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Morpho-Anatomical Characters and Ethylene Production in *Hibiscus rosa-sinensis* L. in Relation to Two-Day Floral Retention

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The cultivars of *Hibiscus rosa-sinensis* are known worldwide for their aesthetic value as garden or potted plants; but they have a short duration or retention of their flowers. This constraint has limited their potential in the floriculture industry because these cultivars have not been used as sources of cut flowers for floral arrangements. The main objective of this research was to find out the factors that affect duration of petal retention in *Hibiscus rosa-sinensis* L. across the four breeds or varieties used, namely, 'Gelia Castillo' (GC), 'Loren Legarda' x 'Estrella F. Alabastro' (LL x EFA), 'Reddy-or-Not' (RON), and 'Wilcox' (WX). Morphological traits such as peduncle diameter, receptacle diameter, peduncle length and petal thickness were related to floral retention of *H. rosa-sinensis* L. Moreover, the anatomy of the abscission zone was observed to provide a tissue-level basis for floral retention. Furthermore, ethylene concentrations were quantified to assess their relation to floral retention. There were no defined abscission zones in flowers of hibiscus; instead, the abscised petals had remnants, suggesting that abscission did not proceed at the petal base where the abscission zone is theoretically located. In addition, ethylene production increased as abscission progressed, typically exhibiting the climacteric pattern and the auto-catalytic nature of ethylene biosynthesis.

Key Words: abscission zone, ethylene, floral retention, *Hibiscus rosa-sinensis*, morpho-anatomy

Abbreviations: FAA – formalin-acetic acid, GC – 'Gelia Castillo', LL x EFA – 'Loren Legarda' x 'Estrella F. Alabastro', PE – polyethylene, RON – 'Reddy or Not', WX – 'Wilcox'

INTRODUCTION

In the Philippines, the hibiscus (*Hibiscus rosa-sinensis* L.) has become a popular ornamental plant with attractive flowers usually adorning many home gardens and public places such as schools, parks and resorts. The beauty of the hibiscus makes it one of the most widely cultivated flowers in brilliant hues of red, orange, or purplish-red, yellow, white, purple, pink and several other color combinations that have either single or double petals. It is used either as a potted plant, fence or hedge plant, and landscape shrub (Magdalita et al. 2009; Magdalita and Pimentel 2010). It is also used for food, feed, industrial and medicinal preparations. The fresh flowers are used as food coloring and as a component of vegetable salad, while the dried flowers are considered as a special delicacy in Mexico.

From a flower bud, the flower emerges in less than 18 h usually in the morning during sunny days. The plant has short-lived flowers, but produces continuing blooms throughout the year. Under natural conditions, the hibiscus rarely produces fruit and seeds (Howie 1980). Since flower retention in the plant is problematic for it to become a cutflower, the control of flowering follows the control of the successive stages of its reproduction, hence it is important to investigate its flowering and longevity.

Hibiscus rosa-sinensis L. has favorable characteristics that are necessary to study floral retention in angiosperms. The reasons for this are the following: i) *Hibiscus* has ephemeral flowers that usually last for 1 d only (Gillman 1999; Woodson et al. 1985; Magdalita 2011); ii) stages of flower retention on the plant could be observed since they are explicitly displayed, having large sizes; iii) they exhibit inter-breed variation in terms of floral longevity (Magdalita 2011); and iv) *Hibiscus* is an economically important crop and it is known worldwide for its aesthetic, food and medicinal values (Rummel 2005;

Kumar and Singh 2012).

Breeding of *H. rosa-sinensis* to produce more economically valuable varieties is continuously being pursued by ornamental breeders in Hawaii, Australia, Denmark, Thailand and the Philippines (Nakasone and Rauch 1980; Australian Hibiscus Society 2007; Kuligowska et al. 2013; Magdalita and Pimentel 2013). The Institute of Crop Science (ICrops) and the Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB) is at the forefront of hibiscus breeding in the country. Aside from flower quality, introgression of genes for resistance to fungal and viral pathogens is being done (Dolores et al. 2014, 2016; Pascual and Magdalita 2012). Since 1995, UPLB has been producing many varieties, which are collectively called the UPLB *Hibiscus* hybrids falling under the different series including the 'Centennial' such as *H. rosa-sinensis* 'Gabriela' and 'Tandang Sora', 'Millenium' such as *H. rosa-sinensis* 'Dolores A. Ramirez' and 'Gelia Castillo', 'Celebrity Star' such as *H. rosa-sinensis* 'Star for All Seasons' and 'Superstar', 'Oblation', e.g. *H. rosa-sinensis* 'Emerlinda R. Roman' and 'Estrella F. Alabastro', 'Women in Public Service' e.g. *H. rosa-sinensis* 'Rosa Rosal' and 'Cynthia A. Villar', 'Women in Science' e.g. *H. rosa-sinensis* 'Fe V. del Mundo' and 'Solita F. Camara-Besa', 'Women in the Arts' e.g. *H. rosa-sinensis* 'Araceli A. Dans', and 'Women Saints and Institutions Named after Them' e.g. *H. rosa-sinensis* 'St. Bridget College'. As of 2016, there were already 46 hybrid varieties that had been released (Pimentel 1999; Magdalita et al. 2009; Magdalita and Pimentel 2010; Magdalita 2011; Magdalita et al. 2011; Magdalita and Pimentel 2013; Magdalita et al. 2016).

However, the flower of these UPLB *Hibiscus* hybrids normally lasts for 1–2 d. This short floral retention on the plant limits the utility of these hybrids either as potted plant or landscaping material. In addition, this constraint limits the use of hibiscus as a cutflower for floral arrangements. While new *Hibiscus* hybrids with 2-d floral longevity have been developed, they should be further hybridized with related *Hibiscus* species to produce interspecific hybrids with longer floral retention in order for *Hibiscus* to be considered as a cutflower. Before this could be achieved, the factors and mechanisms affecting retention of the flowers on the plant need to be investigated. Hence, the aims of this study were to: i) determine the morphological characters of the flower correlated with floral retention, ii) quantify the actual number of hours of floral retention in four breeds of *H. rosa-sinensis* typically identified as 1-d and 2-d retention breeds, iii) investigate the anatomical features at the attachment site of the petals across breeds, and iv) quantify ethylene production of the breeds at specific floral stages. This study was conducted at ICrops and IPB, CAFS, UPLB, College, Laguna, Philippines from April 2014 to May 2015.

MATERIALS AND METHODS

Plant Materials

Thirty flower samples were used in each of the four *Hibiscus* breeds, comprising a total of 120 flower samples for analysis. The four *Hibiscus* breeds with known floral retention of 1 d and 2 d (Magdalita 2011) were the following: 'Reddy-or-Not' (RON) and 'Wilcox' (WX) as 1-d retention, and *Hibiscus rosa-sinensis* 'Gelia Castillo' (GC) and a hybrid between *H. rosa-sinensis* 'Loren Legarda' and *H. rosa-sinensis* 'Estrella F. Alabastro' (LL x EFA) representing the 2-d retention breeds (Fig. 1). Five to 15 replicate plants having luxuriant growth and producing many flowers of each of the above-mentioned varieties generated from the Hibiscus Breeding Program at the ICrops and the IPB under the leadership of the principal breeder, Dr. Pablito M. Magdalita (Magdalita and Pimentel 2010; Magdalita and Pimentel 2013), were used. The four breeds were growing in the *Hibiscus* breeding blocks at the ICrops and IPB, CAFS, UPLB. The standard cultural practices for irrigation, fertilization, pest management and pruning were applied to the experimental plants.

Assessment of Quantitative Morphological Traits of Selected Floral Parts

Morphological characters that could be related to the length of floral retention in the plant were assessed. These included the following: peduncle diameter at attachment site to stem, at median, and at attachment site to the receptacle; peduncle length, receptacle diameter and petal thickness at attachment site and at median of the flower. Thirty flower samples were used for evaluating each character in each of the four varieties. Vernier and micrometer calipers were used to measure these characters. Sepals were removed to expose the receptacle of the flower in order to

Fig. 1. Breeds of *Hibiscus rosa-sinensis* L. used: A) 'Gelia Castillo' (GC), B) 'Loren Legarda' x 'Estrella F. Alabastro' (LL x EFA), C) 'Reddy-or-Not' (RON), and D) 'Wilcox' (WX).

measure receptacle diameter. Petals were excised from the flower to measure their thickness which included petal thickness at attachment site and at median of the flower. The length of floral retention on the plant in terms of number of hours was assessed from full opening or anthesis until the petals abscised from the flower.

Anatomy of the Abscission Zone

Pre-anthesis or bud stage and post-abscission stages were selected to investigate flowers of 1-d and 2-d retention varieties. Five replicate flowers at the abscission stage of the flower were prepared. Immediately after collection, the samples were fixed in 40% formalin-acetic acid (FAA) solution for 1 d before they were submitted for analysis. The fixed samples were sent to the Microbiological Laboratory, Inc. (MicroLab) in Pasay City, Metro Manila, Philippines for microslide preparation. Standard procedures for microtome sectioning and staining using Fast Green and counterstaining with Safranin O were carried out. The prepared slides were viewed under a binocular light microscope (TrueVision tv016b000m, USA) equipped with a digital camera and the ToupView 3.7 software. This software facilitated viewing of the samples through a computer screen to observe and characterize the abscission zone.

Ethylene Production

Ethylene was quantified using a gas chromatography machine (Shimadzu GC 2014, Japan) at the Postharvest Horticulture Training and Research Center (PHTRC), ICrops, CAFS, UPLB. Five replicate flowers of the four varieties were used. The flowers were enclosed in polyethylene (PE) plastic bags. Five cubic centimeters (5 c.c.) of gas samples were taken in each floral stage, including pre-anthesis, anthesis, closure and abscission in the four varieties. The gas samples were collected using a 5 c.c. plastic syringe and then transferred to a 5 c.c. glass vacuutainer (vacuum container). These vacuutainers were submerged halfway in water and were refrigerated prior to transport from ICrops and IPB to PHTRC for gas analysis. From the 5 c.c. vacuutainers, three replicate air samples were obtained, where each replicate was represented by 1 c.c. injection. One c.c. gas sample was injected in the gas chromatograph machine to quantify the amount of ethylene in the samples.

Statistical Design and Analyses

The completely randomized design (CRD) was used for all experiments done in the laboratory. The data were analyzed using Microsoft Excel 2010 and the Statistical Tool for Agricultural Research 1.1 (STAR). One-way analysis of variance (ANOVA) using F-test was used to test differences among varieties, and significant differences among means were subjected to pair-wise mean comparison using the Least Significant Difference (LSD). The Pearson r correlation analysis was used to test the association of morphological characters with the floral retentions of the four breeds.

RESULTS AND DISCUSSION

Varying Length of Floral Retention across Breeds

The varying length of floral retention from full opening until flower abscission across the four breeds was measured (Table 1). In WX, flowers stayed on the plant 13 h starting from full bloom up to full abscission, and flowers of RON stayed for 14 h. *H. rosa-sinensis* 'Gelia Castillo' had the longest (51 h) floral retention, LL x EFA was next to it (47 h), followed by RON (14 h), and WX had the shortest (13 h) petal retention on the plant.

The flowers of *H. rosa-sinensis* usually open before

Table 1. Duration of flower retention (no. of hours) from opening to abscission of the four *Hibiscus* breeds.

Breed	Duration of Flower Retention (h)
'Wilcox' (WX)	13
'Reddy-or-Not' (RON)	14
'Loren Legarda' x 'Estrella F. Alabastro' (LL x EFA)	47
'Gelia Castillo' (GC)	51

daybreak and begin to close in the afternoon, indicating that the plant has only a 1-d floral duration (Purseglove 1968). However, with conventional plant breeding, some varieties have been developed with flowers that could stay on the plant longer, e. g., GC and LL x EFA. Identification of these breeds as 2-d floral duration varieties indicates that a hibiscus cultivar could have more flowers at any one time, making it prolific, and thus it can become an attractive landscaping material. Since 2-d longevity varieties are already available, their hybridization could result in 4-d or 5-d retention varieties, provided that the gene/s for floral retention sustain additive effects. Since this could be possible, it is expected that *Hibiscus* could be used for cutflower production for flower arrangements.

With regard to the biology of *Hibiscus*, floral retention could be considered as a species-level variability allowing the use of this species as a model for determining variation of floral retention. Having in mind this variability will allow anyone to infer how abscission progresses intra- and inter-specifically. Specifically, it will allow anyone to understand how retention varies from one variety to another, and from one species to another in ephemeral flowers such as *Hibiscus*. In addition, this variability will allow the elucidation of the mechanisms necessary to lengthen or prolong the floral retention on the plant. This will also provide a means to understand abscission and exploit possible ways to control it via breeding and selection or by chemical manipulations.

The different varieties of breeds of *H. rosa-sinensis* showed variability in their floral retention length (Table 1). The varieties with greater floral retention length, namely, LL x EFA and GC, had a greater chance of being pollinated, thus increasing their reproductive fitness. Since they can stay longer on the plant, they can be admired more by the on-lookers, making the plant always flowery since the flowers that bloom in a day could be an addition to those that bloom the following day. This in turn increases the value of *Hibiscus* as an ornamental plant. However, due to the variability in floral retention, Marques and Draper (2012) stated that one problem of being an ephemeral flower is the risk of being not pollinated. This condition should be overcome to perpetuate the species, which is fundamental to reproductive fitness. This nature of variability in floral retention is a determinant of their individual chance of being pollinated under natural conditions, which has been proven under experimental conditions (Ratchke 2003; Abdala-Roberts et al. 2007; Giblin 2005).

Morphology and Floral Retention

The eight morphological characters assessed, namely: peduncle diameter at base, median, and attachment to receptacle; peduncle length; receptacle diameter; and petal thickness at attachment site and at the median portion of the four varieties, could be related to floral retention (Table 2). In terms of peduncle diameter at the base (Table 2), GC and LL x EFA had 3.11 mm and 3.00 mm, respectively, indicating that there was no significant difference between the two breeds. RON had a mean diameter of 1.94 mm and WX had 1.35 mm across the base of the peduncle, which resulted in a grouping of A (GC and LL x EFA), B (RON), and C (WX). In terms of diameter at the median of the peduncle (Table 2), variation was observed. There was a significant difference between the four breeds with regard to this trait. For example, the median peduncle diameter in GC was 2.16 mm, 1.90 mm in LL x EFA, 1.16 mm in RON and 0.94 mm in WX. In terms of the diameter at the proximal region of the peduncle going to the receptacle (Table 2), there was also a significant difference between the four breeds. For instance, GC had 3.21 mm for this trait, 3.07 mm for LL x EFA, 1.90 mm for RON, and 1.27 mm for WX. Peduncle length also varied significantly between the four breeds with RON having the longest while GC had the shortest. This result suggests that this trait is a distinct character of each breed that may not be related to the duration of floral retention.

With regard to measurement of the morphological characters of the flowers more directly related to petal retention, significant differences were observed between the four breeds in terms of receptacle diameter and petal thickness at the attachment site and median portion. In terms of

Table 2. Morphological characters of the flowers of the four *Hibiscus rosa-sinensis* varieties WX, RON, LL x EFA and GC. Values presented are means and SD of 30 flower samples of each variety.

Morphological Character		Breed			
		WX	RON	LL x EFA	GC
Peduncle diameter	Base	1.35 ± 0.31	1.94 ± 0.20	3.00 ± 0.44	3.11 ± 0.38
	Median	0.94 ± 0.19	1.16 ± 0.16	1.90 ± 0.22	2.16 ± 0.41
	Attachment	1.27 ± 0.21	1.90 ± 0.20	3.07 ± 0.28	3.21 ± 0.32
Peduncle length		38.95 ± 8.42	61.45 ± 4.64	45.16 ± 10.40	28.36 ± 13.80
Receptacle diameter		3.12 ± 0.32	3.45 ± 0.34	6.99 ± 0.82	8.49 ± 0.57
Petal thickness	Attachment	0.57 ± 0.12	0.84 ± 0.09	1.12 ± 0.14	1.56 ± 0.20
	Median	0.22 ± 0.07	0.30 ± 0.10	0.49 ± 0.15	0.71 ± 0.11

GC – 'Gelia Castillo', LL x EFA – 'Loren Legarda' x 'Estrella F. Alabastro', RON – 'Reddy-or-Not', WX – 'Wilcox'

receptacle diameter, GC had the highest mean diameter with 8.49 mm across the receptacle area, where the petals are attached to, and this was followed by LL x EFA with 6.99 mm. RON had a 3.45 mm mean receptacle diameter, while WX had 3.12 mm. Significant differences between the four breeds were also observed for petal thickness at the attachment site and at the median portion (Table 2). For instance, GC had 1.56 mm petal thickness at the attachment site and LL x EFA had 1.12 mm. In contrast, RON had 0.84 mm and WX had 0.57 mm. For petal thickness at the median area, GC had 0.71 mm and LL x EFA had 0.49 mm, while RON and WX had only 0.30 and 0.22 mm, respectively. These results indicate that 2-d floral retention varieties had thicker petals at the attachment site and at the median portion of the flower, compared with those of 1-d floral retention varieties.

Majority of the values for the different morphological traits, except for peduncle length, showed nearly a uniform decreasing trend with GC having the highest, followed by LL x EFA, RON and WX. However, for peduncle length, RON had the longest (61.45 mm), followed by LL x EFA (45.16 mm), WX (38.95 mm), and GC (28.36 mm) had the least. The variation in morphological characters suggests that the length of floral retention may also vary within the traditional 1-d or 2-d breeds. It was observed that 1-d breeds had 1–2 h and 2-d breeds had 3 h floral retention. Hence, this clarifies the differences between 1-d and 2-d breeds in terms of floral retention on the plant.

Some morphological characters were positively correlated with the length of floral retention of the four breeds (Table 3). These characters were the following: peduncle diameter at the base, median and the attachment site of the receptacle; receptacle diameter; and petal thickness at the attachment site and at the median portion. The measurements or values for these morphological traits tended to increase as the floral retention of the breeds increased from 13 h as observed in WX, 14 h in RON, 47 h in LL x EFA, and 51 h in GC. Generally, the trend for floral retention of the four breeds was as follows: GC > LL x EFA > RON > WX, where GC had both the greatest measurements for morphological characters and the most enduring floral longevity. GC was followed by LL x EFA, then RON, and WX was the last with lowest measurements for the characters and for shortest duration of floral retention. This result suggests that the measurements for these traits are directly proportional to the floral retention in the four breeds. In addition, the results suggest that in particular receptacle diameter and petal thickness are useful morphological traits or good indicators or markers for selection of varieties with longer floral retention in *Hibiscus* breeding work. Moreover, these results suggest that by simply looking at the receptacle of the flower, one can visualize whether the flower will be a 1-d or a 2-d bloom. Furthermore, by just having a feel or touching the petals, one can evaluate whether the flower is thick or thin, and finally, one can tell whether it is a 1-d or a 2-d bloom.

This trend (i.e. GC > LL x EFA > RON > WX) was not just observed in morphological measurements but also in the length of floral retention (Table 1). It suggests that floral retention could indeed have some bearing on morphological traits such as receptacle diameter, petal thickness and peduncle diameter. This finding corroborated an earlier study which indicated that a floral part is correlated with other floral parts. For instance, peduncle diameter could indicate the size of the inflorescence of *Philodendron* (Chonteau and Barabe 2006).

Pearson's correlation analysis showed a more profound relationship between floral retention and the different morphological characters (Table 3). Peduncle diameter, receptacle diameter and petal thickness were correlated very strongly and positively with differential floral retention across the four *Hibiscus* breeds. Only peduncle length had a moderate negative correlation with floral retention.

Table 3. Pearson's correlation showing the r-values across morphological characters versus floral retention.

Morphological Character		r-value	Interpretation
Peduncle diameter	Base	0.8761	very strong, positive
	Median	0.8775	very strong, positive
	Attachment	0.9236	very strong, positive
Peduncle length		−0.4545	moderate, negative
Receptacle diameter		0.9612	very strong, positive
Petal thickness	Attachment	0.8426	very strong, positive
	Median	0.8045	very strong, positive

The results suggest that the characters exhibiting a very strong positive correlation may be used to predict measurements for morphological characters of breeds with longer flower retention on the plant. The results on the morphological characters and their relationships with the length of floral retention in *Hibiscus* are non-existent in recent literatures and are first reported in this study.

Anatomy of the Abscission Zone

There are significant differences in the anatomy of the receptacle area between its bud stage and after its abscission (Fig. 2). Most evidently, there exists some portion of the tissue originally from the attachment of the petals that remained even after abscission. The abscission zone that is being referred to in this study is the petal abscission zone. In the present study, no well-differentiated or distinct abscission zone was observed at the junction of petals and the receptacle prior to, and after abscission in the four breeds of *Hibiscus* (Fig. 2). This result agrees with the findings of Kozlowski (1973) who reported that usually in petals, no distinct abscission zone is apparent but it is still existent. However, in the present study, it is interesting to note the presence of remnants of the petal after abscission, and this portion would then be referred to as the 'petal remnant' (Fig. 3).

The petal remnants of the four breeds varied in length (Fig. 4). A similar observation was reported in *Arabidopsis* petal and sepal abscission by Gubert et al. (2014). In the present study, upon closer analysis of the zone where the petals abscised, it was observed that in the particular area where the petal remnants were located, these remnants were found to be longer in the two breeds, i.e. GC and LL x EFA, which have a longer retention time than the other two breeds, i.e. WX and RON, which have a retention time of only 1 d. In particular, the petal remnant was longest in the 2-d breed GC with 1.22 mm (Fig. 4), followed by LL x EFA with 1.08 mm-long petal remnant at the abscission area of the petal. In contrast, the 1-d retention varieties RON had 0.48 mm petal remnant while WX had the shortest petal

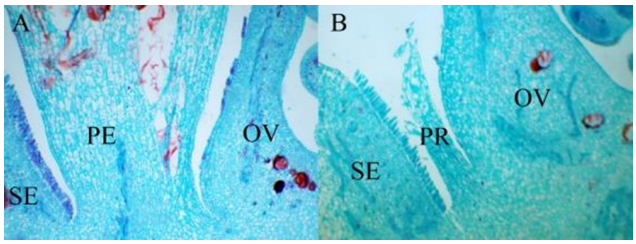

Fig. 2. Anatomy of the receptacle area in *Hibiscus rosa-sinensis* L. during: A) bud stage and B) after abscission. [SE – sepals; OV – ovary; PE – attachment of petals to the receptacle; and PR – petal remnant].

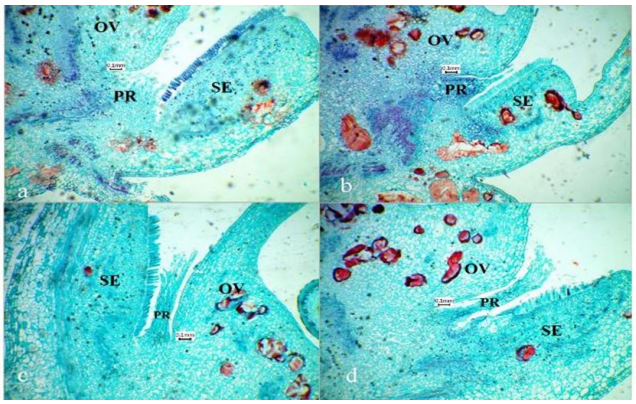

Fig. 3. Petal remnants across *Hibiscus rosa-sinensis* L. breeds visualized through light microscopy (l.s.) aided by ToupView: a) Wilcox (WX), b) Reddy-or-Not (RON), c) Loren Legarda x Estrella F. Alabastro (LL x EFA) and d) Gelia Castillo (GC). [40X l.s.; OV – ovary; PR – petal remnant; SE – sepal; Scale bars = 0.1 mm].

remnant with only 0.22 mm. The length of the petal remnant corresponds to the number of hours of flower retention with GC having the longest (51 h), followed by LL x EFA with 47 h, then RON with 14 h and WX having the shortest with 13 h. This result suggests that the length of petal remnant across breeds has some bearing on floral retention of the plants in the four breeds studied.

The results mentioned above suggest that abscission did not occur completely at the base where the abscission zone is located. At the base of the petal, there were ruptured areas originating at the petal ends. At these areas, abscission perhaps should

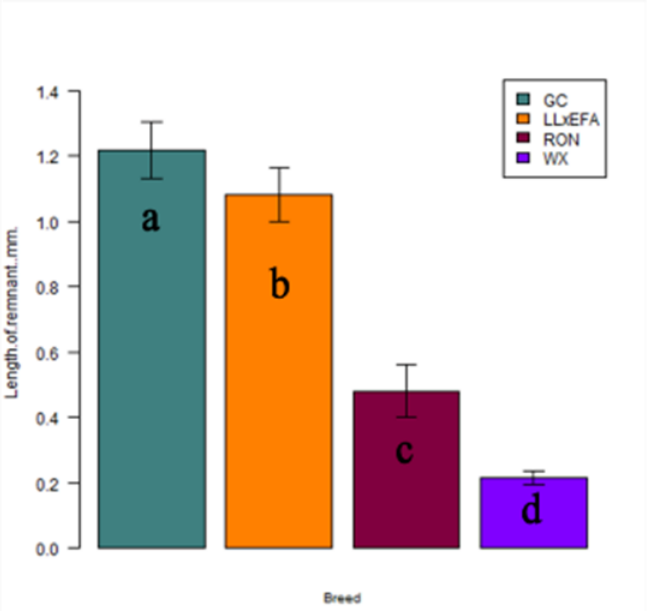

Fig. 4. Mean length of petal remnant across *Hibiscus rosa-sinensis* L. breeds. Data are values of five flower samples per breed and vertical bars indicate CI = 95%. Bars with different letters are significantly different at $LSD_{0.05}$.

have taken place. An alternative explanation to the site of abscission could be explained by the forces acting on the petals. It is possible that there is a greater stiffness in the abscission area where there is a weaker portion that is easier to shear than the base. As one goes along the length of the flower, thickness consequently decreases. This implies that there would be weaker support at the petal end than at the basal portion of the flower which has the strongest support. Consequently, it is possible that in the 1-d floral retention breed, the petal remnants are shorter, implying lower strength of the petal.

Initially at the attachment portion of the petals to the receptacle, there was a broad area of attachment, but as this attachment progressed through abscission, this area became pronouncedly narrow. In the petal remnant, the cells appear elongated with very narrow width. This resultant narrowing of the cells is a characteristic of stretching (Bansal 2009; Bassin et al. 1979). Considering the mechanics of petal attachment, when an elastic material such as the cell wall is subjected to forces such as gravity and even its own weight, which in this case are pulling the petals, the cells tend to stretch due to these forces (Bansal 2009; Bassin et al. 1979).

Furthermore, stretching or elongation of cells is possible due to the forces acting on the petals. It has been known that mechanical forces (especially gravity) acting on the petals help in the process of abscission (Addicott 1973). It was hypothesized that with these forces, the cells were stretched until their stiffness could be overcome by shearing forces causing abscission with the help of degradative enzymes.

In addition, the cells of the petal remnant are longer, which is different from the rest of the surrounding cells (Fig. 5). Moreover, at the base where the petals should have abscised (Berg and Gibernau 2008; Roberts et al. 2000; van Doorn and Stead 1997), there was a severed area which could have been the area of abscission. Around this supposed abscission zone, there is no deposition of lipid-based chemicals. This claim was based on the Safranin O staining where there are no red-stained areas around the supposed abscission zone. Instead, this zone and its surrounding areas were blue-green when stained with fast green, suggesting that they contained carbohydrate-based compounds such as cellulose.

Ethylene Concentration across Breeds

The average amount of ethylene produced varied from one breed to another (Table 4). As the retention of the flowers in each breed increased from 13 h in WX, 14 h in RON, 47 h in LL x EFA to 51 h in GC, the average amount of ethylene produced also increased. However, *H. sinensis* 'Gelia Castillo', which has the longest floral retention of 51 h, had the lowest amount of ethylene production. This result may suggest that ethylene evolution is genotype-specific, regardless of the duration of floral retention.

Regarding the amount of ethylene produced at each floral stage of the four varieties, whether it is a 1-d or a 2-d retention variety, ethylene concentration increased from pre-anthesis to abscission. This result could be one of the factors that contributed to the closure of the flowers followed by abscission. All four varieties produced low levels of ethylene at pre-anthesis, anthesis and closure stages. However, at abscission stage, ethylene tended to rise abruptly. Among the four breeds, LL x EFA, a 2-d retention breed, produced the highest amount of ethylene while WX, a 1-d retention breed, had the lowest amount of ethylene production.

Since there was a constant emission of ethylene from pre-anthesis until closure of the flowers in the four breeds, it is notable that the amount

Table 4. Ethylene concentration (ppm) produced by the four *Hibiscus rosa-sinensis* breeds at each floral stage. Values presented are means with SD.

Breed	Ethylene Concentration (ppm)				
	Pre-anthesis	Anthesis	Closure	Abscission	Mean
WX	0.0484 ± 0.114	0.0577 ± 0.115	0.0856 ± 0.115	0.16890 ± 0.113	0.1334 ± 0.114
RON	0.0601 ± 0.057	0.0808 ± 0.056	0.1000 ± 0.059	0.27160 ± 0.057	0.0901 ± 0.057
LL x EFA	0.02149 ± 0.296	0.03846 ± 0.299	0.06319 ± 0.297	0.63545 ± 0.298	0.1896 ± 0.298
GC	0.03388 ± 0.075	0.04017 ± 0.076	0.04734 ± 0.076	0.17330 ± 0.074	0.0684 ± 0.075

GC – 'Gelia Castillo', LL x EFA – 'Loren Legarda' x 'Estrella F. Alabastro', RON – 'Reddy-or-Not', WX – 'Wilcox'

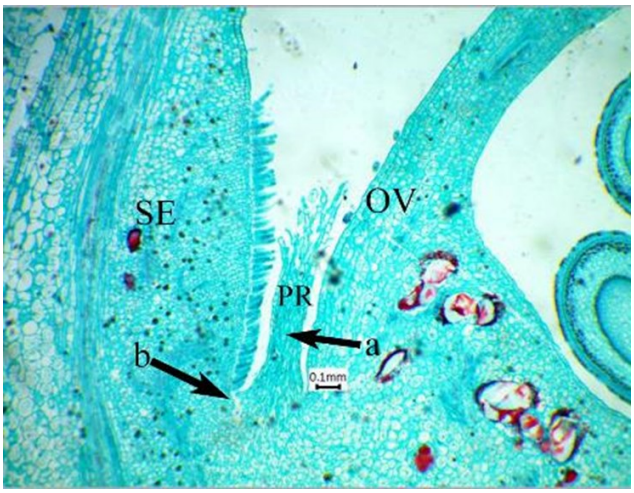

Fig. 5. Appearance of cells in *Hibiscus rosa-sinensis* L. at the petal remnant (arrow a) and at the supposed area of abscission (arrow b). [40X l.s., OV – ovary; PR – petal remnant; SE – sepal; Scale bar = 0.1 mm].

accumulated suddenly peaked after flower closure followed by petal abscission from the receptacle. This peak in ethylene production at the time of abscission could be attributed to the known auto-catalytic synthesis of ethylene wherein the gas utilizes itself to activate the biochemical synthetic pathway for its production through activation of specific genes necessary for its synthesis (Shibuya et al. 2000; Wagstaff et al. 2005).

CONCLUSION

This study investigated the factors feasibly affecting floral retention in 1-d and 2-d retention *Hibiscus* breeds. The morpho-anatomical assessment of characters included factors such as peduncle diameter at base, at median and at nearest site to the peduncle; peduncle length; receptacle diameter; and petal thickness at attachment site and at median. The progression of abscission through longitudinal sections, the anatomy of the abscission zone, and the production of ethylene during the floral life across the four breeds were also assessed.

The four breeds differed significantly in morphological characters such as peduncle diameter, receptacle diameter and petal thickness. In general, these characters had the highest values in GC, followed by LL x EFA, RON and WX which had the lowest values. The trend was GC > LL x EFA > RON >WX. This trend was also observed in terms of duration of floral retention where GC had the longest with 51 h, followed by LL x EFA with 47 h, then RON with 14 h and WX with 13 h which had the shortest.

There was no distinct abscission zone observed for the four varieties. However, there were remnants of the petals after abscission. These petal remnants were longer for the 2-d retention varieties such as GC and LL x EFA than the 1-d retention varieties such as RON and WX. The cells in the petal remnants appeared to be stretched or elongated because of the external forces acting on the petals. Petal remnants were longest in GC with 1.22 mm, followed by LL x EFA with 1.09 mm, RON with 0.48 mm while WX had the shortest with 0.22 mm, following the same trend observed for morphological traits and duration of floral retention.

Ethylene production in 1-d retention breeds, i.e. RON and WX, and 2-d retention breeds, i.e. GC and LL x EFA, increased gradually from pre-anthesis up to the closure stage of the flower. However, ethylene production increased abruptly during abscission following the auto-catalytic pattern of ethylene biosynthesis. The average amount of ethylene evolved by each breed differed from one another, suggesting that ethylene evolution may not be dependent on the duration of floral retention; instead, it could be genotype-specific.

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