’ and ‘Success’ with correspondingly higher L* and b* values ($P < 0.05$) (Table 1). Among the pink-spathed cultivars, ‘Lydia’ had a significantly higher pH value ($P < 0.05$) than ‘Rosa’, which in turn had a significantly higher pH value ($P < 0.05$) than ‘Spirit’. Again, the L* values reflected closely the pH (Table 1). Although b* values were significant between cultivars, they did not correspond to the pH values (Table 1). Neither the pH nor the L*a*b* values were significant among the orange-spathed cultivars. Among the coral-colored cultivars, ‘Venus’ with the higher L* value had a significantly higher pH than ‘KAIRI899’ with a lower L* value. Among the whites, there were significant differences ($P < 0.001$) among L*b* with L* values being positively correlated to pH ($r = 0.97$). Among the green cultivars, cultivar KAIRI709 had the highest L* value followed by ‘Pistache’ and ‘Midori’, in that order. Although all the L*a*b* color space parameters were significantly different among the green-spathed cultivars, none were significantly correlated to pH.

Table 1. The vacuolar pH values and chromospectrometric measurements for 17 cultivars of *Anthurium andraeanum* (Hort.).

Color group ^z	Cultivar ^y	Epidermal peel pH	Chromospectrometric measurements			Mean pH color group
			L*	a*	b*	
White	Cuba	5.51	84.1	-2.2	19.9	5.57
	Pierrot	5.57	88.5	-2.1	10.2	
	Cotopaxy	5.56	89.7	-2.5	11.7	
Green	Midori	5.50	55.2	-17.7	31.4	5.74
	Pistache	5.78	61.2	-9.7	27.1	
	KAIRI 709	5.94	68.3	-18.1	39.6	
Coral	KAIRI 899	5.26	60.5	44.5	28.2	5.38
	Venus	5.49	64.8	43.0	26.3	
Orange	KAIRI 3827	4.50	52.5	54.8	37.2	4.51
	KAIRI 1767	4.53	53.7	48.5	30.9	
	Hawaii	4.49	51.7	53.5	35.1	
Pink	Spirit	5.02	63.7	32.6	14.0	5.20
	Rosa	5.20	69.7	32.7	12.4	
	Lydia	5.40	74.6	23.4	15.4	
Red	Tropical	5.31	41.8	50.4	22.4	5.09
	Success	4.92	38.8	48.7	19.0	
	Mirjam	5.07	37.2	46.7	19.2	
	SE	0.051	1.43	2.14	0.84	0.041

^{z,y}The effect of color group and cultivar were significant on vacuolar pH and L*a*b*.

The relationship between color space parameters a^* and b^* (Fig. 1) showed that various anthocyanin-based color groups could be clearly distinguished. Pinks had low a^* and b^* values, whereas reds have low a^* but high b^* values. The corals and oranges had above average a^* and b^* values and formed a continuum with the corals at the lower end of the continuum. These results indicated that apart from color intensity measured by L^* , which was used to distinguish between red versus pink and orange versus coral, there were differences in a^* and b^* among the color groups.

The effect of spathe developmental stage (6-1, 6-2, and 6-3) significantly influenced pH ($P < 0.05$) in all the three cultivars studied with pH decreasing from Stage 6-1 to 6-2 but not significantly changing thereafter. The cultivar $\times$ stage interaction was not significant, indicating that the effect of stage of spathe on pH was independent of the effect of cultivar (Table 2). The effect of cultivar on pH was again the most profound (F value of 178) with the white-spathed 'Pierrot' (pH = 5.0) having the highest pH followed by red-spathed cultivars Tropical (pH 4.5) and Success (3.7).

The chromospectral data ($L^*a^*b^*$) did not show significant ($P > 0.05$) differences among the three substages within Stage 6 (6-1, 6-2, 6-3), but there was a significant decrease in L^* from Stage 3 to Stage 4, which remained relatively constant thereafter up to Stage 6 (Fig. 2). Similarly, a^* increased significantly from Stage 3 to Stage 4 but remained relatively constant thereafter (Fig. 2). Color space parameter b^* , however, did not show any specific trend with developmental stage (Fig. 2).

Neither the effect of sampling location within the spathe (tip versus base) nor the cultivar $\times$ sampling location interaction were significant ($P > 0.05$) in the six cultivars evaluated. The effect of cultivar differences, however, was highly significant ($P < 0.001$) (data not shown).

Among two cultivars investigated, there was a slight but significantly higher pH ($P < 0.05$) in the abaxial epidermal peel compared with that in the adaxial epidermal peel in 'Sonata', but this was not observed in 'Tropical' ($P > 0.05$). This accounted for the significant cultivar $\times$ surface interaction ($P < 0.01$) (Table 3). The higher pH in abaxial cells was reflected in the significantly higher L^* and small a^* and b^* values in 'Sonata'. Although 'Tropical' showed a small but significant increase in L^* (not significant a^* or b^*) in the abaxial compared with adaxial data, there was no significant difference in pH. The cultivar effect was significant ($P < 0.001$) with the pink-spathed 'Sonata' (pH 5.36) having a higher pH than the red-spathed 'Tropical' (5.02).

In the obake-spathed cultivars, pH was always significantly higher ($P < 0.001$) in the green peripheral regions of the spathe compared with the anthocyanin-containing centers (Table 4). The significant cultivar $\times$ treatment interaction indicates that the degree to which the green peripheral regions had a higher pH varied with cultivars. Difference in pH

Fig. 1. The relationship between color space parameters a^* and b^* in four color groups in *Anthurium andraeanum* Hort.

Table 2. The vacuolar pH and chromospectrometric measurements as influenced by developmental stage and cultivar in *Anthurium andraeanum* Hort.

Developmental stage of spathe ^z	Cultivar	Vacuolar pH ^z	Chromometric measurements ^y			Mean pH stage
			L^*	a^*	b^*	
Stage 6-1	Pierrot	5.00	89.3	-2.9	13.1	4.51
	Tropical	4.65	40.2	50.4	22.6	
	Success	3.89	37.3	48.3	17.5	
Stage 6-2	Pierrot	4.96	89.4	-2.4	12.3	4.33
	Tropical	4.42	41.6	49.3	22	
	Success	3.62	36.7	46.3	16.4	
Stage 6-3	Pierrot	5.00	88.5	-2.1	12.2	4.42
	Tropical	4.54	44.1	46.3	24.2	
	Success	3.71	37.6	46.1	18.9	
SE		0.082	0.53	0.44	0.61	0.047

^zThe cultivar and spathe developmental stage significantly affected pH at $P < 0.05$ and $P < 0.001$, respectively, but the interaction was not significant.

^yThe effect of cultivar, developmental stage, and interaction were significant for $L^*a^*b^*$.

Fig. 2. Spathe chromospectrometric space parameters $L^*a^*b^*$ as affected by spathe developmental stage (the bars represent $\pm$ least significant difference values).

between the green and pigmented centers was particularly high for 'Senator' with a coral center compared with 'President' and 'KAIRI3674', which had a dark pink center (Table 4).

Effects of cultivar, post-harvest age, and the interaction between cultivar and post-harvest age on pH were highly significant ($P < 0.001$). Effect of cultivar on pH was the

highest (highest F value). The white-spathed 'Pierrot' had a significantly higher ($P < 0.001$) pH (5.61) than the red-spathed 'Tropical', 'Success', and 'Mirjam' (average 4.8). The pH increased from 4.96 at picking to 5.27, 24 DAP. Unlike the other cultivars, 'Success' did not show significant variation in pH with age, which would have accounted for the significant cultivar $\times$ age interaction (Table 5).

Table 3. The effect of the sampling location within a spathe (center versus lobe) on pH and chromospectrometric measurements in three obake cultivars of anthurium.

Cultivar	Sampling location	Vacuolar pH ^z	Chromospectrometric measurements ^y			Mean pH cultivar
			L*	a*	b*	
President	Green	5.25	46.1	-15.6	26.3	5.05
	dark pink	4.87	58.3	36.5	16.2	
Senator	Green	5.22	49.7	-17.2	32.7	4.84
	Coral	4.45	76.9	18.3	20.8	
KAIRI3674	Green	4.84	— ^x	—	—	4.66
	dark pink	4.50	—	—	—	
SE		0.047	2.1	2.6	1.7	0.030

^zThe cultivar and spathe location effects significantly affected pH at $P < 0.001$ with significant interaction ($P < 0.001$).

^yThe effect of cultivar, sampling location, and interaction were significant for $L^*a^*b^*$.

^xAdequate blooms were not available for chromospectrometric measurements.

Table 4. The effect of the sampling of spathe surfaces (adaxial versus abaxial) on pH and chromospectrometric measurements in two cultivars of anthurium.

Cultivar	Sampling surfaces	Vacuolar pH ^z	Chromospectrometric measurements ^y			Mean pH cultivar
			L*	a*	b*	
Sonata	Adaxial	5.27	57.6	46.9	14.5	5.36
	Abaxial	5.46	75.0	15.9	9.0	
Tropical	Adaxial	5.07	42.1	50.4	22.1	5.03
	Abaxial	4.98	44.7	50.0	20.8	
SE		0.035	0.46	1.33	0.59	0.024

^zThe cultivar and cultivar $\times$ spathe location interaction effects were significant on pH at $P < 0.001$ and $P < 0.01$, respectively; but the sampling location effect was not significant.

^yThe effect of cultivar, sampling location, and interaction were significant for $L^*a^*b^*$.

Table 5. The effect of time after cut flower harvesting on spathe vacuolar pH and chromospectrometric measurements in four cultivars of anthurium.

Cultivar	Time after picking	Vacuolar pH ^z	Chromospectrometric measurements ^y			Mean pH cultivar
			L*	a*	b*	
Pierrot	0 d	5.58	89.4	-2.4	12.2	5.61
	8 d	5.68	—	—	—	
	16 d	5.59	—	—	—	
	24 d	5.74	41.6	-2.9	12.9	
	32 d	5.46	—	—	—	
Tropical	0 d	4.87	36.7	49.3	22.0	5.06
	8 d	5.00	—	—	—	
	16 d	5.10	—	—	—	
	24 d	5.28	90.3	34.5	1.5	
	32 d	—	—	—	—	
Success	0 d	4.58	42.2	46.3	16.4	4.72
	8 d	4.73	—	—	—	
	16 d	4.75	—	—	—	
	24 d	4.78	36.6	44.5	16.0	
	32 d	4.77	—	—	—	
Mirjam	0 d	4.74	38.8	43.2	17.5	4.89
	8 d	4.80	—	—	—	
	16 d	4.77	—	—	—	
	24 d	4.84	— ^x	—	—	
	32 d	5.28	—	—	—	
SE		0.055	0.51	0.45	0.37	0.025

^zThe cultivar and time of picking significantly affected pH at $P < 0.001$ with significant interaction ($P < 0.001$).

^yThe effect of cultivar and interaction between cultivar $\times$ time of picking were significant for $L^*a^*b^*$. Time after picking was significant for a^* and b^* only. Only 'Pierrot', 'Tropical', and 'Success' were included in the analysis.

^xData on $L^*a^*b^*$ were not collected.

Chromospectral data obtained at 0 DAP and 24 DAP did not show significant ($P > 0.05$) changes in L^* in 'Success' and 'Mirjam' but showed changes in opposite directions in 'Pierrot' and 'Tropical', accounting for the significant interaction. With respect to a^* and b^* , not only were there significant ($P < 0.05$) differences with age, but there was a significant cultivar $\times$ age interaction. For instance, although there were significant reductions in a^* and b^* values in 'Tropical' with

aging, the changes were not significant for the other cultivars. This change in 'Tropical' resulted in a visual bluing by Day 24 after picking, which was not observed in the other cultivars.

Discussion

Most studies on pH have been carried out in floral tissues and limited information currently exists for leaf tissues with the ex-

ception of bracts of *Poinsettia* (Stewart et al., 1975). This is the first study of pH in anthurium spathe, a modified leaf. The pH measurements among the 23 cultivars studied varied from 4.6 to 5.9 in the epidermal peels of spathe, where the pigments are concentrated (Ehrenberger and Kuehnle, 2003). The pH values suitable for developing blue color in floral tissues were found to be above 5.25 in roses (*Rosa hybrida*) (Katsumoto et al., 2007), from 6.6 to 7.7 in morning glory (*Ipomoea tricolor*) (Yoshida et al., 1995), 5.8 to 6.4 in *Petunia hybrida* (Griesbach, 1996), 4.9 to 5.7 in *Phalaenopsis pulcherrima* (Griesbach, 2005), and above 4.1 in *Hydrangea macrophylla* (Yoshida et al., 2003). The values found in this study were within the pH range suitable for blues, identified in three species.

The relationship among various spathe colors and pH was the most striking with the green and white cultivars having the highest average pH (5.65). They were followed by corals (5.38), pinks (5.20), reds (5.10), and oranges (4.50). The differences were significant among color groups, except for differences between reds and pink. Within colors, cultivars with lighter shade (higher L^*) had a higher pH than those with a darker shade. Iwata et al. (1985) demonstrated that coral and pink spathes had lower levels of anthocyanins compared with orange and red spathes. These data suggest that pH is associated with not only the anthocyanin present, but also its concentration.

Evidence of the presence of anthocyanins is associated with a low pH coming from the experiment involving obake (bicolored) anthurium spathes, in which the variously colored segments of the spathe of the same genotype had large divergent pH values. The green and white portions of the spathe consistently had a higher pH than the colored sections of the spathe. This is in conformity with studies in *Fuchsia*, in which epidermal pH values of differently colored parts of the same flower were dissimilar (Stewart et al., 1975).

Similarly, supporting evidence for the effect of concentration of anthocyanins on vacuolar pH comes from the evaluation of two cultivars, Tropical with the same level of pigmentation at the abaxial and adaxial surface and Sonata with a much lighter pigmentation in the abaxial than the adaxial surface. 'Tropical' showed no significant differences in pH between the abaxial and adaxial peels, whereas with 'Sonata', the difference in pH was significant.

Studies in *Petunia hybrida* (Quattrocchio et al., 2006; Spelt et al., 2002) have shown that AN1 coding for a basic helix-loop-helix protein has a pleiotropic effect controlling, in addition to anthocyanin synthesis by regulating dihydro flavonol 4-reductase (DFR), also controls acidification of vacuoles and seed coat morphogenesis. They also showed that different functions of the *an1* gene could be abolished by mutating different domains of the gene. Later work by Quattrocchio et al. (2006) showed that AN1 protein can have differential effects through interacting with different Myb proteins. These results indicate

that the higher pH in whites and greens observed in this study could be the result of pleiotropic effects of controlling elements on anthocyanin biosynthesis. Alternately, Figueiredo et al. (1999) showed that malonic acid residues present in many anthocyanins appear to result in color stabilization by providing a lower pH in the vacuolar solution, which may account for differences among color groups.

There were significant differences in pH between cultivars within each color category. Cultivar differences within each color category varied between 1% to 8%, depending on the color group, indicating the importance of screening a large number of cultivars within each color group to identify those with high pH for use in bioengineering of blue flowers as suggested by Griesbach (2005) for orchids. Similarly, considerable within cultivar variation in pH was seen in roses. A total of 169 cultivars was screened for pH and flavonoid content in roses with six cultivars (pH values varying from 4.85 to 5.46) being selected as candidates for transformation (Katsumoto et al., 2007).

There was a significant decrease in pH between the developmental Stages 6-1 to 6-2, which remained constant through Stage 6-3. However, chromospectral analysis showed a significant decrease in L^* and increase in a^* up to Stage 4, beyond which the differences were not significant. These results in combination suggest that the decrease in pH from Stage 6-1 to 6-2 may reflect that pH changes with development that may be associated with factors other than anthocyanin concentration. In addition, although the development age of the base is regarded as older than the tip of the spathe (Hartmann et al., 2001), there were no significant pH differences between tip versus base in any of the cultivars tested.

The pH of spathe increased with time after harvest of 'Tropical' and 'Mirjam' but not 'Success'. Furthermore, the change in pH was not associated with L^* but rather with changes in a^* and b^* , which mirror changes in color rather than intensity. Loss of vase life in anthurium is as a result of loss of spathe glossiness, spathe, and spadix browning and spathe bluing (Elibox and Umaharan, 2008b; Paull, 1982). Spathe bluing is attributed to an increase in pH from 5.2 to 5.6 caused by an increase in ammonium ions resulting from protein breakdown (Paull et al., 1985).

In roses, blue color was obtained (Katsumoto et al., 2007) by silencing the endogenous DFR and expression of iris DFR and *viola* F3'5'H into a high pH, high flavones genetic background. Prior studies on anthurium (Collette et al., 2004; Elibox and Umaharan, 2008a) that elucidate the genetic control of anthocyanin biosynthesis in spathe tissue paves the way for engineering blue hues. Nevertheless, it is first important to identify an appropriate pH background. This study suggests that corals, which have the highest pH among the color groups, may be best suited as targets for transformation, because they contain lower levels of pH-reducing anthocyanins

(Iwata et al., 1985). Alternately, red anthurium should be screened for genotypes with high pH values.

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